VI Chemical and Biological Engineering Doctoral Symposium

BIOTECHNOLOGY IN TRANSFORMATION

Shaping Science and Industry to Global Challenges

BOOK OF ABSTRACTS

2nd - 3rd of October 2025
University of Minho, Braga, Portugal



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"Biotechnology in Transformation: Shaping Science and Industry to Global Challenges"

Braga, Portugal
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SCHEDULE

2nd October

08:30	Registration and Welcoming
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Food & Feed Panel	
09:30	"From Science to Industry: How Colab4Food Drives Food Innovation" - Alexandra Azevedo
09:50	"Aptamer-Based Strategies for Ensuring Food Safety under Climate Change" - Ricardo Oliveira
10:15	"Antibiotics in honey: a comprehensive analysis in occurrence and risk assessment" - Helena Rodrigues
10:30	Coffee Break
Environmental Panel	
11:00	"Starting Over and Discovering: A True Story of Scientific Life" - Zlatina Genisheva
11:20	"From Waste to Resource: Biophysical Strategies to Respond to Global Challenges in Agriculture and Biomedicine" - Margarida Fernandes
11:45	"Ultrasound-Assisted Processes for Sustainable Removal of Contaminants of Emerging Concern in Wastewater: A Case Study with Bio-Based Adsorbents" - José Fernandes
12:00	Lunch Break
Health Panel	
14:00	"Target-based antibiotic discovery" - Tiago Beites
14:20	"New 2D-nanomaterials for Biomedical Applications" - Artur Pinto
14:45	"Shaping patient-specific bioengineered intestinal niches" - Mafalda Neto
15:00	Coffee Break & Poster Session
15:30	Workshop "The art of the science pitch: Tools and techniques to make your reaseach stand out with" - Carlos E. Costa
16:30	End of the Day

3rdOctober

09:00	Registration and Welcoming
Industrial Biotechnology Panel	
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09:35	"Navigating the Crossroads of Bioengineering on the Path to ndustrial Biotechnology" - Tatiana Aguiar
10:00	"Peroxisome engineering towards de novo y-decalactone production in *Ashbya gossypii*" - Miguel Francisco
10:15	Coffee Break
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15:30	"Commercial solutions for biotechnology" - Tiago Patinha (SOLÍTICA)
16:00	Awards Session & Closing Session – Bianca Marques
16:30	End of the Day

Acknowledgments

The editors and organizing committee would like to express their gratitude to all participants and organizations for their involvement, particularly:

- Centre of Biological Engineering (CEB).
- University of Minho (UM).
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- The PhD Program Director, Dr. Joana Azeredo.
- The Associate Professor, Dr. Lígia Rodrigues – moderator of roundtable.
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- All the members of the scientific evaluation committee.
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- All the sponsors and media partners.
- To all those who supported us—before or during the event, directly or indirectly!

This Book of Abstracts for the VI CBEDS 2025 reflects the outstanding contributions of young scientists and PhD students engaged in diverse research areas of Biotechnology.

The organisers also hope that the VI CBEDS 2025 served as a fruitful platform for the exchange of ideas and will be remembered as a significant milestone in the ongoing tradition of Chemical and Biological Engineering Doctoral Symposium editions.

Preface

Chemical and Biological Engineering has been significantly growing as an interdisciplinary biotechnological and scientific field, providing enormous challenges and opportunities for both academy and companies.

The Chemical and Biological Engineering Doctoral Symposium (CBEDS) has been organized annually by the students of the PhD Program in Chemical and Biological Engineering of the University of Minho, since 2020. It aims to promote the exchange of knowledge between students, teachers and researchers from the Chemical and Biological Engineering fields.

The 2025 event celebrated the 6th edition with the theme "Biotechnology in Transformation: Shaping Science and Industry to Global Challenges" and took place at the University of Minho (Gualtar Campus) on the 2nd and 3rd of October 2025. This edition aimed at showcasing the challenges and innovations in the diverse field of Biotechnology (Health, Food, Environmental and Industrial), both in Portugal and worldwide.

The program included oral and poster communications, along with a round table, aiming to promote active discussion and constructive criticism, lectures by invited speakers and workshops on Science Communication.

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FOOD & FEED

KEYNOTE LECTURES

ALEXANDRA AZEVEDO, COLAB4FOOD

From Science to Industry: How Colab4Food Drives Food Innovation

With a PhD in Food Science and Technology, Alexandra Azevedo has over 10 years of experience in research, innovation and product development. She has a background in biochemistry and bioengineering and worked on vitamin encapsulation, bioactive coatings, and lipid nanosystems. She joined Colab4Food in 2023 at Innovation and Value2Market Department, focusing on food innovation.

RICARDO OLIVEIRA, INIAV

Aptamer-Based Strategies for Ensuring Food Safety under Climate Change

Ricardo Oliveira holds a PhD in Chemical and Biological Engineering (FEUP, 2023), an MSc in Biotechnology (UM, 2017) and a BSc in Biochemistry (FCUP/ICBAS, 2015). He is currently a researcher at the National Institute for Agrarian and Veterinary Research (INIAV, I.P.), specialising in food safety and diagnostic/therapeutic solutions.

LIST OF ABSTRACTS

ORAL COMMUNICATION

Antibiotics in honey: a comprehensive analysis in occurrence and risk assessment

H. Rodrigues[1,2], R. Soares[1,2], L. Moumeni[1,3,4], M. Leite[3], M.B.P.P. Oliveira[1], A. Freitas[2,3]

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Monitoring antibiotic presence in honey samples is crucial to ensure a safe consume of this natural product being a way to prevent toxic effects and the development of antimicrobial resistance. The occurrence of antibiotic residues in honey may result from the overuse of these medicines in beekeeping practices, but also from indirect environmental sources. Unlike other foods of animal origin, no Maximum Residue Levels are defined for honey, which makes it difficult to assess compliance in honey samples and to establish clear regulatory thresholds for consumer protection. The present study aimed to evaluate the safety of honey through a multi-class approach that included compounds from several different families, namely β-lactams, quinolones, macrolides, sulphonamides, tetracyclines, lincosamides, and diaminopyrimidines. Antibiotics were extracted using an optimized and simple QuEChERS method, with 0.1% Formic acid in ACN:H_2O (80:20, v/v) as the extraction solvent, followed by a salt addition step (1 g of NaCl and 2 g of $MgSO_4$). Analytical determination of the target antibiotics was carried out using two detection approaches: Ultra High-Performance Liquid Chromatography coupled to Time-of-flight mass spectrometry (UHPLC-ToF-MS) and UHPLC coupled to Tandem mass spectrometry using a QTrap detector (UHPLC-QTrap-MS/MS). The methods were validated according to the performance criteria established in Commission Implementing Regulation 2021/808. Two distinct groups of samples were studied for antibiotic contamination: one included ten samples obtained directly from beekeepers in ten regions of Bejaia Province, Algeria, while the other consisted of fifty-four commercially available samples purchased from local supermarkets in Portugal. All Algerian samples were positive for at least one antibiotic, with cefazolin, cloxacillin, difloxacin, enoxacin, lincomycin, and norfloxacindetected in all samples. Average concentrations ranged from 0.65 to 42.8 $\mu g \cdot kg^{-1}$, and maximum concentrations ranged from 0.75 to 57.45 $\mu g \cdot kg^{-1}$ (for trimethoprim and cloxacillin, respectively). Different contamination levels were found in the Portuguese samples, with four different antibiotics detected. These included two positive samples for flumequine (3.70 and 8.28 $\mu g \cdot kg^{-1}$) and tildipirosin (5.25 and 5.50 $\mu g \cdot kg^{-1}$), while erythromycin and oxolinic acid were each detected in one sample at 0.45 and 7.86 $\mu g \cdot kg^{-1}$, respectively. A risk assessment analysis was subsequently performed to evaluate the potential health hazards for consumers and to provide scientific evidence to support regulatory decision-making.

POSTER COMMUNICATIONS

New Protein-rich Foods: Nutritional value and impact on human health

Cordeiro, David M[1,2]; Pereira-Wilson, Cristina[1,2,3]; Teixeira-Guedes, Catarina[2,3]

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The growing global population and increasing demand for sustainable protein sources are driving the research for alternative solutions to meet future nutritional challenges. This study investigated various emerging protein sources, including insects (*Tenebrio molitor* and *Alphitobius diaperinus*), fermented foods (Tempeh), mycoproteins (Quorn), and pulses (Cowpea and Sprouted Cowpea). Their nutritional composition, digestibility, effects on cell growth and antioxidant activity were evaluated.

Chemical analysis showed high protein content in all samples, with insects, Tempeh, and Quorn having the highest concentrations (48%-56%). Germination increased protein content in Cowpea.

Osborne fractionation further highlighted clear differences in protein distribution: insects presented elevated albumins and prolamins, with *A. diaperinus* also showing a notable glutelin-like fraction; cowpea and sprouted cowpea were rich in albumins, with sprouting markedly increasing globulin levels; tempeh contained the highest albumin and prolamin concentrations, while Quorn exhibited a balanced profile across protein fractions.

Samples were submitted to INFOGEST *in vitro* digestion, which demonstrated high digestibility and the bioactive peptides confirmed by SDS-PAGE and FPLC analyses. These findings were corroborated with an increased antioxidant activity, as determined by ABTS, DDPH and FRAP colorimetric assays. Digested samples significantly promoted cell growth in human cell lines (RKO, HEP-G2, and CCD 841), with varying responses across cell types. Additionally, in HEP-G2 cells treated with digested extracts from *T. molitor*, *A. diaperinus*, and Tempeh, a marked reduction in baseline reactive oxygen species (ROS) levels and H_2O_2-induced ROS production was observed, likely attributable to bioactive peptides.

Overall, these results underscore the potential of alternative protein sources as nutrient-dense, functional foods with health-promoting properties. They offer promising avenues for supporting cellular health, mitigating oxidative stress, and contributing to the development of sustainable food systems.

Personalised Nutrition for Type-2 diabetes: Unlocking Bioactive Compounds Potential Through Encapsulation

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Type 2 diabetes (T2D) is a serious chronic health condition, affecting over 537 million people worldwide. The use of natural compounds such as bioactive peptides and polyphenols (*e.g.*, resveratrol) in personalized functional foods show promise for managing T2D. However, incorporating these compounds into food matrices remains challenging due to their low stability and bioavailability, which hindering a targeted release. Our research focuses on developing nanoencapsulation systems to overcome these limitations by protecting bioactives from degradation, enhancing their functionality, and enabling controlled gastrointestinal release. Enzymes such as α-glucosidases play a key role in glycaemic control by regulating carbohydrate absorption and modulating blood glucose levels. Bioactive peptides inhibiting these enzymes help reduce intestinal carbohydrate uptake and support blood glucose regulation. Polyphenols are the most abundant antioxidants in the human diet and have demonstrated potential in reducing the risk of developing type 2 diabetes (T2D). Resveratrol, a non-flavonoid polyphenol, is widely studied for its potent anti-diabetic effects, particularly its ability to modulate hyperglycaemia-induced oxidative stress. But, to optimise their efficacy and preserve sensory qualities, polyphenols require appropriate encapsulation to prevent degradation and non-specific interactions during food processing. Emulsions can be an excellent alternative to improve bioactives solubility and stability, and stable protein emulsions may offer protection from oxidation and improve bioavailability. However, nanoemulsions can be unstable in the liquid form, limiting their application in food formulations. Drying the nanoemulsions could be a good method to increase their stability and storability, and facilitate dosage, enabling them to be applied in broader food applications. We have successfully developed innovative nanocapsules providing gastric resistance and targeted intestinal release of α-glucosidase inhibitory peptide and resveratrol. These findings offer promising potential for the development of functional foods specifically designed for the management and treatment of T2D.

Thermal tolerance of *Talaromyces trachyspermus* ascospores: a comparison of ascocarp-protected and free spores

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Heat-resistant moulds (HRM) are microorganisms known for their ability to endure high temperatures encountered during heat treatment [1,2]. HRM exhibit both an asexual phase, characterized by the production of non-heat-resistant spores called conidia, and a sexual phase, which results in the production of heat-resistant ascospores [3]. These ascospores are formed inside asci, many of which are enclosed in a large fruiting body called an ascocarp [4]. In their natural state, ascospores remain dormant and require activation by an external trigger, such as heat or high pressure, to germinate [5]. The spoilage caused by HRM is a major challenge for the fruit juice industry, resulting in considerable economic losses, and a key issue in this sector is the presence of *Talaromyces trachyspermus*. The aim of this study was to investigate the influence of ascocarp structure on the thermal activation and inactivation of *T. trachyspermus* ascospores. Specifically, we compared the heat resistance of ascospores when they are still enclosed within ascocarps to heat resistance after being released. The fruiting bodies of *T. trachyspermus* were produced on malt extract agar under different incubation periods of 2 months and 6 months at 25 °C. Thermal treatments of 80 °C and 85 °C for up to 15 minutes were carried out to determine the temperature required to activate or inhibit heat-resistant ascospores. The results showed that mechanical disruption of ascocarps prior to heat treatment led to significantly higher activation of ascospores compared to when the ascocarps were only broken after thermal exposure. This suggests that the ascocarp structure may act as a protective barrier, reducing the effectiveness of heat activation when ascospores are still enclosed during treatment.

Acknowledgements: Diana Sousa gratefully acknowledges the financial support from the Foundation for Science an Technology (FCT) through the PhD fellowship 2023.04050.BDANA.

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Sweet Waste, Smart Food: Valorizing Carob Pulp into Functional Cereals

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The pursuit of healthier diets and sustainable food systems has intensified research on functional ingredients obtained from agro-industrial by-products. Carob pulp, a sucrose- and polyphenol-rich residue from locust bean gum production, is a promising substrate for prebiotic enrichment. Fructo-oligosaccharides (FOS), derived from sucrose via enzymatic or microbial routes, selectively stimulate beneficial gut microbiota and contribute to digestive and metabolic health. This study explored in-situ strategies for converting carob sugars into FOS while enhancing antioxidant potential, with the goal of creating clean-label flours suitable for application in functional cereal products.

Carob pulp was subjected to aqueous extraction, optimized through response surface methodology considering pulp-to-water ratio, extraction time, and temperature. FOS synthesis was assessed by (i) enzymatic transfructosylation using four commercial blends (Novozym® 960, Viscozyme® L, Pectinex® Ultra SP-L, Catazyme® 25 L), and (ii) submerged fermentation with FOS-producing fungi (*Aureobasidium pullulans* CCY 27.1.94 and *Aspergillus ibericus* MUM 03.49). Extracts were analyzed for sugar and FOS composition, phenolic and flavonoid content, and antioxidant activity (FRAP, ABTS). FOS gastrointestinal stability was evaluated using the standardized INFOGEST *in-vitro* digestion protocol.

Optimized conditions enabled sucrose extraction of 85.7 ± 1.0 g/L. Among enzymatic systems, Novozym® 960 achieved the highest FOS yield (0.423 ± 0.013 $g_{FOS}/g_{initial\ sucrose}$; purity 29.6 ± 0.3%). Fermentation with *A. ibericus* also generated promising results (0.347 ± 0.003 $g_{FOS}/g_{initial\ sucrose}$). Carob extracts displayed high antioxidant capacity (FRAP: 72.1 ± 0.2 $\mu mol_{TE}/g_{DW}$; ABTS: 5.35 ± 0.05 μg/mL) and notable phenolic (8.57 ± 0.04 mg_{GAE}/g_{DW}) and flavonoid (2.74 ± 0.02 mg_{CE}/g_{DW}) content. Enzymatic treatment enhanced bioactive release, whereas fermentation modified the phytochemical profile. *In-vitro* digestion confirmed strong FOS resistance (<10% hydrolysis). Incorporation of FOS-enriched carob flours into extruded cereals was successful, producing products that retained prebiotics with acceptable texture and flavor. Stability of FOS post-extrusion will be further evaluated.

Carob pulp represents a valuable, sustainable substrate for producing prebiotic- and antioxidant-enriched flours. Enzymatic and microbial strategies allow its valorization into functional ingredients, which can be effectively incorporated into extruded cereals, supporting innovation in clean-label, health-promoting foods.

Upcycling of food industry by-products for both FOS and white *Chlorella sorokiniana* production

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The constant need for food represents a huge challenge worldwide. Moreover, the increasing search for more sustainable and yet nutritious products, has resulted in higher demand for alternative protein sources. Given their rich composition in proteins and other natural compounds, microalgae are commonly presented as a promising alternative food source to overcome such challenges. Some of these microorganisms, although photosynthetic, can grow under heterotrophic conditions in the absence of light, with high growth rates associated. However, these processes are usually expensive due to the costs associated with the use of an external sugar source, namely glucose, which hinders its application at industrial scale. Therefore, the integration of low-cost carbon sources from food industry by-products, especially those with high sugar content, present a viable alternative.

The objective of the present work was the use of sugar-rich by-products of the food industry (deriving from caramel, menthol candy, chewing gum, and sugar almonds production processes) as substrate for both the heterotrophic growth of the white microalgae *Chlorella Sorokiniana* and the production of fructooligosaccharides (FOS). To do so, enzymatic hydrolyses were carried out in pure sucrose and the by-products using a Novozym® 960 enzymatic complex, originating hydrolysates containing sugars (Fructose, Glucose, Sucrose) and different forms of FOS (1-Kestose, Nistose and 1F- Frutafuranosyl Nistose). Afterwards, diluted hydrolysates were tested as carbon source for microalgae with initial concentrations of glucose (obtained from sucrose hydrolysis) ranging from 20 to 40g/L. When using pure sucrose hydrolysates, those with glucose at 20g/L showed a growth (μ=1.432±0.028 d^{-1}, n=3) comparable to the control (μ=1.411±0.007 d^{-1}, n=3) (p>0.05), which was not verified at 30 (μ=1.337±0.027 d^{-1}, n=3) and 40 (μ=0.605±0.012 d^{-1}, n=3) g/L. Among the by-products, only almond assays presented growth (Abs (750nm)=4.583±0.215, n=3), although lower comparing to the control (Abs (750nm)=6.327±0.184, n=3) (p<0.05).

Despite the clear need for optimization, the successful growth of *Chlorella sorokiniana* using sugar almonds as low-cost substrate marks a significant breakthrough, opening the possibility for commercial medium replacement and supporting a circular economy.

Natural-based solid lipid nanoparticles suitable for vitamin E encapsulation for food applications

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Developing micro/nanostructured release systems can increase the effectiveness of food ingredients, ensure the correct dosage, and broaden their range of application. Solid lipid nanoparticles (SLNs) are promising carriers for improving the stability and bioavailability of lipophilic bioactives like vitamins. Natural ingredients such as Candelilla wax (CW) and soybean lecithin (SL) offer sustainable, safe and efficient options for SLNs formulation compared with synthetic materials. Thus, the present study focuses on optimisation of vitamin E-loaded SLNs formulations, on evaluation of their stability and vitamin E bioaccessibility under *in vitro* digestion, aiming to support their application in functional food systems.

A Central Composite Rotatable Design was implemented to optimise CW (1.5% - 3%) and SL (1.5% - 3%) concentrations. The stability and physicochemical properties of SLN, namely particle size, z-potential, PDI, and morphology, were evaluated over a month.

The vitamin E-loaded SLNs were subjected to an *in vitro* digestion process using the harmonised INFOGEST digestion method. Vitamin E-loaded SLNs bioaccessibility (*i.e.* vitamin E fraction found in the micellar phase) and stability were measured after the *in vitro* digestion process.

Optimised vitamin E-loaded SLNs displayed z-potential values around -22 mV, particle size around 220 nm, and PDI around 0.180. Additionally, SLNs' stability over the storage period was validated (z-potential, particle size and PDI approximately -25 mV, 230 nm, and 0.160, respectively).

This study was inspired by the need to create sustainable, safe, and natural SLNs for functional food applications. The findings indicated that SL and CW may be used as SLNs wall materials, which would serve as carriers for bioactive substances as vitamin E.

Veterinary Medicines In Animal Farm Samples: Food Safety In A One Health Perspective

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The growing intensification of livestock production has been associated with the widespread use of veterinary pharmaceuticals, particularly antibiotics and anti-helminthic. While essential for animal health management, their frequent application raises concerns regarding the persistence of drug residues in food products and their dissemination into the environment. Such contamination may contribute to the emergence of antimicrobial and antiparasitic resistance, representing a major challenge for human, animal, and ecosystem health under the "One Health" perspective.

To investigate these issues, broad-spectrum analytical approaches were established, allowing the simultaneous detection and quantification of multiple veterinary drug residues in both animal-derived products and environmental matrices from farming systems. As part of this assessment, 10 raw milk samples collected directly from animal farms were analyzed, providing critical evidence of potential consumer exposure. The findings contribute to a more comprehensive understanding of contamination pathways, support risk evaluation, and emphasize the importance of implementing sustainable and responsible practices in intensive food production to minimize pharmaceutical pollution and its associated risks.

Acknowledgements: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. This work was also supported by Plano de Recuperação e Resiliência (PRR) through the project PRR-C05-i03-I-000190 – RumiRes: Epidemiological Surveillance and Awareness of Antimicrobial Resistance and Drug Residues in Small Ruminants in the Central Region.

Identification of novel high-affinity aptamers targeting *Salmonella Enteritidis*

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Salmonellosis is one of the most reported foodborne diseases, with the consumption of eggs and egg products being the main source of outbreaks. *Salmonella* infections are estimated to cause an overall economic loss exceeding €3 billion per year, including productivity losses for poultry farmers, supermarket recalls, and veterinary treatment costs. The prophylactic use of antibiotics was one of the strategies to control *Salmonella spp*. infections in poultry. However, the spread of antimicrobial resistance and the consequent restrictive legislation implemented by the European Union have severely limited the use of antibiotics in this field. Thus, the food industry is seeking alternative control measures to prevent *Salmonella spp.* contamination and reduce the incidence of human salmonellosis. Meanwhile, aptamers have emerged as promising antimicrobial agents, showing strong neutralizing activity against pathogenic microorganisms. These single-stranded oligonucleotides fold into defined three-dimensional structures, allowing them to bind target molecules with high affinity and specificity.

Aptamers specific for FimH protein (*S. Enteritidis* adhesion protein, responsible for the initial contact of *Salmonella spp*. with hens' intestinal cells) were generated by Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Carboxylated magnetic beads were functionalized with the target protein through EDC/NHS coupling and incubated with an ssDNA library. DNA sequences that bound the target were separated, PCR-amplified, and used in the next SELEX round. After 12 iterative rounds, the enriched ssDNA pool from the 3rd, 7th and last round was subjected to NGS and analyzed with the bioinformatics solution AptaSUITE.

In the final ssDNA pool, five major sequence-similarity clusters were identified, and the most abundant sequences from each cluster were selected for further analysis and aptamer production. The *in silico* analysis of 8 selected aptamers yielded promising results for the aptamers' stability, with Gibbs free energy (DG) values ranging from - 12.67 to -20.73 kcal/mole, as well as high GC content and the presence of G-quadruplexes structures.

In this work, aptamers targeting the FimH adhesin were successfully selected, indicating strong enrichment of high-affinity binders over 12 SELEX rounds. These findings pave the way for potential applications in the control of *Salmonella* infections.

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Electrohydrodynamic jet printing for the development of HPMC-based microstructures: design and optimization

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Electrohydrodynamic (EHD) jet printing is a new micro-additive manufacturing technology that uses electric fields to precisely deposit material through a nozzle, achieving high resolutions with high viscosity inks. Seeing as it is a recent technology, bio-based inks have yet to be designed and optimized. Hydroxypropyl methylcellulose (HPMC) stands out as a biodegradable biopolymer with excellent compatibility, paving the way for sustainable smart packaging sensors.

In this work, solutions with different concentrations of HPMC (1%, 2% and 3%) in ethanol (from 0% to 90%) were evaluated and characterized in terms of viscosity, surface tension and conductivity, and used in printability tests using a home-made EHD jet printer. Certain parameters of the EHD jet printer were fixed, such as flow rate (28.28 $\mu l\ h^{-1}$, corresponding to a shear rate of 10 s^{-1} in the nozzle type), working distance (1 mm), substrate (glass with 100 nm layer of tungsten-titanium) and the nozzle diameter and material (200 μm, stainless steel). The speed was varied between 1 mm s^{-1} and 15 mm s^{-1} and the voltage was manipulated between 1.5 kV and 2.5 kV until Taylor's Cone formation.

Then, a printability ternary graph (water-ethanol-HPMC) was obtained, selecting HPMC-based bioinks that achieved higher resolutions (dots and lines as small as 50 μm, determined by microscopy), showed less clogging and reproducible results. The printable zone was obtained from concentrations between 1 and 2% HPMC in 10%-50% ethanol. On this range, the bioinks present viscosities of 13 mPas to 100 mPas, surface tension of 29 mNm to 42 mNm and conductivities of 16 $\mu S\ cm^{-1}$ to 69 $\mu S\ cm^{-1}$.

Overall, the results show the potential of using HPMC to develop bioinks compatible with EHD jet printing, foreseeing their use on food and biomedical applications.

Algal Lipids-Loaded Nanoemulsions: Formulation, Stability, and Oxidation Profile

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Algal lipids, particularly those rich in polyunsaturated fatty acids, have attracted interest as functional ingredients due to their health benefits and potential as sustainable alternatives to fish oil. However, their use in foods is limited by poor stability and high susceptibility to oxidation. Nanoemulsions (NEs) have emerged as promising strategies to overcome these limitations. In this study, we developed and characterized NEs for the encapsulation of a food-grade algal lipid extract (LE) obtained from a commercial algae blend for food applications. LE was produced by ultrasound-assisted extraction with ethyl acetate and characterized for its composition. NEs were formulated using medium-chain triglycerides (MCT) or sunflower (SUN) oil and stabilized with rhamnolipids (0.05 wt%) as biosurfactant. NEs with 1 wt% of lipid phase were prepared through pre-homogenization followed by high-energy ultrasonication. Control NEs, without LE, were also prepared. Physicochemical properties, including droplet size (DS), polydispersity index (PDI), zeta potential, and pH stability, were evaluated. The degree of lipid oxidation after production was assessed using the thiobarbituric acid reactive substances (TBARS) assay. LE–NEs showed favorable physicochemical characteristics, with DS<200 nm (MCT: 137.4 ± 2.2 nm; SUN: 163.8 ± 2.9 nm), low PDI (MCT: 0.205 ± 0.026; SUN: 0.237 ± 0.014), and highly negative zeta potential (MCT: –70.9 ± 3.2 mV; SUN: –70.2 ± 3.3 mV), suggesting good colloidal stability. Notably, LE–NEs exhibited a smaller DS compared to controls, indicating an effect of the LE within the system. Additionally, LE–NEs demonstrated superior resistance to pH-induced destabilization (pH 2.5–9) compared to controls, supporting their potential for incorporation into diverse food matrices. Lipid oxidation, assessed by TBARS, showed values of 2.80 ± 0.03 and 2.48 ± 0.05 μmol MDA L^{-1} emulsion for MCT–NE and SUN–NE, respectively, indicating a low to moderate level of oxidation and confirming that formulations preserved lipid integrity, with slightly higher oxidation in MCT–NE. In conclusion, the encapsulation of algal lipids in NEs resulted in stable formulations with favorable physicochemical properties and preserved lipid integrity, highlighting the potential of LE–NEs as versatile delivery systems for functional food lipid ingredients.

ENVIRONMENTAL

KEYNOTE LECTURES

ZLATINA GENISHEVA, CVR

Starting Over and Discovering: A True Story of Scientific Life

Zlatina Genisheva is a Principal Investigator at CVR. Holding a PhD in Chemical and Biological Engineering, she has extensive experience in chromatography, wine fermentations, and sustainable extraction processes. She is involved in several international projects focused on waste valorisation.

MARGARIDA FERNANDES, CMEMS

From Waste to Resource: Biophysical Strategies to Respond to Global Challenges in Agriculture and Biomedicine

Auxiliary Researcher at Centre for MicroElectromechanical Systems (CMEMS) at UM, is a Principal Investigator of 3 on-going projects for the development of stimuli-responsive biomaterials for advanced applications in agriculture and health. She is currently the coordinator of a Horizon-RIA (HORIZON-CL4-2023-RESILIENCE-01-TWO-STAGE) project VINNY: “Advanced nanoencapsulation of bio-based pesticides and fertilizers for a circular and sustainable viticulture" (10.3030/101130039). She counts with 80 publications (h-index: 29), 1 patent, 12 project participations (4 as PI) and is currently supervising 7 PhD students. Previously, was a MSCA Individual Fellowship holder (ID Grant Agreement:331416), she was also awarded with the L’Oréal Portugal Medals of Honor for Women in Science in 2018.

LIST OF ABSTRACTS

ORAL COMMUNICATION

Ultrasound-Assisted Processes for Sustainable Removal of Contaminants of Emerging Concern in Wastewater: A Case Study with Bio-Based Adsorbents

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The presence of emerging contaminants (CECs), including pharmaceuticals and trace metals, presents an increasing challenge in wastewater treatment, as conventional methods often struggle to effectively remove these compounds. These pollutants threaten aquatic ecosystems and human health, underscoring the need for innovative and sustainable treatment solutions. Ultrasound (US) offers a promising approach, producing acoustic cavitation and the rapid formation and collapse of microbubbles, which create extreme local conditions that improve chemical reactions and pollutant degradation. This review explores the current challenges of managing CECs in wastewater and assesses advanced sono-based treatment techniques. This work places a special focus on the state-of-the-art in ultrasound-based treatment processes, with particular attention to recent research exploring the combination of ultrasound with organic and bio-based adsorbents. This approach leverages cavitation effects to accelerate adsorption kinetics and improve pollutant removal efficiency. In this context, organic compost (OC) was evaluated as a low-cost adsorbent under ultrasonic exposure at 20 kHz. The results show that pollutants are rapidly and substantially removed: within one minute, US-assisted adsorption increased copper removal by 4.4 times and the adsorption of the antibiotic ciprofloxacin by 1.7 times. Overall, 70% of CIP and 89% of Cu were removed within 1-5 minutes, compared to traditional adsorption, which requires much longer contact times to achieve similar removal. These findings demonstrate that ultrasound acts as a catalytic driver, boosting adsorption kinetics and treatment performance. Integrating ultrasound with adsorption offers a sustainable and efficient approach consistent with circular economy principles by valorising organic waste. Besides adsorption, US can support other advanced treatment technologies by reducing chemical use and treatment time. Ultrasound-assisted processes present a pathway towards greener and more effective wastewater management, capable of addressing the limitations of conventional treatment plants on CECs removal.

POSTER COMMUNICATIONS

3D printing membranes for antimicrobial and aquatic remediation applications

Rodrigues, Soraia C.[1,3]; Alves, Dinis C.[1,2,3*]; Alves, Mariana F.[2,3,4]; Queirós, Joana M.[1,2,3]; Martins, P. M.[2,3], Lanceros-Mendez, S.[1,5,6]

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The strong technological and industrial development has generated an increase in pollutants that are released into the environment. These emerging pollutants are found in products used in everyday life, they are resilient and very stable in the environment, making current conventional water treatment methods obsolete for their removal.

New technological concepts have proven effectiveness in removing these contaminants, in this way the solution to this problem may involve advanced oxidation processes such as photocatalysis and membrane technology. Thus, the present work focuses on the production, characterization and application of photocatalytic membranes produced by additive manufacturing. Direct ink writing (DIW) was the technique used in this work, testing the influence of various printing parameters. DIW allows us to produce multilayer membranes with high reproducibility, reduced material waste and control their shape and morphology.

As a result, a composite by DIW for water treatment applications was developed, where TiO_2 and Ag-TiO_2 nanoparticles were immobilized in a matrix of fluoride-co-hexafluoropropylene polyvinylidene (PVDF-HFP) and sodium chloride (NaCl). It`s efficiency was tested under UV radiation in degradation of the antibiotic ciprofloxacin (2.5 mg/mL) and disinfection of the bacteria *E. coli*. After evaluation it was shown that, among the nanocomposites immobilized with photocatalytic active material, the membrane with 10% TiO_2 showed the best efficiency in CIP degradation under UV radiation, with a value of 74%. Furthermore, the same material showed a reduction in cell viability of, approximately, 100% of the bacterial cells under UV radiation. Additionally, SEM analysis revealed an interconnected porous membrane and a stable polymeric structure, despite the immobilization of different nanocomposites, as confirmed by FTIR-ATR.

A new green alternative for photocatalytic nanomaterials

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The introduction of mesoporosity in amorphous silica using surfactants was a turning point in the silica materials field. This innovation broadened its applications, making mesoporous silica a valuable material for drug delivery, opto-electronics, catalysis, photocatalysis and beyond [1], [2]. Yet, industrial production of synthetic mesoporous silica still relies on resource-intensive global supply chains [3]. Sustainable and locally produced alternatives are therefore urgently needed. Diatoms, the most abundant single-celled microalgae group on Earth, offer such an alternative. Their intricate silicon dioxide cell walls - or frustules - are naturally porous and highly organized. With over 100 000 estimated species [4], diatoms display remarkable morphological diversity in size (2 - 500 μm), shape (pennate and centric), and nanopore architecture. Harnessing this natural diversity as a nanomaterial source has already been proposed for advanced applications [5], [6]. One of the most promising methods currently available for environmental remediation is photocatalysis, valued for low activation energy requirements, low-cost chemicals, and recyclability [7]. Mesoporous silica, facilitating a wide range of photocatalytic applications, is a state-of-the-art field of research. Diatom biosilica has been suggested as photocatalytic support material [8], [9], however the exploitation of species-specific characteristics for targeted applications was only barely studied. This project aims to develop a new class of innovative silicon dioxide nanodevices by utilizing the unique species-specific characteristics of diatoms. The focus is on photocatalytic applications, reducing reliance on synthetic and environmentally costly materials. By utilizing the natural ability of diatoms to integrate elements from the environment, the biosilica will be functionalized with relevant photocatalytic agents. These nanodevices will be fabricated and evaluated through proof-of-concept experiments to demonstrate their potential – primarily as novel template materials for photocatalytic platforms, with the capacity to advance emerging technologies beyond the current state of the art for societal benefit. This research is supported by the FCT – Foundation for Science and Technology, under PhD studentship number 2024.01564.BDANA.

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Innovative hair conditioning: cationic lignin as a sustainable alternative to conventional polymers

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The use of cosmetic products is a highly popular practice. However, the increasing use of these products has raised concerns about their environmental impact. For example, some compounds commonly found in hair care formulations, such as cationic conditioning agents, are reported to be toxic to aquatic organisms [1]. The rinsing of hair care products results in their entry into wastewater systems, and, eventually, their discharge into rivers and oceans. These problems, and the increasing preference for natural products with low environmental impact, have motivated the research on renewable feedstocks for the development of cosmetic formulations [2]. Biopolymers, such as lignin, are excellent candidates to be used in bio-based formulations. Lignin is a natural polyphenol that has been reported as a multi-functional cosmetic ingredient for hair care, offering antibacterial, antioxidant, and sun-protective activities [2]. Lignin's hydrophobic nature can help to restore the hydrophobic barrier characteristic of healthy hair, and the presence of its functional groups allows for chemical modifications, namely by introducing cationic groups, which will enhance the interactions with hair. In this work, lignin was extracted from acacia wood and then chemically modified to prepare cationic derivatives that could act as hair conditioning agents. The effect of the cationization conditions on the degree of substitution and on the surface charge of the prepared polymer were evaluated. The ability of these new lignin-based conditioning agents to efficiently repair damaged hair were assessed by evaluating their deposition into model surfaces mimeting the hair surface. Lignin-based conditioning agents showed lower ecotoxicity to aquatic organisms of different trophic levels compared to a conventional commercial polymer (poliquaternium 11). Lignin derivatives with low toxicity were then incorporated into some preliminary formulations and their stability and sensorial properties were evaluated.

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HEALTH

KEYNOTE LECTURES

TIAGO BEITES, I3S

Target-based antibiotic discovery

Tiago Beites obtained his PhD degree in Biology from the Faculty of Sciences of University of Porto in 2013. In 2015, he joined Weill Cornell Medicine (New York, USA) as a postdoctoral associate to work on fundamental biology of the human pathogen Mycobacterium tuberculosis. From 2020 until 2023, he held junior faculty positions in the same institution. In 2023, he joined i3S - University of Porto and currently leads a team under the umbrella concept of pathogens target biology.

ARTUR PINTO, FEUP

New 2D-nanomaterials for Biomedical Applications

Artur M. Pinto, obtaining his PhD in Biomedical Engineering from the Faculty of Engineering - University of Porto (FEUP), visiting U. Washington (USA). Afterward, Artur was appointed as a Post-Doctoral Researcher at Eindhoven University of Technology, the Netherlands. Following his Post-doc, Artur has been awarded a long-term Researcher position at LEPABE-FEUP. He is the Principal Investigator of collaborative projects between FEUP, i3S, and UT Austin (USA) focused on developing new 2D-nanomaterials and adjusting their properties for biomedical applications, such as phototherapy of cancer, involving chemotherapy and immunotherapy, and 3D-printing.

LIST OF ABSTRACTS

ORAL COMMUNICATION

Shaping patient-specific bioengineered intestinal niches

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Organoids provide superior physiological relevance than traditional *in vitro* models due to their cellular diversity and self-organisation, yet their closed architecture restricts apical access, limiting functional studies [1]. Although organoid-derived monolayers established using 2D culture systems enable direct access to the apical surface, they continue to fall short in matching the cell renewal and differentiation capacity intrinsic to 3D organoids [2]. To address this, we designed an organ-on-chip device integrating spatiotemporal environmental control and a tissue- and patient-specific decellularized extracellular matrix (dECM), emulating the *in vivo* milieu for organoid-derived culture. Organoids were generated from human colonic crypts and cultured in Matrigel [3]. Prior to differentiation, intestinal organoids displayed cystic morphology and a polarised epithelial barrier. Differentiation for 7 days induced budding structures and expression of markers for specialised cells, including goblet (MUC2) and enteroendocrine cells (chromogranin A). To establish monolayers, expanded organoids were dissociated and seeded on culture inserts for 21 days. They similarly exhibited secretory cells by day 7, maintained a polarised barrier with a dense apical brush border, and expressed intercellular junctions (ZO-1). Transepithelial electrical resistance increased until day 14, indicating enhanced barrier integrity. The apparent permeability of Lucifer Yellow was closer to values found ex vivo compared to immortalised cell co-cultures. As expected, both differentiated 3D organoids and their derived monolayers showed decreased stem cell marker expression and increased differentiation markers, except for Lysozyme, a marker for Paneth cells, which reside in the stem cell niche. While both showed similar loss of stem cells, 3D organoids had a stronger increase in differentiation markers, particularly Chromogranin A. These findings demonstrate robust formation and characterisation of 2D monolayers from 3D organoids, while still evidencing their limitation in recapitulating the full complexity of 3D organoids. Ongoing work involves the optimization of the decellularization of the intestinal tissue to develop the hydrogel for culturing these monolayers under dynamic conditions within an organ-on-chip device comprising a central chamber accommodating the dECM.

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POSTER COMMUNICATIONS

Bioprospection of novel antimicrobial compounds from the abandoned mine of São Domingos

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The rise of multi-drug-resistant pathogens has been recognized as a major public health concern by the World Health Organization. In response to this growing issue, it becomes increasingly important to find new substances capable of tackling these infections.

Extreme environments force microorganisms to develop unique metabolisms to survive, often producing interesting compounds that help them adapt to such hard conditions. Thus, these species can be a source of several novel bioactive molecules, such as antimicrobial compounds.

This study focuses on exploring the potential of the acid mine drainage from the abandoned mine of São Domingos to identify compounds with antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

To achieve this goal, a combined metagenomic and culture-dependent approach was employed. Soil and water samples were collected from the mine's open pit and from the stream originating from it. For metagenomic analysis, DNA was extracted from samples using commercial kits. To try to improve the concentration of DNA, glucose and yeast extract supplementation was also tested.

Shotgun metagenomic sequencing and bioinformatic analysis of the extracted DNA from both soil and water samples revealed a high proportion of reads assigned to unidentified species, confirming the presence of unique microorganisms in this environment.

Culture-based techniques employed to the soil samples allowed to isolate microorganisms that were subsequently tested against the selected pathogens using the top agar diffusion method, evaluating their capacity to produce antimicrobial compounds. The results showed promising inhibitory activity against *S. aureus* for one of the isolates.

This study demonstrates that São Domingos mine harbours unique microorganisms with the potential to produce antimicrobial compounds, highlighting extreme environments as valuable sources for novel bioactive molecules.

Improving Hygiene in Hospital Laundry: Microbial Load Reduction on Non-Porous and Textile Surfaces

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Healthcare-associated infections (HAIs) remain a major challenge for hospitals, raising costs and threatening patient safety. Cross-contamination between surfaces is a key transmission route, with hospital textiles acting as potential vectors of infection. Therefore, the role of hospital laundry in reducing the microbial load on textiles and preventing recontamination through contact with transport carts and vehicles is vital.

This study compared the antimicrobial efficacy of conventional disinfectants (Products A and B) with that of a novel formulation (*Care Us and Care Us Breath*) under different operational conditions. The aim was to assess their ability to reduce microbial loads and consequently minimize the risk of HAIs. Samples were collected from carts and vehicles after disinfection with Products A and *Care Us*. Additionally, textiles that had previously been disinfected with Product B and *Care Us* Breath were inoculated with microorganisms identified via MALDI-TOF, including *Staphylococcus aureus* and *Enterobacter hormaechei*, in order to compare the products' antimicrobial activity. Microbial reduction was also measured directly on textiles following their use and disinfection with both products at varying concentrations.

For non-porous surfaces, manual application of *Care Us* at room temperature and undiluted achieved the greatest efficacy, with reductions exceeding five logarithms. Textile inoculation confirmed the superiority of *Care Us Breath* over Product B, with *Care Us Breath* achieving greater microbial reductions when both were applied at equal concentrations.

In summary, *Care Us* and *Care Us Breath* have demonstrated superior antimicrobial efficacy to that of currently used disinfectants. Importantly, they can be used under less demanding conditions, such as at low temperatures on non-porous surfaces, which could lower hospital operating costs without compromising infection control. These findings highlight their potential to enhance infection prevention practices, minimize cross-contamination from hospital textiles and surfaces, and ultimately reduce the risk of healthcare-associated infections (HAIs).

Novel antisense molecules-based vaginal capsules to inhibit *Candida albicans* filamentation

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Vulvovaginal candidiasis (VVC) is an increasing public health concern with significant clinical, social, and economic implications. The misuse of antifungal agents has contributed to the emergence of multidrug-resistant fungi, particularly *Candida albicans*. The pathogenicity of *C. albicans* is closely linked to its ability to transition from a yeast to a filamentous form, facilitating the invasion of vulvovaginal tissues and the host's immune system. Antisense therapy has emerged as a promising strategy to control *C. albicans* filamentation, particularly through the use of the cationic lipid–2'-O-MethylRNA EFG1 oligomer complex. Vaginal drug delivery in gel capsules ensures a local action and allows controlled drug release.

Thus, this study aims to develop an innovative therapeutic approach for the treatment of VVC by integrating the cationic lipid–2'-O-MethylRNA EFG1 oligomer complex into chitosan-coated capsules.

The research focused on developing vaginal gel capsules incorporating the cationic lipid molecules antisense oligomers (ASO) designed to inhibit *C. albicans* filamentation. The chitosan-coated capsules were evaluated through stability and swelling assays, morphological characterization by Scanning Electron Microscopy (SEM), and physicochemical analyses using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). In addition, their cytotoxicity and the effect on *C. albicans* filamentation were also evaluated.

The capsules exhibited uniform morphology with a porous surface, enabling gradual release of the ASO over time. Improved release of free ASO was observed at 6 and 24 hours, while lipoplex with ASO showed enhanced release at 24 and 48 hours. *In vitro* assays confirmed a significant reduction in *C. albicans* filamentation for up to 48 hours. Moreover, no significant signals of toxicity were observed.

This delivery system represents a safe and effective alternative for the treatment of VVC, offering a novel strategy to control the escalation of *C. albicans* infections.

Phage isolation and therapeutic potential against multidrug-resistant *Staphylococcus pseudintermedius* and *Enterococcus* spp. in dogs

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Staphylococcus pseudintermedius is a Gram-positive, coagulase-positive commensal of canine skin that can cause opportunistic infections such as urinary tract infections, otitis, and surgical site infections [1]. Alongside *Enterococcus* spp., increasingly reported as opportunistic pathogens in dogs, these bacteria present a growing concern due to their multidrug-resistant (MDR) profiles [2]. The rise of methicillin-resistant *S. pseudintermedius* (MRSP) and MDR *Enterococcus* strains has severely limited therapeutic options, as many conventional antibiotics are ineffective or restricted in veterinary medicine. In this context, bacteriophage (phage) therapy emerges as a safe, targeted, and clinically relevant alternative [3]. This study aimed to develop and characterize a functional collection of bacteriophages active against MRSP and *Enterococcus* isolates from canine patients.

Phages were isolated from canine-associated samples and screened for lytic activity using phagograms. Morphological characterization was performed by Transmission Electron Microscopy (TEM). Genomic DNA was extracted by phenol chloroform, sequenced on the Illumina MiSeq platform, assembled, and annotated. Functional analyses included prediction of coding sequences and screening for antimicrobial resistance genes, virulence factors, and lysogeny-associated markers. *In vitro* assays assessed bacterial susceptibility and antibiofilm activity, while *in vivo* evaluation was performed by phage application in canine patients.

Seven phages were isolated - five targeting *S. pseudintermedius* and two targeting *Enterococcus* spp. - demonstrating lytic activity against 57% of 24 MRSP and 18 *Enterococcus* clinical isolates. Notably, three phages exhibited a polyvalent host range. Genomic analyses confirmed that all sequenced phages were strictly lytic and lacked genes associated with lysogeny or virulence, supporting their therapeutic potential. Importantly, one phage (ASFA_094) was applied in a clinical case of generalized MRSP- associated osteomyelitis in a dog, leading to complete resolution of clinical signs, and evidence of bone remodeling.

These findings highlight the therapeutic promise of phage therapy for MDR bacterial infections in dogs and underscore its broader relevance within a One Health framework. Future efforts will focus on strengthening veterinary collaborations to support clinical translation and to establish multicentric case documentation, advancing phage therapy as a viable alternative in veterinary medicine.

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Disrupting Hospital Biofilms: Microbiological Validation of *PipeUCare*

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The hospital environment is a hotspot for the proliferation of pathogenic microorganisms, with many of them resistant to conventional antibiotics. In Portugal, healthcare-associated infections affect around 11% of hospitalized patients, which is well above European average. Greywater systems are an underestimated source of these infections, often serving as reservoirs and dissemination routes for antibacterial-resistant bacteria. This study presents the microbiological validation of *PipeUCare*, the first antimicrobial disinfectant specifically designed for use on the non-porous surfaces of greywater systems. This product addresses a critical regulatory and technological gap.

The antibacterial and antibiofilm performance of *PipeUCare* was evaluated on three commonly used surfaces in hospital piping: rigid and flexible PVC and stainless steel (INOX). The disinfectant was compared with two conventional products (Product A and B) using mono and multi-species biofilm models. The selected microorganisms — *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus hirae* — are key agents in hospital-acquired infections and are to form biofilms on surfaces. Biofilms were developed under simulated greywater flow conditions, including on the referred surfaces with and without fluid accumulation and after up to 5 water passages.

PipeUCare demonstrated a >5-log reduction in viable cell counts for all tested strains after five water flow cycles. It completely eradicated preformed biofilms in dry zones and significantly reduced biomass in wet zones. A clear inhibition halo was observed for all surfaces 48 hours post-treatment, indicating sustained antibacterial activity. Furthermore, effectively prevented bacterial adhesion, demonstrating a preventive effect against biofilm formation that is not observed with conventional products A and B.

This study provides strong microbiological evidence of the effectiveness of *PipeUCare* in the control of clinically relevant biofilms over critical infrastructure surfaces. The results emphasize the importance of integrating biofilm-targeted disinfection strategies into hospital sanitation protocols and highlight the importance of developing materials and agents that are specifically adapted to the complex microbiological challenges of greywater systems.

Commercial wound dressings for the treatment of pressure injuries: characterization and health professional perspectives

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Pressure injuries remain a frequent and challenging clinical complication, with a significant impact on patients' quality of life and on healthcare systems. Their prevention and treatment require a multidimensional approach, including pressure relief, wound debridement, and the use of appropriate wound dressings. However, currently available products present limitations, both in terms of antimicrobial effectiveness and in comfort or ease of use. In this context, incorporating the perspective of healthcare professionals, particularly nurses, becomes essential in the evaluation and development of more effective solutions. This study aimed to characterize, both structurally and in terms of antimicrobial activity, 9 commercial wound dressings frequently used in the prevention and management of pressure injuries, while also meeting healthcare professionals experiences, opinions, and preferences, with the goal of contributing to a more functional and comfortable design allowing more effective dressings.

The structural analysis showed that hydrofiber wound dressings displayed high absorption capacity and low thickness, making them suitable for highly exudative wounds, whereas foam wound dressings offered greater comfort but lower absorption. Wound dressings incorporating antimicrobial agents (silver/honey) demonstrated, in general, antimicrobial efficacy.

About healthcare professionals' perspectives, functionality, comfort, and ease of application were highly valued features. In particular, the Allevyn Gentle Border wound dressing was highlighted for its multilayer construction, while difficulties in the removal of certain products, such as Suprasorb Liquacel and Aquacel, were reported.

The critical evaluation of dressings commonly used in clinical practice contributes to the identification of their limitations and strengths, which is essential for the development of more effective, user-friendly, and clinically adapted products.

Application of MALDI-TOF MS in the identification of microorganisms in pressure injuries in the domiciliary context

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Pressure injuries (PIs) are localised lesions on the skin and soft tissue caused by unrelieved pressure, shear or friction over bony prominences, especially in people with reduced mobility and advanced age. They represent a major health concern in these patients, impacting their overall health, and quality of life, and have significant associated health care costs. Rapid and accurate microbiological identification of pathogens is crucial for selection of more targeted therapies. This study aimed to access the microbial profile of PIs in the home care context using Matrix-Assisted Laser Desorption/ Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS).

In this study, samples were collected in the north and centre of Portugal. A total of 43 PI samples from 34 patients were analysed, with an average age of 84 years, and equally distributed between masculine and feminine genders. The microorganism present in the samples were isolated using selective and differential agar media and subsequently identified by MALDI-TOF MS.

Stage 4 PIs, one of the most advanced tissue damage categories, were the most encountered lesions. The predominantly affected areas by PIs among these individuals were the trochanter, coccyx, sacrum, leg and ankle. The most frequently isolated microorganisms were *Staphylococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., and *Proteus* spp.

This study underlines the clinical importance of analysing the microbiome of PIs in the context of home care context, providing essential data for clinical practice and research. The more advanced stages of PIs in this setting reflets the complex nature and risk of the domiciliary setting. MALDI-TOF MS proved to be a rapid and efficient method, while providing valuable data for microbiological analysis in this research. The results contribute to the continuous improvement of health care and for guiding future therapies.

A novel phage-derived protein from *Acinetobacter baumannii* bacteriophage as a potent antimicrobial agent

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The overuse and misuse of antibiotics have led to the rise of multidrug-resistant pathogens, creating a global public health crisis, with pathogens such as *Acinetobacter baumannii* at the forefront of this crisis. *A. baumannii* is a gram-negative bacterium associated with severe hospital-acquired infections, particularly in immunocompromised patients, and has been classified as a critical priority for the development of new antimicrobials by the World Health Organization (WHO).

Bacteriophages (phages), viruses that infect and kill bacteria, have emerged as a promising alternative to antibiotics. During the early stage of infections, phages evolved to express proteins that inhibit or divert key host metabolic functions. Recent research highlights the potential of phage-derived proteins, particularly those expressed during the early stages of infection, responsible for hijacking bacterial metabolism and inhibiting growth. However, the mechanistic basis of these early phage-host interactions remains largely unexplored.

This study utilises transcriptomic methodologies, such as RNA-seq, to profile early-gene expression from the *A. baumannii* phage Highwayman. We identified 27 early phage genes of unknown function and individually evaluated their ability to inhibit bacterial growth. Among these, only one gene exhibited pronounced toxicity and growth inhibition against *A. baumannii*, reducing CFU/mL counts by up to 5 logs. Additionally, while the phage is only able to infect the host's specific capsular type, we confirmed that this gene can inhibit bacterial growth in different *A. baumannii* strains, while remaining non-toxic to other species, such as *E. coli*. The phage-derived protein was successfully overexpressed and purified from *E. coli*, and characterised for structure and thermostability.

Collectively, these findings underscore the promise of phage-derived early proteins as innovative therapeutics for combating drug-resistant *A. baumannii* infections. Further functional and mechanistic studies are warranted to advance this promising alternative antimicrobial strategy towards clinical application.

TGF-β1 Biosensor: A Tool for Identifying Inflammatory Conditions

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Inflammation plays a vital role in the human body in response to various potentially harmful stimuli, such as pathogens, cell injuries, or toxic compounds [1]. While essential for tissue repair and defence, chronic or dysregulated inflammation can contribute to the development of numerous pathological conditions such as degenerative diseases, fibrosis, cancer, infections, and even ageing [1-2]. Transforming Growth Factor beta 1 (TGF-β1) is a context-dependent cytokine with anti-inflammatory properties, essential for immune homeostasis and, during pregnancy, for maternal-fetal tolerance, placental development, and immune regulation [3-5]. Its dysregulation is linked to several conditions, including cancer [1], pregnancy complications [5], renal disorders [6], and autoimmune diseases [7]. Given its clinical relevance, the development of rapid, sensitive, and user-friendly methods to detect biomarkers such as TGF-β1 at the point of care is crucial for improving the diagnosis and monitoring of inflammation-related pathologies and enabling more precise evaluation of disease progression. This work describes an immunosensor based on Electrochemical Impedance Spectroscopy (EIS), specifically designed and developed for detecting and measuring TGF-β1. The biosensor was validated in various clinically relevant human body fluids, including urine, amniotic fluid, blood serum, and also in cell culture media, demonstrating reliable performance and flexibility. Compared to traditional gold-standard methods such as ELISA, the proposed sensors offer several benefits: significantly shorter assay times, elimination of labelling steps, smaller sample volume requirements, increased sensitivity, and suitability for point-of-care applications.

This biosensor provides a label-free platform for real-time monitoring of inflammatory biomarkers, allowing precise evaluation of molecular signatures associated with immune and inflammatory responses. Owing to its sensitivity and versatility, it holds a strong potential for diverse clinical applications, including early diagnosis, prognostic assessment, and therapeutic monitoring, in conditions such as autoimmune diseases, infections, cardiovascular disorders, and pregnancy-related complications.

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A closer look at CeO2NP: Integrating skin model for toxicological assessment and regulatory implications

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Cerium oxide nanoparticles (CeO_2NP) are widely used in cosmetics, such as sunscreens, and in various industrial applications due to their antioxidant and redox properties. However, despite their biomedical potential, existing studies on CeO_2NP cytotoxicity often show conflicting results, largely due to a lack of standardized data and experimental conditions. This inconsistency creates a significant challenge for regulatory bodies, especially in countries like Brazil, which lack specific regulations for these nanomaterials. This study aimed to provide robust evidence on the biological effects of CeO2NP by assessing their cytotoxicity in physiologically relevant dermal exposure models. We utilized both conventional 2D cell cultures (Ntert and Hs-27) and a more advanced 3D reconstructed human epidermis. The NP were characterized by Dynamic Light Scattering (DLS), Transmission and Scanning Electron Microscopy (TEM/SEM), zeta potential and X-ray diffraction.

Our multiparametric analysis in 2D models involved evaluating cell viability (PrestoBlue), proliferation (PDL), and oxidative stress (ROS), with no signs of cytotoxicity found across a concentration range of 1 to 100 μg/mL over 72 hours. Similarly, the 3D reconstructed epidermis model—assessed via histology, viability (PrestoBlue), transepithelial electrical resistance (TEER), and permeability—also showed no evidence of cytotoxicity under the tested conditions.

Our findings underscore the critical importance of using multiparametric strategies and comparing 2D and 3D models to generate a comprehensive safety profile for nanomaterials. These robust data are crucial for developing informed regulatory policies and ensuring the responsible use of CeO_2NP in a regulatory context that requires standardized safety criteria.

Targeting PDR1-mediated antifungal resistance with antisense oligonucleotides: a novel approach to control *Candida glabrata* infections

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The increasing prevalence of *Candida glabrata* infections, driven by the fungus' high intrinsic and acquired resistance to azole antifungals, poses a significant therapeutic challenge worldwide. PDR1 is a gene that regulates the overexpression of efflux pumps in response to antifungal pressure and also organizes broader cellular processes that interfere with membrane permeability and stress resistance. In order to overcome the rising levels of resistance, new alternative therapies are urgently needed. Antisense therapy (AST) has emerged as a promising gene-targeted approach capable of modulating gene expression at the mRNA level with high specificity. However, its application in pathogenic fungi remains largely unexplored. This study investigates whether silencing PDR1 using an antisense oligomer (ASO) interferes with *C. glabrata* viability by disrupting membrane permeability and other adaptive mechanisms under the control of this transcription factor. The ASO was validated in silico and was shown to retain binding capacity despite mutations detected in clinical strains. It reduced *C. glabrata* growth by ~20% and lowered PDR1 expression by ~30% *in vitro*. To evaluate its effect in a living infection context, the *Galleria mellonella* model was used. The ASO was previously encapsulated in cationic liposomes to protect the oligomer and ensure its delivery to the inside of cells. To achieve this, two liposomal formulations were tested, DOTAP:DOPC:PEG and DOTAP:DOPE:PEG. Both formulations improved larval survival after infection, with increases of 14–17% at 24 h, 21–23% at 48 h, and up to 30–32% at 72 h post-infection. The formulation with DOTAP:DOPC:PEG showed slightly better performance. These results demonstrate that liposomal delivery enhances ASO cellular uptake, enabling effective PDR1 silencing. Overall, this work shows that AST is an effective way of combatting *C. glabrata* infections.

Development of Titanium-Gold Thin Films for Flexible Dry Electromyography Electrodes with Tailored Electrical Resistivity

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With the global ageing population, the demand for innovative healthcare solutions to diagnose motor disabilities in the elderly is increasing. Electromyography (EMG), which records muscle activity, is essential for detecting neuromuscular disorders and enabling personalised treatment. While Ag/AgCl wet electrodes are commonly used, their dependence on conductive gel can lead to discomfort and hinder long-term use. In contrast, dry electrodes provide a more comfortable and practical solution for extended monitoring, since they don't require conductive gels. However, they must meet strict requirements such as flexibility, electrical conductivity, and biocompatibility. This study aims to investigate the use of titanium-gold (Ti-Au) thin films as potential dry electrodes, due to their biocompatibility and promising flexibility and electrical conduction. The films weredeposited on silicon and glass substrates by magnetron sputtering using the glancing angle deposition (GLAD) technique to be fully characterised. Ti-Au films with an Au/Ti ratio ranging from 0 to 0.80 were deposited in various geometries: conventional, inclined, and zigzag, using deposition angles from 0° to 90°. This study aims to evaluate these Ti-Au thin films as potential dry electrode materials for EMG applications, focusing on how their composition and growth geometry influence electrical performance. The electrical resistivity of the thin films was measured using the four-point probe method, and their crystalline structure and microstructure were characterised by X-ray diffraction and transmission electron microscopy, respectively. Results seem to indicate that the small tested variations in the Au/Ti ratio tend to reduce electrical resistivity compared to pure Ti films, regardless of the deposition angle. Films deposited at angles of 70° or higher exhibited significantly increased electrical resistivity. Despite this trend, the films continue to show high electrical conductivities while allowing for tailored mechanical flexibility. Therefore, both microstructural effects and chemical composition greatly impact the films' electrical performance. Future work will assess these films' mechanical and biocompatibility properties to further support their development as dry EMG electrodes.

Exploring the Role of Essential Oils in Treating *Staphylococcus pseudintermedius* Skin Infections in Companion Animals

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Staphylococcus pseudintermedius is a key etiological agent of skin infections in companion animals, particularly dogs, and has gained special attention due to the emergence of antibiotic-resistant strains, including methicillin-resistant *S. pseudintermedius* (MRSP). These strains pose a significant challenge in clinical settings, as traditional antibiotic treatments are often ineffective, especially against bacteria capable of forming biofilms. This common trait of *S. pseudintermedius* contributes to its resistance. Therefore, there is growing interest in identifying alternative therapeutic strategies with simultaneous antimicrobial and anti-biofilm effects.

In this study, we evaluated the antimicrobial and anti-biofilm properties of 15 essential oils (EOs) against clinical isolates of *S. pseudintermedius*. An initial screening, using the agar diffusion method, identified EOs with promising antimicrobial activity. These were subsequently tested to determine their ability to disrupt pre-formed *S. pseudintermedius* biofilms. To assess safety, *in vivo* toxicity assays were performed using the *Galleria mellonella* larvae model.

In vitro assays demonstrated that the selected EOs exhibited significant antimicrobial and antibiofilm activity, reducing both biofilm biomass and culturability of biofilm cells. *Cymbopogon citratus* showed the highest inhibitory effect, completely eliminating culturability of biofilm cells. Furthermore, the EOs exhibited low cytotoxicity at therapeutically relevant concentrations, supporting their potential safety for topical use. In conclusion, our study demonstrated that the tested EOs have strong potential to inhibit planktonic and biofilm forms of *S. pseudintermedius*, positioning them as promising alternatives or adjuvants to antibiotics in veterinary practice.

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Point-of-care technology towards the non-invasive screening of early gastric cancer

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Early detection of gastric cancer is challenging due to the asymptomatic nature of precursor lesions, such as low- and high-grade dysplasia [1]. However, Western countries have no efficient screening method to facilitate early diagnosis [1]. Identifying accurate biomarkers may provide better risk assessment and stratification of individuals, enabling more timely interventions [2]. In this context, saliva-based protein research holds promise due to its potential for non-invasive diagnosis [2]. Furthermore, point-of-care (PoC) devices offer great potential for rapid, accurate, accessible, and cost-effective detection of cancer biomarkers, improving patient outcomes, particularly in resource-limited settings [3]. A case-control study was conducted at the Gastroenterology Department of IPO Porto. Saliva samples are being collected from patients with confirmed gastric lesions who underwent endoscopic submucosal dissection, including low-, high-grade dysplasia, and early-stage adenocarcinoma, as well as from healthy individuals. A proteomics analysis (n=14) was conducted using mass spectrometry, and the top differentially expressed proteins will be validated using conventional protein detection and quantification techniques (ELISA). In a preliminary analysis, over 5000 proteins were detected in saliva from cases and controls, and more than 30 were significantly dysregulated in gastric lesions.

To translate these findings into clinical practice, we developed a paper-based lateral flow assay (LFA) for the detection and quantification of selected salivary biomarkers. For targets that are difficult to tag, an integrated microfluidic module is employed for sample purification [4]. As proof of concept, matrix metalloproteinase-9 (MMP-9) was selected. Optimization steps included evaluation of detection methods, membrane selection, concentrations of capture and biotinylated antibodies, and protein immobilization on nitrocellulose for control line formation. The feasibility of the sandwich assay was confirmed by ELISA. Using the LFA, MMP-9 was successfully detected at 10 g/mL.

Future work will focus on increasing assay sensitivity to lower detection limits at the ng per milliliter level and extending the platform to additional candidate biomarkers.

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From Library to Lead: a Roadmap for Bioactive Peptide Discovery

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Peptide-based therapeutics hold significant promise for addressing complex diseases, due to their wide variety of biological functions, including antimicrobial activity, immune modulation, and cell regulation. They often exhibit higher potency and selectivity for their targets and low toxicity, making them attractive candidates for drug development [1,2].

This communication presents an educational overview of the systematic process for identifying bioactive peptides from a rationally designed peptide library. The process begins with the selection of a clinically relevant disease model, guided by well-established genetic markers and cellular systems that accurately reflect key pathophysiological mechanisms, which serve as the foundation for designing effective assays to evaluate peptide activity.

From this foundation, we describe the design principles of the peptide library, including pertinent source proteins linked to the disease or biological pathway of interest, sequence diversity, structural motifs known to influence activity, stability, or receptor binding, and computational predictions, including in silico screening for activity, stability, and low toxicity [3,4].

High-throughput screening, synthesis, and characterization / biological assays are then employed to evaluate peptide activity, stability, and selectivity, leading to the identification of promising lead compounds. This approach highlights the potential of bioactive peptides and demonstrates a rational, systematic strategy for their discovery and development.

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ELISA-Based Biosensor for Apoptosis Detection

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Apoptosis is a crucial biological process that ensures cellular turnover, tissue remodelling, immune system function, and the elimination of harmful, damaged and unwanted cells [1-3]. Unlike necrosis, which is uncontrolled and chaotic, apoptosis proceeds in a regulated manner [4]. Apoptosis is a process indispensable for embryonic development, immune tolerance, and homeostasis of tissues, and its dysregulation is implicated in the pathogenesis of numerous diseases, including cancer, autoimmune conditions, and neurodegeneration [3-5]. Caspases, a family of cysteine proteases, are key regulators of apoptosis [6]. These enzymes can be divided into three main groups: i) initiator caspases, like caspase-8, which trigger the apoptotic pathway; ii) effector or executioner caspases, such as caspase-3, which execute cell death by dismantling the cell's components; and iii) inflammatory caspases, like caspase-1. Specifically, caspase-3 plays a central role in executing apoptosis, making it a critical biomarker for detecting abnormal cell death by both extrinsic and intrinsic pathways [2,4,7,8]. This work aims to develop and optimise an ELISA method for the detection and quantification of apoptotic biomarkers, with a focus on caspase-3, to provide a more robust tool for monitoring apoptosis. The method is based on a direct ELISA format, using an antibody conjugated with a fluorescent dye, thereby establishing a fluorescence-based technique for the quantitative analysis of caspase-3. Currently, no commercial ELISA for caspase-3 employs a direct approach. Most available kits are based on sandwich formats with pre-coated plates and chromogenic substrates, which increase costs and involve multiple procedural steps. In contrast, the customised assay developed here uses a direct ELISA with a fluorescently labelled antibody, simplifying the workflow, reducing reagent consumption, and enabling sensitive quantitative readouts.

To validate its applicability across different biological contexts, the method was tested in multiple body fluids, including human serum, urine, amniotic fluid and cell-culture medium. This strategy provides a reliable platform for quantifying a key apoptotic biomarker, facilitating a more accurate assessment of apoptotic processes that may be influenced by drugs or pathological conditions.

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New protein hydrogel for wound healing

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The aim of this project was the development of thermosensitive hydrogels incorporating bioactive natural compounds as advanced wound dressings. The hydrogel matrix included a blend of Pluronic F-127 and F-68, FDA-approved PEO-PPO-PEO triblock copolymers, known for their temperature-triggered sol-gel transformation and biocompatibility. This system was loaded with heather honey (*Erica* sp.), a natural product characterized by its inherent acidity (pH 3–5), high osmolarity, and non-peroxide antibacterial factors like methylglyoxal, which provides broad-spectrum antimicrobial activity. Also, an extract from the heather plant, traditionally used for its anti-inflammatory and antiseptic pharmacological effects, will be incorporated. To promote tissue regeneration, the hydrogel was functionalized with an Elastin-Like Polypeptide (ELPs) and keratin. The ELP exhibit a reversible phase-transition behaviour, being also biodegradable and biocompatible. Keratin improve dermal elasticity and remodeling, key factors in wound healing. Also, it strongly influences the activation of keratinocytes, promoting reepithelialization, and granulation tissue formation. This multifunctional design aimed to synergistically address the major challenges of chronic wounds – persistent inflammation, bacterial infection, and impaired healing – by combining a "smart" moisture-donating hydrogel with the concerted action of antimicrobial, antioxidant, and regenerative natural agents.

The ELP was successfully expressed and purified, with a yield of 36.9 mg/L. The heather extract demonstrated exceptional antioxidant capacity (DPPH assay), showing potent radical scavenging activity (EC50 = 0.499 mg/mL, TEAC = 0.404). In contrast, the honey sample showed significantly weaker antioxidant capacity (EC50 = 6.559 mg/mL, TEAC = 0.031), with minimal DPPH inhibition. Nevertheless, the honey exhibited potent broadspectrum antimicrobial activity, with MIC/MBC values of 25% (w/v) for *S. aureus* and *P. aeruginosa*, and 30% (w/v) for *E. faecalis*.

Formulation studies revealed that the gelation temperature is highly tunable, and optimized formulations underwent a $T_{sol\text{-}gel}$ transition at physiologically relevant temperatures (25-37°C). All hydrogels presented a slightly acidic pH (4-6), beneficial forwound healing.

In conclusion, the developed hydrogels can be formed at body temperature and successfully integrate the potent antioxidant activity of the heather extract, the antimicrobial action of honey, and the moisture and pro-regenerative properties of ELP and keratin. Thus, the bioactive hydrogels show great potential for treating infected wounds while promoting regeneration.

What makes *Candida glabrata* a formidable vaginal pathogen? Correlating biofilm ability, pH adaptation, antifungal susceptibility and host risk factors

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Vulvovaginal candidiasis (VVC) is a globally significant infection caused by commensal *Candida* species that can become pathogenic in presence of various risk factors. *Candida glabrata* is increasingly concerning due to its rising incidence, low susceptibility to firstline antifungals and ability to acquire resistance [1]. It also exhibits aggressive virulence factors like biofilm formation, which complicates treatment and raises the risk of reinfection. Expanding our understanding of this species' virulence traits and its adaptation to the vaginal environment is therefore crucial.

This study aimed to analyse and correlate biofilm formation, pH adaptation, antifungal susceptibility and host risk factors of vaginal *C. glabrata* strains. We examined 37 vaginal strains previously isolated from women with VVC or colonized [1]. Biofilm formation was tested in synthetic vaginal fluid at pH 4 (normal) and pH 7 (abnormal). Susceptibility to fluconazole, ketoconazole and caspofungin was assessed using E-tests. Risk factor data were obtained through participant questionnaires.

The results showed that all strains were capable to form biofilms, with biomass levels varying by strain. Biofilm biomass was significantly higher at pH 4 in all strains, and this effect became more pronounced as biofilm biomass increased, suggesting that vaginal acidity promotes C. glabrata virulence traits. Among the 37 isolates, 6 were fluconazole resistant and 25 were resistant to at least one antifungal. Interestingly, infected and colonized women showed similar proportions of resistant and strong biofilm producer strains, suggesting these traits are inherent rather than infection-driven. A potential correlation between strong biofilm production and fluconazole resistance was found, indicating women with resistant strains may face higher biofilm formation risk. Additionally, risk factors like previous infections, menopause and comorbidities were linked to antifungal resistance.

This study allowed a better comprehension of the *C. glabrata* vaginal virulence, contributing to the development of more effective management strategies for complicated VVC.

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Selection of DNA aptamers to block enterotoxigenic *Escherichia coli* in a *Galleria mellonella* larval model

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Enterotoxigenic *E. coli* (ETEC) infections lead to significant costs for the swine industry worldwide. Among other virulence factors, fimbriae are essential for the initial adhesion of ETEC to intestinal epithelial cells. In particular, F4-type (K88) fimbriae are commonly associated with neonatal infections and most post-weaning diarrheal infections. These infections are traditionally prevented or treated with antibiotics, but their use is highly restricted due to antimicrobial resistance. Therefore, DNA aptamers emerge as a promising approach to block the initial adhesion of F4-ETEC due to their target-binding capacities.

The present study was divided into four main steps: (1) selection of specific aptamers against F4-ETEC bacteria using the cell-SELEX approach; (2) evaluation of dissociation constant (Kd) for the selected aptamers (AptF4_1, AptF4_2, AptF4_3, AptF4_4) (3) evaluation of *in vitro* specificity for these aptamers using two different bacterial strains: F4-ETEC and *E. coli* K12 (commensal *E. coli*); (4) evaluation of the anti-ETEC performance of the most specific aptamers (AptF4_1, AptF4_2) using the *Galleria mellonella* model. From the cell-SELEX methodology, the four most representative aptamers were selected from a total of 2.5 million sequences sequenced by NGS. These aptamers presented low Kd values, indicative of high binding affinities with F4-type fimbriae. Regarding the *in vitro* specificity evaluation, one aptamer (AptF4_2) stood out due to its higher specificity to F4-ETEC in comparison with *E. coli* K12. The *in vivo* tests corroborated the anti-ETEC potential of AptF4_2 because this aptamer at 500 nM increased the survival and health index of *Galleria mellonella* compared to the positive control (F4-ETEC inoculation).

Therefore, this work demonstrates the potential of aptamers in the treatment of ETEC infections as an alternative to antibiotics. However, for future swine applications, these aptamers will be modified with nucleic acid mimics and encapsulated for oral administration.

Receptor-Binding Proteins as Biorecognition Tools for Targeted Detection of *Listeria monocytogenes*

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Listeria monocytogenes (LM) is a highly virulent pathogen responsible for the vast majority of foodborne listeriosis, a life-threatening infection particularly affecting pregnant women, newborns, older adults, and immunocompromised individuals. *L. monocytogenes* shows remarkable environmental resilience and a high mortality rate, emerging as a persistent public health threat with the potential to cause meningitis, septicemia and fetal loss. However, standard detection techniques are often slow, resulting in delayed targeted therapy and increased reliance on broad-spectrum antibiotics. As a solution to limitations in current diagnostic approaches, receptor binding proteins (RBP) from bacteriophages (the viruses of bacteria) offer highly specific and rapid pathogen recognition, supporting improved diagnostic approaches for *L. monocytogenes*.

Genomic sequences of virulent phages and lysogenic *Listeria* strains were computationally examined using bioinformatics approaches (BLASTp,HHpred) and domain prediction resources (Motif Search, Pfam, InterProScan) to select potential RBPs. From these analyses, phage-derived genes encoding RBPs were engineered as mTagBFP2 fusion constructs and expressed in *Escherichia coli*. The resulting proteins were subsequently evaluated for binding specificity and sensitivity through epifluorescence microscopy.

The candidate RBPs demonstrated binding affinity for *Listeria* species, displaying high specificity toward virulent *L. monocytogenes*.

Tail fiber RBPs show high specificity for pathogenic *Listeria* monocytogenes, positioning them as promising biorecognition probes for next-generation diagnostics and potential therapeutic applications.

Influence of Hemodynamic Forces on Vascular–Tumour Cell Interactions Regarding Brain Metastasis from Lung Cancer

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Cancer metastases account for 90% of cancer-related deaths [1], showing the devastating impact of metastasis on patients and highlighting the importance of studying its mechanisms. In lung cancer, metastasis occurs through the dissemination of circulating tumour cells (CTCs), which mostly target the brain (15% to 43%) [2]. When circulating tumour cells (CTCs) arrive at the blood–brain barrier (BBB), they undergo extravasation to exit the bloodstream [3]. Yet, this mechanism remains poorly understood due to the unique structural and physiological properties of the BBB, as well as the complexity of *in vivo* systems. In order to evaluate the specific impact of hemodynamic forces during CTC extravasation, the development of new dynamic and biomimetic models to study lung CTC extravasation to the brain is crucial, though also challenging. Microfluidic systems are promising platforms to mimic physiological microenvironments under controlled conditions [4]. The development of a microfluidic model of brain metastasis facilitates the study of how hemodynamic forces influence the extravasation of lung CTCs into brain parenchyma, one of the key processes within the metastatic cascade. My master's thesis work consists of developing a microfluidic device to explore the interaction between brain endothelial cells and lung CTCs under distinct hemodynamic forces. In this work, we propose to fabricate a single-channel PDMS-based microfluidic device with main dimensions of 400 μm height, 16 mm length and 0.25 cm^2 of working area functionalised with collagen type I and fibronectin. An endothelial cell monolayer was established under low shear stress conditions (2x10-5 Pa) to ensure consistent nutrient supply, often insufficient in device cultures. Ongoing assays are focused on evaluating endothelial cell response to various levels of shear stress through Live/Dead assay, growth kinetics and immunocytochemistry for expression of membrane proteins, and assessment of cell-cell interactions between endothelial and lung cancer cells. Overall, we believe this study can contribute significantly to advancing personalised medicine approaches for cancer treatment and prevention.

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Mapping epithelial-mesenchymal transition in lung cancer

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Metastases drive systemic dysfunction in multiple organ systems, contributing significantly to patient mortality [1]. During dissemination, cancer cells undergo phenotypic transitions that enable their survival, adaptation to secondary sites and initiation of metastatic outgrowth. In lung cancer, cellular plasticity associated with epithelial-mesenchymal transition (EMT) facilitates organ-specific metastasis through mechanisms such as chemotherapy resistance [2] and collective invasion patterns [3]. However, the impact of EMT on the mechanical properties of cancer cells and subsequently their extravasation dynamics at secondary sites remains largely unexplored. Here, we demonstrate that induction of EMT in A549 or HCC827 non-small cell lung cancer cells, treated with 5 ng/mL TGF-β in DMEM medium with 2% FBS for one week, is accompanied by a diversity of cell line and exposure-time specific cellular adaptations. Such cellular changes depend on the initial cell phenotype, thus highlighting the heterogeneity of cancer cells within the primary tumour. Morphologically, cancer cells undergoing EMT acquire a spindle-like shape, in contrast to the round shape observed in non-treated cells, independently of the cell line. During EMT induction, cells undergo cytoskeleton protein remodelling, namely vimentin and cytokeratin. Experimental evidence from live-cell atomic force microscopy using Quantitative Imaging mode (cantilever spring constant of 0.25 N/m, tip radius of 15 nm, z length of 4000 nm, imaging area of 60 x 60 μm^2, 4096 data points per sample), at 37 °C, suggests the EMT increases the elastic modulus of both cells transitioning from epithelial to mesenchymal phenotype. Pixel-wise force-spectroscopy mapping at a maximum indentation force of 0.4 nN, reveals a stiffer nuclear region compared to the cytoplasm, likely providing mechanical protection to the nuclear contents during hematogenous dissemination. Ongoing studies are addressing the extravasation dynamics and invasiveness characteristics depending on their phenotype.

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Biosensing Approach for Biomarker Detection in Infectious Diseases

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Accurate and timely diagnosis of infectious diseases remains a critical challenge in clinical practice. Biomarkers have emerged as valuable tools for improving diagnosis, enabling early detection, monitoring disease progression, and guiding appropriate treatment decisions. Alongside direct pathogen identification, the simultaneous detection of host-response biomarkers provides valuable insights into infection severity and aetiology. Several biomarkers that are upregulated during the infection process have proven useful in predicting clinical outcomes and in distinguishing between bacterial and viral infections [1]. Among them, interleukin-6 (IL-6) plays a key role in the inflammatory response and has been widely recognised as a clinically relevant biomarker for infection diagnosis, risk stratification, and disease monitoring [2]. Microfluidics shows immense promise for biomarker detection due to the fact that it offers advantages such as faster analysis, enhanced sensitivity in bioassays, lower consumption of samples and reagents, cost reduction, and the ability to miniaturize and combine complex procedures into compact systems [3]. In this work, we present a novel biosensing microfluidic system capable of detecting and quantifying infection-related biomarkers, with a particular focus on IL-6. Due to the typically low concentration of IL-6 in clinical samples, magnetic nanoparticles were employed to selectively separate and concentrate the biomarker from complex biological samples. The magnetically captured IL-6 was then detected and quantified using a fluorescence-based approach by measuring the signal generated from an anti-IL-6 antibody labelled with a high-intensity fluorescent dye. The assays demonstrated high sensitivity and specificity, highlighting its potential to replace conventional ELISA as a faster and more efficient alternative. This approach is being integrated into an optofluidic system previously shown to efficiently concentrate bacterial pathogens from blood [4], to enable automated and multiplexed detection of both pathogens and host-response biomarkers. Together, this approach holds strong promise as an advanced diagnostic tool for infectious disease management.

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Evaluation of the Antitumor Potential of Microalgae Extracts in Different Genetic Profiles of Colorectal Cancer Cell Lines

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Marine resources are a rich source of bioactive compounds with significant biotechnological potential. Among them, microalgae stand out for their ability to produce sulphated polysaccharides, polyphenols, carotenoids, proteins, and phycobiliproteins, which have been associated with antioxidant, anti-inflammatory, and anticancer activities. Recent studies have shown that microalgae extracts can inhibit tumor growth and induce apoptosis, highlighting their promise in cancer prevention and treatment. Given its high global incidence, colorectal cancer has been one of the main focuses in the search for algae-derived compounds with greater therapeutic efficacy and safety profiles.

This study aimed to obtain aqueous and hydroethanolic extracts (50:50 and 80:20 ethanol:water) from selected microalgae species namely, *Phaeodactylum tricornutum*, *Chlorella vulgaris, Tetraselmis chuii*, and *Arthrospira* spp., using ultrasound-assisted extraction. The extracts were subsequently characterized regarding extraction yield, chemical composition (proteins, lipids, carbohydrates, ash, pigments, and phenolic compounds), and antioxidant potential (DPPH, ABTS, and FRAP assays). Furthermore, their anticancer potential was evaluated by assessing cytotoxic effects on colorectal cancer cell lines (HT29, RKO, HCT116 ^{p53} wild-type and HCT116 $^{p53\text{-}null}$) using the MTT assay.

The results showed that extraction efficiency was strongly dependent on solvent polarity, with more aqueous solvents favoring the recovery of polar compounds (*i.e.* polysaccharides and proteins), while hydroethanolic mixtures enabled more effective extraction of lipophilic and bioactive metabolites. From the 12 extracts obtained, an initial screening on colorectal cancer cell lines revealed that the 80:20 ethanol:water extracts of *P. tricornutum* (PT) and *C. vulgaris* (CV) exhibited the strongest cytotoxic effects. These extracts were therefore selected to proceed with studies and determined IC50. The extracts exhibited higher cytotoxic activity against both HCT116 $^{p53\ wild\text{-}type}$ and HCT116 $^{p53\text{-}null}$ cell lines, suggesting their potential effectiveness against cells harboring KRAS mutations, one of the most common alterations in colorectal cancer, regardless of p53 status.

Although preliminary, these findings highlight the potential of selected microalgae extracts as sources of bioactive compounds with antioxidant and anticancer properties, supporting their further development as novel therapeutic agents for colorectal cancer.

Hospital Greywater Systems as Reservoirs of Biofilms: Microbiological Validation of a Novel Antimicrobial Agent

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Greywater systems in hospital settings can contribute to healthcare-associated infections due to the accumulation of microorganisms, particularly within nosocomial biofilms that adhere to the inside of the pipes. These biofilms are difficult to eradicate using conventional disinfectants, particularly in hospital sink drainage systems composed by stainless steel and PVC components. This study evaluates the performance of *PipeUCare*, a novel developed antimicrobial agent, under real-world conditions for the disinfection of hospital sink drainage systems.

PipeUCare was applied in the sinks of eighteen hospital rooms, in three different zones: S – sink drain, D – adjacent dry surface, and W – adjacent wet surface. Samples were collected after application and microbial analysis were conducted to determine the total culturable bacteria ($CFU \cdot mL^{-1}$) and cell viability (OD690). The investigation focused on three surface types commonly found in healthcare water systems: stainless steel, rigid PVC, and flexible PVC.

The results showed that *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterobacter asburiae* were the most frequently identified microorganisms in biofilm samples. *PipeUCare* demonstrated a >5-log reduction in the microbial load at most sites, especially in the dry zone (D) that showed almost total biofilm eradication across all tested materials. The wet zone (W) exhibited a strong but slightly lower biofilm reduction, highlighting the product's effectiveness even under humid conditions. Even though, results in zone S, the drain, were more variable, *PipeUCare* was still more effective than conventional products in both disinfection and recolonization prevention.

These findings support the effectiveness of *PipeUCare* in realistic contamination scenarios and highlighting its considerable potential for integration into infection prevention protocols, particularly in critical healthcare settings.

Exploiting Bacteriophages for Targeted Control and Rapid Detection of *Klebsiella pneumoniae*

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Antimicrobial resistance remains a critical global health challenge, with *Klebsiella pneumoniae* classified by the World Health Organization as a high-priority pathogen due to its prominent role in healthcare-associated infections. Novel strategies for both infection control and rapid detection are urgently needed to counter this threat.

In this study, we characterized a newly isolated *K. pneumoniae* bacteriophage with potential applications in therapeutic intervention and diagnostic development. The phage was isolated from wastewater using clinical *K. pneumoniae* strains and characterized. TEM analysis showed a myovirus phage that was able to infect 31% of the tested K. pneumoniae isolates. The phage has a latent period of 10 minutes and a burst size of 41 PFU/cell. Whole-genome sequencing and annotation identified candidate RBP that were cloned and expressed in *E. coli* Arctic Express (DE3) fused to a fluorescent protein. This enabled to assess their ability to recognize and bind to *K. pneumoniae* cells through fluorescence microscopy. This functional analysis identified two binding proteins.

Overall, this work highlights the dual potential of this phage: as a candidate for therapeutic applications and as a source of proteins with high potential for the development of phage-based detection tools targeting *K. pneumoniae*.

Evaluation Of Pmc79 In Colorectal Cancer Using A Vascularized Crc-On-A-Chip Model

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Colorectal cancer (CRC) represents the third most prevalent and the second most lethal malignancy worldwide, accounting for approximately 520,000 new diagnoses and 245,000 deaths annually in Europe. Despite significant progress in screening and therapeutic strategies, CRC remains a major clinical burden, largely due to pronounced tumor heterogeneity and frequent resistance to frontline regimens such as 5-fluorouracil (5-FU). Traditional two-dimensional (2D) culture models insufficiently recapitulate the complex tumor microenvironment, limiting translational predictability. In contrast, vascularized CRC-on-a-chip platforms provide physiologically relevant conditions for preclinical drug assessment.

In this study, the antineoplastic efficacy of a polymer–metal conjugate, PMC79, was investigated using a vascularized CRC-on-a-chip system composed of HUVECs, NHLFs, and GFP-tagged CRC cells (SW480-GFP and RKO-GFP) cultured within PDMS-based microfluidic devices simulating tumor vasculature. Drug perfusion was conducted over a 48-hour period. PMC79 exhibited a robust cytotoxic effect, achieving a 2.7-fold reduction in GFP signal in SW480-GFP cells, thereby surpassing the efficacy of 5-FU, and a 2.51- fold reduction in RKO-GFP cells. Furthermore, at 40 μM (the SW480 IC50), PMC79 directly disrupted endothelial organization, evidenced by a 2.45-fold decrease in RFP-HUVEC signal relative to medium controls.

In summary, PMC79 demonstrated pronounced anticancer activity within a physiologically relevant CRC-on-a-chip framework, outperforming 5-FU and additionally exerting concentration-dependent effects on vascular integrity. The effect of PMC79 on the vasculature is particularly relevant given that tumor angiogenesis and vascular remodeling are central to CRC progression, metastasis, and drug resistance. The tumor vasculature not only sustains cancer growth by supplying oxygen and nutrients but also constitutes a dynamic barrier influencing drug delivery and therapeutic efficacy. These results position PMC79 as a compelling candidate for CRC therapy and emphasize the translational importance of integrating advanced 3D and microfluidic models into preclinical drug development pipelines.

INDUSTRIAL BIOTECHNOLOGY

KEYNOTE LECTURES

CARLA SILVA, CITEVE

Biotechnology @ Textiles: Technology Achievements, Opportunities and Challenges

Carla Silva (Director Chemistry and Biotechnology Department at CITEVE) holds a degree in Biological Engineering, a M.Sc. in Industrial Biotechnology and a PhD in in Textile Chemistry. With more than 25 years of experience in industry-oriented research, she has been responsible for the coordination of R&D projects in biotechnology and bio & circular economy.

TATIANA AGUIAR, CEB

Navigating the Crossroads of Bioengineering on the Path to Industrial Biotechnology

Assistant researcher at CEB, Tatiana integrates the Molecular Biotechnology and Precision Fermentation (BioPreFer) research group. With over 15 years of experience in engineering microbial platforms for the precision fermentation of bio-based products, her work is dedicated to advancing sustainable bioproduction through engineered microbes, bridging fundamental research and applied innovation in molecular and microbial biotechnology. Since 2018, her primary focus has been the development of eco-sustainable and innovative protein-based and protein-engineered materials for traditional industrial sectors.

LIST OF ABSTRACTS

ORAL COMMUNICATION

Peroxisome engineering towards de novo γ-decalactone production in *Ashbya gossypii*

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Lactones are highly valuable cyclic esters used as fragrances and flavor enhancers, possessing high industrial value. They are mainly produced by chemical synthesis or bioconversion strategies, leading to high production costs and the need for a more sustainable process. Lactones are converted from intermediate compounds derived from the β-oxidation pathway, a process that provides acetyl-CoA through degradation of fatty acids (FA) in the peroxisome. Compartmentalization of metabolic pathways in organelles can improve target compound production, as shown in yeast with peroxisome enzyme surface display in conjunction with enlarged membrane surface area that successfully increased acetyl-CoA levels and derived products. *Ashbya gossypii* is a filamentous fungus and a natural de novo producer of lactones, making it an interesting target for peroxisome targeted engineering. Metabolic engineering of *A. gossypii* was carried out to modulate expression of genes involved in peroxisome formation. Developed strains were characterized, γ-decalactone production was analyzed by GC-FID and lipid profiling and quantification were achieved by GC-MS. An *A. gossypii* peroxin for peroxisome formation expressed under different promoters was successfully integrated in the genome as well as a construction of a transmembrane peroxin for peroxisome surface display fused with a reporter gene. Microscopy observation allied with timelapse imaging revealed physiological changes in timed events of initial growth, and a fluorescence analysis revealed localization of reporter gene signal, suggesting the potential of endogenous peroxin for peroxisome surface display in *A. gossypii*. Developed strains conserved biomass production yields, suggesting no increase in cellular metabolic burden despite no increase in γ-decalactone production. These results showed the feasibility of peroxins for peroxisome modifications and organelle surface display of enzymes in *A. gossypii*, while exhibiting new metabolic engineering approaches to organelle compartmentalization towards optimizing γ-decalactone production for a more biobased sustainable process.

POSTER COMMUNICATIONS

Exploring the combination of enzymatic treatment and ultrasound-assisted extraction of biochemical compounds on *Microchloropsis gaditana*

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Microchloropsis gaditana (formerly known as *Nannochloropsis gaditana*) is a marine microalga of high biotechnological interest, rich in biochemical compounds such as polyunsaturated fatty acids, pigments and antioxidants. It is mainly used as feed in aquaculture, but it has many other potential applications such as nutraceuticals, cosmetics and biofuels. However, its rigid and complex cell wall significantly limits the accessibility to these intracellular compounds, presenting a major challenge for their extraction.

Therefore, this work aims to study the application of ultrasonication and enzymatic treatment on the disruption of *M. gaditana* and extraction of biochemical compounds.

For this, three different treatments were applied under optimized conditions: (1) enzymatic treatments with cellulase, pectinase and viscozyme at a concentration of 40 mg gdw^{-1}, incubated at 55 °C for 72 hours; (2) ultrasonication, using a 40 kHz probe in pulse cycles; and (3) combination of ultrasonication with enzymes as post-treatment. The samples were analysed by cytometry and the extraction yield and intracellular organic matter released were assessed. Proteins, lipids, carbohydrates, pigments and antioxidant activity were also quantified.

The results suggested that 5 minutes of ultrasonication with pulse cycles of 4 s on and 2 s off provided the higher value of extraction yield without degradation of biochemical compounds. Cellulase was the most effective enzyme in most of the tests performed, both in (2) and (3), providing an extraction yield 2-10% higher than the other enzymes.

Regarding all the treatments applied, the best results were obtained when ultrasonication and enzymatic treatment were combined, suggesting a possible synergistic effect between these treatments. The combination of ultrasonication for 5 minutes, followed by the incubation with cellulase at 55 °C for 72 hours resulted in the highest extraction yield of 31,3 ± 0,4 %. Although the treatments allowed the extraction of some intracellular compounds, cytometry analysis indicated partial permeabilization of the cells rather than complete cell lysis, since all the treatments led to a decrease in cell integrity, but values remained above 50%.

Cosmetic potential of a structural protein co-polymer based on silk, elastin and keratin

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The hair cosmetic industry is constantly searching for new ingredients and innovative materials that can help enhance properties such as softness, shine, shape, and color. However, conventional products often contain harmful chemicals. In this study, we developed a bio-based alternative using a recombinant protein composed by repetitions of consensus motifs from the structural proteins silk, elastin and keratin, termed SELP::KP. This protein was expressed in *Escherichia coli* and purified by a non- chromatographic method to high purity. Fourier-transform infrared spectroscopy (FTIR) confirmed the β-sheet-rich structure, characteristic of the silk and SELP-like proteins.

Calorimetry studies confirmed the high thermal stability of SELP::KP. When applied to hair, SELP::KP co-localized at both the cuticle and cortex, indicating strong penetration capacity. Mechanical testing of treated virgin hair showed significant improvements: Young's modulus increased by 34.9%, and extensibility improved by 12.4% compared to untreated controls, indicating enhanced strength and elasticity. Additionally, protein's potential as a safer perming agent was evaluated, maintaining a curling effect similar to the chemical treatment, but with lower damaging effects. Furthermore, SELP::KP showed to reduce to half the combing strength needed, acting as an effective conditioning agent.

This research highlights SELP::KP as a promising bio-based co-polymer for hair cosmetics, offering both styling benefits and improved hair health, as a leave-in treatment or a hair mask that aids in combability and perming service.

Unveiling hypersaline environments: novel biosurfactants and pigments unlocking new opportunities in biotechnology

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Extreme environments, such as saline and hypersaline sites, are promising sources of biomolecules with potential for biotechnological innovation. Among them, biosurfactants emerge as sustainable alternatives to petroleum-based surfactants, offering low toxicity, high biodegradability and comparable or even superior performance.

In this study, hypersaline sites in Portugal, Spain and Cape Verde were explored using culture-dependent approaches to assess their potential for biosurfactant production. Water and soil samples were chemically characterized and used for microbial isolation, resulting in a collection of 450 isolates. This collection was screened for biosurfactant activity using the Blood Agar and Victoria Pure Blue BO (VPBO) assays. A total of 52 isolates showed positive results and the most promising isolate from each sampling site was selected for further validation.

Biosurfactant production was validated through surface tension reduction, emulsion index and VPBO assays. We further investigated the nature of the biosurfactants (extracellular or cell-bound) and optimized their biosynthesis in three culture media (MGM, LB and MB) supplemented with 1% n-hexadecane and with salinity adjusted to the conditions of the sampling site. Additionally, some isolates exhibited emulsifying capacity, and one was able to produce a pink pigment.

Our findings demonstrate the biotechnological potential of hypersaline environments as sources of novel biomolecules like biosurfactants and pigments, supporting their relevance as green alternatives for diverse industrial fields.

Bioprospecting extreme environments through metagenomics for the discovery of promising biomolecules

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Extreme environments, such as hypersaline environments, composting sites and mines, harbour a high diversity of highly adapted microorganisms called extremophiles. These ecosystems are also promising sources of unique bioactive molecules with high biotechnological potential. This study aims to explore these environments at both taxonomic and functional levels based on metagenomic analyses using databases such as COG, CAZy and CARD.

Focusing on taxonomic analysis, the study observed a high presence of bacteria in all environments, with high concentrations of archaea in hypersaline environments, high diversity in composting sites and acidophilic communities that are resistant to heavy metals and to the low pH generally find in mines. The COG functional profile revealed similar percentage distributions of the main categories across the environments, though variations were observed that reflect adaptations to adverse conditions, such as a greater presence of metabolic genes in composting sites, genes involved in information storage and processing in mines, and genes involved in cellular processes in hypersaline environments. The CAZy database revealed the abundance of Glycoside Hydrolases (involved in lignocellulose degradation) and Glycosyltransferases (involved in the transfer of sugar moieties to diverse biomolecules) in all environments studied, as well as the presence of the other classes of enzymes in auxiliary activities, with slight variations depending on the environment. CARD analysis revealed the presence of antibiotic resistance genes common to all environments, thus suggesting the possible presence of antibiotic classes such as tetracyclines, macrolides, and glycopeptides.

Overall, our results reveal the significant biotechnological potential of the three environments for the identification of interesting biomolecules for various applications, including bioremediation, sustainable agriculture, healthcare, food and industrial processes.

A Revolution in the Textile Industry: Sustainable Innovation with Cardoon Fibre

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The textile industry is one of the world's oldest and currently faces significant environmental challenges. It is characterised by high energy and water consumption, produces a lot of waste and contribute to biodiversity loss. Water contamination is another critical issue, caused by monoculture practices and fertilisers, as well as the discharge of industrial waste such as dyes and chemicals. There is an urgent need to adopt innovative solutions that favour the transition to a circular and sustainable economy, that can reduce environmental pressure.

One alternative is the use of biomass plantations, which can help to regenerate degraded soils and create new market opportunities. In this context, cardoon (*Cynara cardunculus)*, a perennial herbaceous plant, shows great potential as its ability to grow on marginal and degraded land makes for combatting desertification and restoring ecosystems. However, to ensure true sustainability and economic viability, the by-products must be utilised, particularly the stem fibre.

This study analyses the potential use of cardoon stems as a sustainable alternative to cotton in the textile industry. The research explores the fibre extraction process and the technical, economic, and environmental viability of the resulting material. Fungal tests were conducted to ascertain whether cardoon and cotton fibres are equally susceptible to colonisation by *Aspergillus niger* (MUM 28645), in both planktonic and biofilm forms. Additionally, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were used to characterise the fibre.

Cardoon fibre has been shown to be more resistant to the fungus in both its free and biofilm forms. FTIR data revealed a similar chemical composition, highlighting the presence of lignin in cardoon fibre and its absence in cotton fibre. The fibre diameter was similar for both fibres, as observed from the SEM images.

This approach enables us to evaluate the potential of cardoon to contribute to the sustainable transformation of the textile sector by reconciling innovation, competitiveness and environmental responsibility.

Optimizing the biosurfactant/bioemulsifier production by halophilic isolates from Rio Maior salterns

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Microbial biosurfactants have gained emphasis as sustainable alternatives to synthetic surfactants due to their biodegradability, low toxicity, and potential applications in industries, such as cosmetics, pharmaceuticals, and food. However, the costs associated with production and recovery are still limiting their large-scale use. Hypersaline environments are important reservoirs for discovering novel biosurfactants, as the extreme conditions drive microorganisms to produce unique surface-active biomolecules that enable them to adapt to high salt stress.

The main goal of this study was to improve the production of biosurfactants/bioemulsifiers by halophilic microorganisms (*Salicola salis* and *Halomonas sabkhae*) sourced from Rio Maior salterns. For this purpose, different culture media, temperature, agitation, and aeration conditions were evaluated. The microbial growth, surface tension reduction, emulsification index, and the presence of biosurfactants able to solubilize the Victoria Pure Blue BO (VPBO) dye were used to establish the optimal conditions. The nature of the biosurfactants/bioemulsifiers (extracellular or cell-bound) was also analyzed. For both microorganisms, the most suitable conditions included LB culture medium supplemented with 15% of NaCl and 1% of *n*-hexadecane, 100 rpm, and higher aeration. However, some differences were found for the optimal temperature, namely 30 °C and 37 °C for *S. salis* and *H. sabkhae*, respectively. The cell-bound biomolecules proved to be more promising, showing a surface tension reduction of about 20 mN/m, an emulsification index (24h) of 25%, and a critical micellar concentration around 4 g/L. The VPBO also suggested the presence of biosurfactants for both microorganisms. Overall, this work emphasizes hypersaline environments as powerful sources of underexplored high-value biomolecules.

Designing recombinant seed storage proteins for textile applications

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The growing demand for functional textiles with greater strength, hydrophobicity, and durability, has driven interest in incorporating new biomolecules, particularly proteins, in textile innovation. Plant storage proteins are distinguished by their ability to form films, biodegradability, and amphiphilic nature. These properties, together with the cost-benefit ratio in their production and the reduced environmental impact, make plant storage proteins a new class of biomolecules with high potential for the development of advanced functional textiles.

Cruciferin, the main storage protein of canola seeds, presents a hexameric structure, in which each subunit contains an acidic (-α) and a basic chain (-β), linked by a disulfide bond. In this work, three recombinant proteins derived from canola cruciferin (Cruc, Cruc-α, and Cruc-β) were designed and explored for the functionalization of cotton substrates. After expression, purification and characterization, cotton fabrics were functionalized with the cruciferin-based proteins by exhaustion and padding methods. To further improve the bioactivity of the functionalized cotton fabrics, the proteins were combined with a grape hydroethanolic extract. Functionalization was confirmed by SEM, with the proteins forming a film on the cotton fibres. The coating also increased the hydrophobicity of the fabrics, particularly for the samples functionalized with Cruc protein by padding. Moreover, no significant differences were observed on coating ability and fabrics hydrophobicity when the grape extract was also used.

Technical Guide to study the Chemical and Physical Properties of Human Hair

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Hair has a biological purpose of protecting the scalp, maintaining the thermoregulation equilibrium of the body and with a key role in humans' physical appearance. Hair has distinctive morphological, mechanical and chemical properties that distinguish it from other body tissues. The structural complexity of hair fibers demands a multidisciplinary approach to its analysis. The health of hair and the tests of potential products should be studied with high accuracy and reproductively. This work provides a comprehensive overview used to evaluate several measurable characteristics of hair, divided in five categories: the aesthetic and sensory parameters, the surface morphology, chemical structure, mechanical and thermal properties. This includes several techniques and properties as classical as shine and mechanical strength to newer methods as differential scanning calorimetry and elemental composition. A deep understanding of hair's complex properties combined with the application of advanced analytical techniques is crucial for developing effective hair care products and compare them. The need for standardization of hair analysis techniques intensified the importance and motivation to write an article that thoroughly explores highly described techniques that are crucial for hair characterization.

Exploring *Palmaria palmata* hydrolysates as substrates for biomass and value-added compounds production by *Yarrowia lipolytica*

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The growing expansion of seaweed farming for human consumption and as a source of bioactive compounds for several industries, along with accidental releases, has led to the spread of invasive species that threaten coastal ecosystems. The dual role of seaweeds, as a renewable resource of bioactive compounds and as an ecological management challenge, underscores the need for strategies that both exploit and manage seaweed biomass. The relatively high carbohydrate content of *Palmaria palmata* (a red seaweed from the Atlantic coast of Europe) makes this biomass suitable as feedstock for microbial bioprocesses within a biorefinery framework. This study explored the potential of *P. palmata* hydrolysates as substrates to produce *Yarrowia lipolytica* NCYC 2904 biomass and value-added compounds in stirred-tank bioreactors. Hydrolysates from non-washed *P. palmata* (PH) and washed *P. palmata* (WPH) were obtained after hydrothermal pretreatment in an autoclave (121 °C, 20 minutes), followed by enzymatic hydrolysis with Cellic® CTec3 (15 FPU/g of solid). The yeast grew without inhibition in undiluted hydrolysates, consumed significant amounts of xylose (60 g/L), and produced erythritol, a feature not previously reported. The highest erythritol concentration (40 g/L) and protein content in yeast biomass (29 %, w/w, dry basis) were obtained in PH cultures, whereas the highest biomass concentration (43 g/L) and lipids accumulation (15 %, w/w, dry basis) were attained using WPH. Furthermore, pressurized stirred-tank bioreactor experiments at 2.5 and 4 bar of total air pressure enhanced protein synthesis to 53 % (w/w), representing a 3.8-fold increase compared to the protein content in crude seaweed. Lipids produced under all conditions were composed of mono- and polyunsaturated fatty acids, and the yeast biomass exhibited a well-balanced amino acid profile, including essential amino acids. These results demonstrate the potential of *Y. lipolytica* as a microbial platform to valorize seaweed biomass into protein-rich biomass (approved for human consumption), lipids, and erythritol.

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Process-Oriented Strategies for Recombinant Protein Production in *Escherichia coli*

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Escherichia coli remains one of the most established microbial platforms for recombinant protein production, combining fast growth kinetics, simple nutritional requirements, and scalability from laboratory to industrial settings. Despite these advantages, the efficient production of correctly folded and biologically active proteins often requires careful optimization of upstream and downstream processes (DSP). Key strategies include tight control of culture conditions such as temperature, aeration, induction timing, and feeding regimes in high-cell-density fermentations, which strongly influence protein solubility and yield. Process engineering approaches, such as fed-batch, as well as the application of controlled stress conditions, can enhance protein productivity while reducing aggregation. In the upstream process, the strain, culture media composition and pre-culture can be altered in accordance with fine tuning or with statistical methods to improve final productivity. Furthermore, cell disruption, purification and concentration methods can be altered in the DSP process to improve the yield. Moreover, advances in bioprocess monitoring and modeling have facilitated a more rational design of production strategies, improving reproducibility and scalability. Together, these process-oriented optimizations further establish *E. coli* as a versatile and cost-effective system for the large-scale production of recombinant proteins with applications across biotechnology, biopharmaceuticals, cosmetics and agrofood industry.

Enzyme Production by Solid-State Fermentation of Brewer's Spent Grain: Ohmic Heating Versus Autoclaving Pretreatment

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The industrial utilization of enzymes facilitates sustainable bioprocessing and enhances product quality. Although enzyme production is predominantly achieved via submerged fermentation, solid-state fermentation (SSF) constitutes a more environmentally sustainable alternative. SSF is characterized by minimal free water and the use of moist solid substrates, enabling microbial metabolism with reduced energy and water inputs. The valorization of agro-industrial residues, particularly brewer's spent grain (BSG), further reinforces the sustainability of SSF systems. BSG, a major by-product of beer production, is frequently discarded or employed as low-value livestock feed despite its rich composition of hemicellulose, cellulose, lignin, and protein, making it an attractive substrate for microbial
growth. Pretreatment of BSG enhances the bioavailability of its constituents. While chemical methods are traditionally used, environmentally benign strategies such as hydrothermal processing and ohmic heating (OH) are emerging as promising alternatives. OH generates internal heat by passing alternating current through a semi-conductive matrix, potentially serving as both pretreatment and sterilization step.

In this work, BSG underwent autoclave and OH pretreatments, followed by scanning electron microscopy (SEM) characterization and use as substrate for SSF with *Aspergillus niger*. After seven days of incubation, phenolic compounds, antioxidant activity, soluble proteins, reducing sugars, and enzymatic activities were quantified.

SEM analysis revealed that autoclaved BSG largely preserved its structural integrity, whereas OH induced pronounced matrix disintegration and fiber exposure, indicative of improved compound accessibility. Consistently, OH treatment significantly enhanced solubilization of phenolics, proteins, and reducing sugars compared with autoclaving. SSF of OH-pretreated BSG resulted in superior enzyme production, with xylanase and protease activities reaching 540 U/g and 49 U/g, corresponding to 1.5- and 1.6-fold increases relative to autoclaved controls.

These findings highlight OH as an effective pretreatment for enhancing enzyme yields and underscore its potential as a sustainable strategy for by-product valorization aligned with circular economy principles.

PDMS surface wettability modification for microfluidics applications

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Polydimethylsiloxane (PDMS) is commonly used in microfluidic devices due to its optical clarity, biocompatibility, and ease of fabrication. However, its hydrophobic nature (surface contact angle of 110°, [1]) limits its effectiveness in applications that rely on fluid transport and small molecule detection. Therefore, it is of high interest to develop long-term PDMS surface modification methods. Surface modification techniques (e.g, surface coatings, chemical activation, bulk modification) [2] have been explored to address these limitations, to enhance the wettability of the PDMS surface. In particular, microfluidic devices used for organ-on-a- chip, inadequate surface wetting leads to the inefficient flow of fluids through channels, a lack of biocompatibility for cell culture, and surface adsorption of biomolecules, leading to difficulties in accurate molecular analysis. This study investigates the use of the surfactant PEO, (C2H4O)n [3] (Polyethylene oxide, Polysciences Europe GmbH, Hirschberg an der Bergstraße, Germany) at 1% and 2.5 % (w/v), incorporated in bulk in the PDMS mixture at 10:1 (w/w) ratio to improve its hydrophilicity [4]. The bulk modification method refers to a process where the selected surfactant is blended with a PDMS mixture while all these components are still in a liquid form [5]. Water contact angle (WCA) measurements were used to assess the effectiveness of these modifications for 14 months. Initial results indicated that PDMS modified with PEO demonstrated a significant reduction in WCA to values around 27°, suggesting improved hydrophilicity immediately after fabrication. In addition, it was also tested samples modified with the combination of surfactant- bulk modification and oxygen plasma treatment (15W and 25 W for 30s or 2 min). The results demonstrated that the addition of PEO combined with the oxygen plasma treatment reduced the values of CA, *i.e.* increased the PDMS hydrophilicity, maintaining the value constant over several months and keeping PDMS optical property, which is of high importance when microscope visualizations within the devices are needed. The outcomes of this work were encouraging, especially when the 2.5% PEO surface modification and oxygen plasma were used. The samples exhibited improved wettability over 14 months, a great advancement for improving the fabrication of organ-on-a-chip devices.

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ROUNDTABLE & WORKSHOPS

ROUNDTABLE

LÍGIA RODRIGUES, CEB, UM (MODERATOR)

From Passion to Profession: the realities of a scientific career

Ana Machado
Area Manager for R&D and Innovation at MC SONAE

Cristina Rocha
Assistant Researcher (tenured) at Centre of Biological Engineering, University of Minho

Débora Ferreira
Junior Researcher at Centre of Biological Engineering, University of Minho

Joana Alves
Former Centre of Biological Engineering Researcher

Marisol Dias
PhD student at International Iberian Nanotechnology Laboratory

WORKSHOPS

CARLOS COSTA, LABBLES

The art of the science pitch: Tools and techniques to make your research stand out with

Carlos E. Costa is a science communication officer at LABBELS. With a PhD in Chemical and Biological Engineering, he has coordinated initiatives such as the Image and Video Team of Pint of Science Portugal (2019–2021), and is the creator and producer of the science podcast PhD Chain Reaction. He combines his scientific communication work with ongoing projects in music, sound engineering, and independent film production. He specialises in bridging the gap between science and society through creative communication grounded in clarity, visual storytelling, and a strong sense of aesthetics.

The workshop occurred on 2nd October and aimed to improve communication skills of scientists, with several advices and interactive examples for relaxing and captivating attention when speaking.

RICARDO MALHEIRO, CEO OF READYTOPUB

Scientific Writing - General Principles for Obtaining a Scientific Publication.

The extra workshop occurred on 1st October and aimed to guide the participants in before-writing tasks, such us analyse the journals to publish their work, learn about impact factor, and impact factor limitations, know how to analyse the journals metrics and how to select the most appropriate journal for their work. Also, one of the main goals was to demystify the idea that publishing in high impact journals is an impossible task.

Topics: (1) What is an impact factor journal? (2) Why to publish in indexed journals? (3) Limitations of the impact factor; (4) Excuses and mentality changes; (5) Selection of the most appropriate journal: The Journal of Citation Reports (JCR) as an essential tool.