

### **ABSTRACT BOOK**





# LEbiotec

Abstract Book

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TALK

#### Innovative method for plant biostimulants production

Chiara Anglana<sup>1-2</sup>, Piergiorgio Capaci<sup>1</sup>, Fabrizio Barozzi<sup>1</sup>, Egidio Stigliano<sup>2</sup>, Gian Pietro Di Sansebastiano<sup>1</sup>

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Biostimulants have a high economic value and the global market is continuously expanding since their use contributes to increasing the production, yield and quality of plants with great advantages in the agri-food sector. Development of biostimulants from waste has an important valorization potential in a context where there is ever-increasing research of ecosustainable cultivation methods, combined with the concept of circular economy which has the purpose of converting waste materials and by-products into new valuable resources.

Recently, we have filed a patent application (No. 102024000012364) disclosing a system for the innovative use of aquatic mosses secretion as plant biostimulant to be used in agriculture and *in vitro* culture. We observed how the biomasses of aquatic mosses grown in temporary immersion bioreactors or open vessels, actively secrete into the water several molecules that we characterized as different classes of phytohormones, small amounts of peptides and other secondary metabolites with biostimulatory effects on several plant species.

After growth cycle in bioreactors, the basic culture medium, a byproduct to be discarded, was instead used in several experiments also in complex formulations. For example, we observed an increase in root length of 35% and fresh weight of 43% in *Nicotiana tabacum* plants treated in *in vitro*. Experiments had excellent results even under stress conditions. In greenhouses experiments, carried out on various vegetables such as Foeniculum vulgare, we recorded an increase of 64% in fresh weight and 51% in dry weight, while, in field experiments on Zea mays, final productivity was 34% higher in dry grain weight compared to the untreated control.

The entire production line is a zero-waste process opening very interesting perspective in the industrialization of an innovative method to produce phytohormones to be used as biostimulant and beyond.

# Saccharomyces cerevisiae as a model organism to evaluate the antioxidant potential of dietary phytochemicals: a promising approach for therapeutic discovery

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Consuming natural foods, particularly fruits and vegetables, can help alleviate oxidative stress, a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants. Oxidative stress plays a significant role in the development of various acute and chronic diseases as well as the aging process.

Flavonoids such as quercetin, apigenin, luteolin, naringenin, and genistein, along with the stilbene resveratrol, are abundant in fruits and plants and have demonstrated promising antioxidant properties *in vitro*. However, their efficacy *in vivo* remains uncertain due to extensive metabolism.

This study investigated the antioxidant activity and underlying mechanisms of these compounds *in vivo*, using *Saccharomyces cerevisiae* as a eukaryotic model organism. After confirming the non-toxicity of these compounds to yeast cells within a specific concentration range, various assays, including growth curves, spot assays, drug drop tests, and CFU assays, demonstrated the protective effects of polyphenols against  $H_2O_2$ -induced oxidative damage.

Polyphenol-treated cells showed reduced intracellular ROS levels and protein carbonylation, indicating effective antioxidant properties. Additionally, treatment with these compounds restored catalase activity and decreased oxidized glutathione levels, thereby supporting the maintenance of redox homeostasis.

These findings suggest that the tested natural compounds effectively protect yeast cells from oxidative stress-induced damage by scavenging intracellular ROS and enhancing cell viability.

This research suggests the potential of these polyphenols as dietary supplements or therapeutic agents in mitigating oxidative stress-related disorders.

### Green synthesis of Chitosan Nanoparticles as Locked Nucleic Acid Delivery Systems

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Nanoparticle delivery systems, such as chitosan nanoparticles (Ch-NPs), hold great potential for enhancing therapeutic efficacy and safety. Ch-NPs, derived from the natural polysaccharide chitosan, offer several benefits, including increased stability, improved cellular uptake, and superiori ability for targeted delivery. Ch-NPswere synthesized via the ionic gelation method, with tripolyphosphate (TPP) acting as a cross-linker obtaining a size between 150 and 300 nm. Successively, Ch-LA were functionalized with hyaluronic acid (HA). This synthesis involved encapsulating negatively charged locked nucleic acid (LNA) oligonucleotides targeting miR-210 (LNA-210) through ionic interactions with the polymeric components. The resulting NPs exhibited high entrapment efficiency of LNA-210 and provide effective protection of the genetic material from RNAse degradation. Ch-NPs loaded with LNA-210 were biocompatible without intracellular reactive oxygen species (ROS) production in cardiosphere-derived cells (CDCs). Moreover, treatment with Ch-LNA did not result in any detectable alterations in the organization of cortical actin fibers and mitochondria morphology. Additionally, no significant changes were observed in cell area or nuclear circularity. These groundbreaking findings unlock new opportunities for research and innovation, setting the stage to fully harness the potential of Ch-NPs in revolutionizing targeted drug delivery and advancing precision medicine.

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### Development of inorganic nanoparticles for multimodal imaging applications

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In recent years, nanotechnology has made possible the development of new contrast agents with increasingly exceptional performance. In this regard, inorganic nanoparticles are one of the most studied materials thanks to the unique physicochemical properties that depend on their nanoscale dimensions.

Various synthetic approaches have been proposed for the preparation of inorganic nanoparticles with different compositions and sizes, which can be tailored according to their applications, such as solid tumor diagnosis, imaging, drug delivery and theranostics. In addition, their surfaces can be functionalized with biomolecules or polymers to improve pharmacokinetics and biodistribution, thereby increasing diagnostic efficiency and effective drug delivery by reducing adverse side effects associated with nonspecific delivery, as well as increasing drug concentration at the desired site of action. [1,2]

An accurate diagnosis is the first vital procedure to effectively improve disease outcome, and it largely depends on the development of imaging technology. In this field, there has been enormous interest in the development of new tools for multimodal molecular imaging, which combines different techniques to assess anatomical and physiological processes in vivo. [1]

Different imaging modalities, such as magnetic resonance imaging (MRI), computed tomography (CT), fluorescence imaging (FLI), nuclear medical imaging (PET, SPECT), and photoacoustic imaging (PAI), can provide anatomical and spatial information about site and progression stage of pathologies. Within this frame inorganic nanomaterials, thanks to their adjustable compositional and physicochemical characteristics, enable detailed cellular and molecular characterization. [2,3]

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### Next-generation sensing: towards a real application of the MIP-sensors based on Green Analytical Chemistry

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Green analytical chemistry (GAC) is a branch of modern analytical chemistry that focuses on minimizing the environmental impact of chemical processes. With the aim of reducing the use of hazardous reagents, limiting waste production and promoting energy-efficient processes, GAC is proposed as a solution to the growing demand for sustainable and ecofriendly approaches [1].

Chemical sensors based on molecularly imprinted polymers (MIPs) have proven to be tools that meet the requirements and principles of green analytical chemistry, opening new, more environmentally friendly and efficient pathways [2]. Designed to recognize specific target molecules, MIPs function similarly to natural receptors in biological systems. Their high selectivity, sensitivity and robustness therefore make them ideal candidates for applications in environmental monitoring, medicine and food safety. The advantages of MIP-based sensors include the use of electrochemical techniques where miniaturized transduction elements can be used, allowing limited use of reagents and solvents, with the possibility of regeneration approaches that reduce their overall environmental impact while enabling regular monitoring at lower cost and in the shortest possible time.

This presentation discusses the integration of MIP-based sensors with GAC principles and show some recently developed sensors [3 - 5] optimized for monitoring in complex matrices such as milk, tap water, seawater and surface water. By combining these tools with miniaturized devices, research is getting closer to the next generation of sensors, making monitoring of environmental pollutants, early diagnosis in hospitals and checking for adulterants in food a daily routine.

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# Harnessing Green-Synthesized TiO<sub>2</sub> Nanoparticles for Superior photocatalytic Degradation of Pollutants in Aquatic Environments

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In the last years the aquatic environment has been increasingly contaminated by organic chemicals. Beside conventional methods used for the removal of toxic pollutants, other technologies are needed to treat wastewaters, such as advanced oxidation processes (AOPs) that are usually used in combination with biological treatments. Among different materials, Titanium dioxide (TiO<sub>2</sub>) is the most studied photocatalyst due to its unique physical-chemistry properties including high photoactivity. To this end, the photocatalytic activity of TiO<sub>2</sub> NPs was evaluated by investigating the degradation of methylene blue (MB) under UV light. In detail, the TiO<sub>2</sub> NPs used in this work were synthesized by means of two different synthesis methods: a green route, exploiting the *Aloe vera* leaves extract properties, and a conventional approach, using in both cases Titanium(IV) isopropoxide (TTIP) as TiO<sub>2</sub> precursor. Both synthesized NPs were characterized by means of XRD, TEM and  $\zeta$ -potential analysis. The both types of TiO<sub>2</sub> NPs showed a degradation efficiency value of (50 ± 3)% and of (16 ± 3)% after 180 min, respectively for green and conventional TiO<sub>2</sub> NPs. This result was confirmed by the reaction rate constant: it was found to be about 5 times higher for TiO<sub>2</sub> NPs synthesized by green approach than that of NPs obtained by conventional route.

### Development of optical and electrochemical ultra-sensitive sensors for the detection of biomolecular markers for application in clinical and food analysis

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The analytical chemistry field is currently focused on enhancing the already established technologies and creating innovative methods for analyte assessment. Chemical sensors, comprising a recognition element for the target binding and a transducer for the processing of a reliable signal, represent a valid alternative offering fast and specific responses. This led our group to the development of optical and electrochemical sensors based on artificial recognition elements for the detection of analytes of food and clinical relevance. The designed artificial recognition elements were molecularly imprinted polymers (MIPs), deriving from the polymerization of functional monomers around target templates whose removal leaves complementary cavities, and receptors based on metal ion-mediated interaction.

A MIP-based electrochemical sensor was developed for the amperometric detection of tyrosine, obtained from the electropolymerization of a thiophene-derivative around the target molecule. A linear response was achieved in the clinical concentration range 15 – 200  $\mu$ M with a LOD of 1.04  $\mu$ M. Selectivity, stability and real sample analysis finalized the analytical performance of the sensor.

An optical sensor based on metal-ion receptor was developed, exploiting the metal ion affinity for analyte functional moieties. Particularly, a reconfiguration strategy was established, allowing the sensor tuning towards different analytes. This resulted in the detection of target analytes such as the dipeptide carnosine and the nucleotide ATP by interchanging between metal ions on the same optical transducer.

Generally, our sensor development strategies led to the production of feasible, cost-effective and sensitive devices, contributing to the increasing demand for innovative instruments involved in clinical and food analysis.

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# Polyethylene terephthalate (PET) nanoplastics affect the behavior and physiology of *Artemia franciscana*

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Nanoplastics (NPs) are increasingly recognized as a significant environmental and health concern. Studying the responses of living organisms to nanoplastic exposure is crucial for understanding the environmental and health impacts of NP pollution. The present study aims to evaluate the behavioral and physiological response of the model microcrustacean organism *Artemia franciscana* to exposure to polyethylene terephthalate (PET) nanoparticles, which are widely diffused in the environment due to the extensive use of PET products and the degradation of larger plastic items.

The study used environmentally relevant model PET NPs characterized by an intrinsic autofluorescence. They were obtained from PET bottle degradation by a fast top-down approach based on mechanical fragmentation, a process close to the mechanical abrasion of microplastics occurring in the environment. The study was carried out by real-time integration of behavioral recordings with measurements of physiological outcomes during acute exposure (24h and 48h) of *A. franciscana* at two different life stages, newborn and adults, to several concentrations of PET NPs ranging from 0.5 to 50 ng/L. PET NPs exerted a significant impact on the motile behavior of the studied organism such as alterations in mean acceleration and trajectories of movement. Results contribute to elucidating how nanoplastics affect neurological and physiological functions in invertebrates.

### Lab-on-chip for Xylella fastidiosa study

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*Xylella fastidiosa* subsp. *Pauca* is a particularly perilous gram-negative bacterium that caused the devastation of the landscapes in Salento peninsula and triggered a crisis in olive oil production [1], [2].

In response to this epidemic outbreak, our research group has developed two different Labon-chip. The first is a miniaturized qPCR system for an on-field detection: a printed circuit board (PCB) integrates a miniaturized heater, temperature sensors and a fan to perform the Real-time PCR thermal cycling. With an intuitive software in Labview, it is possible to control all process steps and detect *X. fastidiosa* by a laser and a detector integrated in the sample miniaturized system like a common thermal cycler would do.

The second is a biomimetic microfluidic device designed to replicate the pathogen's habitat, proposing a new tool to facilitate the study and evaluation of the therapeutic efficacy of novel drugs. The design aims to faithfully reproduce the physiology and geometry of olive tree xylem vessels including 16-45 µm wide and 40-60 µm high channels. The fabrication of the device involved the use of photolithography to produce an SU-8 master, created with a mask designed using CleWin software and produced by a specialized company. Utilizing the Mask Aligner MA6 Suss Microtech, the design was transferred from the mask to the photoresist through exposure to UV light. Subsequently, soft lithography was employed to create a PDMS replica of the chambers and channels, affixed to a glass slide through plasma treatment in order to conduct microfluidics tests.

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### Aptamer-Enhanced Electrochemical Biosensor for the Detection of *Listeria monocytogenes*

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Keywords: Aptamers, Electrochemistry, Sensors-BioSensors, Polymers

A wide variety of biosensors has been developed and investigated in recent years, and electrochemical biosensors are obtaining an increasing attention from the scientific community. Among the different electrochemical biosensors, spacing from amperometric to potentiometric and impedentiometric sensors, an upcoming class has risen up: Aptasensors. These biosensing platform exploit aptamers, which are complex biological species obtained through gene sequencing [1]. These custom-made oligonucleotides grant very useful capabilities, such as high stability, reproducibility, selectivity and ease-to-use. Being custommade species, these can be fully tailored to detect specific species such any bacteria/pathogens of interest. In the case of the present work, the aptamer was custommade to detect Listeria monocytogenes, a food-related bacterium responsible of Listeriosis, an invasive infection that can be lethal for weak people and pregnant women. The biosensor platform consisted of the aptamer and a polymer (Polydopamine and its derivatives) to which the aptamer is anchored. Different concentrations of polymer and aptamer were investigated. The entire biosensor was investigated step-by-step through Cyclic Voltammetry, Impedance Spectroscopy, Fourier-Transform Infra-Red spectroscopy and Raman Spectroscopy.

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## Revealing Melanoma Cell Proteome Transformations with Silver Nanoparticles: An Innovative Comparative Study

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Malignant melanoma represents the most lethal form of skin cancer, also due to its resistance to drug-induced apoptosis. Here, we present a comprehensive analysis of global proteomic changes induced in the human melanoma cell line (A375) upon exposure to AgNPs synthesized through two different routes, at two time points (24 and 48 hours). The traditional method of AgNPs colloidal synthesis is compared with a "green" method that uses polyphenols present in the *Laurus nobilis* extract as reducing and stabilizing agents. After a careful physico - chemical characterization of AgNPs with different techniques, the cellular response, observed through proteomic changes, offers valuable insights into the affected cellular functions and pathways. The results show that green AgNPs are less toxic to normal cells compared to colloidal AgNPs, showing a selective impact on oncoproteins and reducing the viability of cancer cells. After 24 hours of exposure to green AgNPs, a downregulation of proteins crucial for cell proliferation, survival and differentiation is observed, highlighting a cellular response to DNA damage, programmed cell death and autophagy. All the treatments with green AgNPs led to a decrease in a series of oncogenes, demonstrating a cancer cell growth inhibition that is even higher over time.

Furthermore, prolonged exposure (48 hours) leads to an increase in the metabolism of carboxylic acids, related to an improved carbohydrate metabolism via aerobic glycolysis rather than anaerobic glycolysis, thus indicating a prominent feature of cancer state. Lastly, the study highlights the reaction of melanoma cells to AgNPs in the context of dysregulated copper homeostasis, a key process in the angiogenesis of solid tumors. To the best of our knowledge, this is the first study to report the effects of differently synthesized AgNPs on melanoma cells using an integrated proteomic approach.

### Electrochemical and spectroscopical characterization of apta-sensor based on gold electrodes functionalized with poly-L-DOPA film

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Keywords: Aptamers; Biosensors; Electroanalysis; Electropolymers.

**Abstract**: The development of new biotechnological integrated and miniaturized devices is an interesting aspect of biosensors application in different biomedicine fields, for example for detection of microorganisms responsible for food poisoning, such as Salmonella, *Clostridium botulinum* and *Listeria monocytogenes*. A fast and easy detection of a particular target is the aim of these kind of new biosensors, which promise to be fast and reproducible portable devices. In this work, gold screen printed electrodes (SPEs) are modified by electrochemical deposition of a polymer, able to work as linker for a specific aptamer that will detect the target of interest. The polymer used to functionalize this biosensor is L-Dopa, a chiral amino acid known for its adhesive properties. Because of L-Dopa electropolymerization has not been well characterized yet <sup>[1]</sup>, the experiments conducted in this work are aimed at better characterization of L-Dopa polymeric film, trying to understand the functional groups that could bind the suitable aptamer for detection of Internalin-A, a Listeria monocytogenes surface protein. Gold SPEs were first electrochemically modified with poly-L-Dopa film, then InIA aptamer was drop-casted on working electrode. The modified SPEs characterization was performed by Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) in ferrocyanide; to appreciate polymerized L-Dopa characteristics, Infrared (IR) and Raman Spectroscopies were performed too.

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### Chirality induction in porphyrin films for enantioselective sensing applications

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An achiral and hydrophobic porphyrin (figure 1) was co-assembled at the air-water interface with mesoscopic silica nano-helices functionalized with -NH<sub>2</sub> groups. In particular, right-handed (RHH-NH<sub>2</sub>) and left-handed (LHH-NH<sub>2</sub>) were dispersed in the aqueous subphase of a Langmuir trough, and the co-assembled composite film was obtained without forming covalent bonds between the components.



Figure 1. Chemical structure of the achiral free base porphyrin (Pp)

This method facilitated the transfer of the porphyrin/nano-helices co-assemblies onto a solid support as a thin hybrid layer, using the Langmuir-Schaefer (LS) method. The interaction between the two species was characterized using spectroscopic techniques and atomic force microscopy, confirmed the formation and interaction of the composite films, revealing that the porphyrin film's morphology differs significantly from the hybrid layer. CD measurements performed directly on the solid films demonstrated that the chirality of the porphyrin aggregates could be tuned according to the chirality of the silica nano-helices, leading to the formation of chiral co-assemblies. When the co-assemblies were transferred onto surface plasmon resonance (SPR) slides and exposed to histidine enantiomer solutions, selective chiral discrimination was observed. This discrimination was determined by the alignment or mismatch between the chirality of the analyte and the helicity of the SPR signal named  $\Delta$ AOI, was calculated after ultrapure water flux and analyte solution flux on both films obtained using both helices.  $\Delta$ AOI was used to quantify the sensitivity of the co-assembled layers to the different histidine enantiomers.

### Enhanced Antibacterial Efficacy of PMMA Nanostructures Fabricated via Electron Beam Lithography: A Study on Nanograting and Nanopillar Arrays

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Nanostructured antibacterial surfaces are gaining the scientific community's attention due to their ability to mechanically disrupt bacteria. In this study, an effective fabrication method, based on Electron Beam Lithography, of PMMA nanostructures i.e. nanograting and nanopillar arrays with pitches of 160 to 200 nm is presented. Atomic Force Microscopy (AFM) was used to characterize the structures. Successively, *E. coli* was incubated on these surfaces and a significant reduction in bacterial adhesion, especially on nanopillars, was observed. Bacteria exhibited shape changes and damage, indicating cellular disruption. AFM analysis confirmed reduced major axis length, decreased surface area, and increased roughness, likely from membrane rupture. Nanomechanical studies revealed decreased Young's Modulus and increased Adhesion Forces, particularly in lower-pitch nanogratings and higher-pitch nanopillars. These findings highlight the potential of nanostructured surfaces as advanced antibacterial materials.

#### The Oxidative Potential of Particulate Matter: A case study in Lecce

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The study of oxidative potential of particulate matter (PM) is a key element in understanding its potential adverse effects on biological systems. Acellular assays help indicate the ability of particulate matter to produce reactive oxygen species, which are linked to cellular damage and oxidative stress, providing useful information on health risks. In 2022, 371 monthly samples of PM10 and PM2.5 were collected using smart samplers (low flow) at various sites in the city of Lecce and its province (24 sites in total). The collected samples were subjected to different analytical techniques to obtain information on the chemical composition of PM. In particular: TOT (Thermal-Optical Transmittance) analysis to determine the carbonaceous fraction (OC/EC) using the EUSAAR2 protocol; ED-XRF to determine the content of major elements (using a Spectro Xepos analyser); DTT assay to estimate the oxidative potential; and High-Performance Ion Chromatography (IC) for the determination of soluble ions. After acquisition the complete dataset of chemical characterisation, a Source Apportionment analysis was conducted using the EPA PMF5.0 receptor model. This made it possible to identify the emission sources and quantify their contribution to PM and OP. The results show that, in the area studied, the sources contributing largest to both PM and OP are combustion sources, in particular combustion from vehicular traffic and biomass burning.

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### Influence of natural extracts to Improve photobiomodulation effects on HaCaT keratinocytes

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Photobiomodulation is a non-invasive therapy that uses light, generally in the red (320-750 nm) or near infrared (750-1400 nm)1 spectrum, to stimulate biological processes aimed at increasing cellular energy production, reducing inflammation and promote tissue repair<sup>1,2</sup>. Although favorable results on the effectiveness of this therapy have been reported, its long-term effects remain under study, as does the use of natural extracts that can improve or complement the therapy. For example aloe vera<sup>3</sup>, resveratrol<sup>4</sup>, turmeric<sup>5</sup> and green tea<sup>6</sup> which provide antioxidant and anti-inflammatory protection<sup>8</sup>.

Taking advantage of a commercial 808 nm diode laser used for hair removal, two conditions were compared; the commercially used treatment (T1) and the maximum laser power (T2) to analyze the influence in cell viability and morphology in human epidermal keratinocytes (HaCaT). For a subsequent study four natural extracts (pomegranate, tomato, walnuts and squid ink) as complementary agents.

At first glance, the parameters of laser therapy have been optimized: frequency, time and power; as well as the optimal concentrations of each extract. According to the results obtained with the MTT technique, three of the four extracts improved cell viability after laser treatment, without observing any morphological changes at the cellular level.

Studies are underway that use different combinations to identify the maximum possible action, as well as proteomic analyzes that identify the active pathways that lead to this increased cell viability. With this, we seek to obtain a complex matrix as a candidate for enhancing the effects of photobiomodulation in the treatment of different dermatological conditions.

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### Gold Nanoparticles from *Laurus Nobilis* Leaves Extract as radiosensitizers in cancer radiotherapy

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Breast cancer remains the most prevalent cancer affecting women, with over a million new cases diagnosed annually. This underscores the significant burden it places on global health systems and the substantial costs involved. Standard treatments, such as chemotherapy and radiotherapy, are commonly administered following mastectomy, but their effectiveness can vary depending on the specific cancer subtype.

Combining these therapies can improve patient outcomes, yet radiotherapy faces challenges such as radiation resistance and a lack of specificity in targeting cancer cells.

Nanotechnologies, in particular metallic nanoparticles (NPs), offers promising solutions to improve radiotherapy outcomes. In particular, gold nanoparticles (AuNPs) are particularly suitable due to their high atomic number, which enhances the radiation effect through the photoelectric process, thereby increasing damage to cancer cells. Many researches shown that AuNPs can serve as effective radiosensitizers, boosting tumor destruction by elevating the local radiation dose.

Furthermore, the use of green chemistry methods, provide a safer and more sustainable approach to obtain high stable NPs using Laurus Nobilis leaves extract. Then, their potential as radiosensitizers in MCF-7 breast cancer cells were investigated exposing them to various doses of X-rays. Cell viability, cell morphology alterations and DNA damage were assessed. The results indicated that green AuNPs significantly enhanced the effectiveness of radiotherapy at lower doses, suggesting they could be a valuable addition to breast cancer treatment protocols.

### Plant Based Scaffolds and potential applications

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The plant-cell wall is organized as concentric layers that vary among them on the concentration of cellulose, hemicellulose, pectin, lignin, and soluble proteins. Interestingly, plant cell walls have a natural continuous porosity with specific patterns and pore size ranges that could host animal cells and favor the exchange of nutrients and the clearance of waste. In the present work, we explored the effect of given desiccation protocols on the microstructure of several decellularized plant-based matrices, their microstructure, composition and preliminary results regarding their potential for cell culture and tissue engineering applications.

Through the exploration of physical and chemical possible modifications to the decellularized matrices we were able to conclude that the heat treatment was the one with the least volumetric reduction. Via SEM imaging we were able to verify the distribution and size of the pores from the tested matrices with an average of 72-90  $\mu$ m but with sizes ranging from 50  $\mu$ m up to 120  $\mu$ m. Thanks to the FTIR analysis we explore the differences between the native, decellularized, and two of the treated matrices. Finally the viability assay showed cytotoxic activity, therefore at least from the biological point of view there is space for improvement.

### Characterization of bacterial cellulose-neem-hypericum oil wound care paste *in vitro* and in *Galleria mellonella in vivo* model

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Surgical wound infections represent a global health emergency because of the spread of antibiotic-resistant bacteria. In this context, nanotechnologies help the optimization of strategies for managing skin lesions with antibacterial and antibiofilm activity.

In this work, we developed a biobased formulation made with bacterial cellulose nanofibers obtained through a green fermentation process, combined with a mixture of neem and hypericum oils, commonly used in the cosmetic field (1).

The resulting bacterial cellulose-based paste has been physicochemical characterized. *In vitro* antibacterial assays showed that the bacterial cellulose-based paste has biofilm detachment capabilities against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The efficacy of the treatment was also evaluated *in vivo* using *Galleria mellonella* larvae as a burn wound infection model, observing an increase in the survival percentage of the injured and infected larvae treated with our formulation, compared to untreated individuals. The innovative aspect of this formulation is the detachment action of bacterial biofilms, which contributes to a faster tissue regeneration process without inducing antibiotic resistance. Furthermore, in this study, the involvement of *G. mellonella* larvae as a model of burn wound infection allowed a preliminary evaluation of the safety and efficacy of the formulation, contributing to the development of a more complex system than traditional *in vitro* ones, but limiting the involvement of mammalian models, in line with the 3Rs principle.

These results, supported by further investigation, could lead to the development of a biobased formulation to be applied on skin lesions for antibacterial treatments.

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### POSTER

### Greed and altruism: how quorum sensing mediates between these two behaviors

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Greed and altruism are two sides of the same coin, and represent opposite antagonistic mechanisms that may lead communities of social beings to extinction. Bacterial strains consisting of unconditional cooperators (UC) or defectors (DC) in relation to the production of public goods, have been analyzed under different experimental conditions<sup>1</sup> and both die out quickly. UC are characterized by a low growth efficiency and become extinct due to aging even in presence of leftover resources. DC are greedy, grow fast, and rapidly run out of food. On the other hand, well-mixed UC and DC populations survive longer. Quorum sensing (QS)<sup>2</sup> is the ability of bacteria to cooperate when a density threshold is reached, and was proposed as the *deus-ex-machina* that presides over this phenomenon <sup>1</sup>. In previous works <sup>3,4</sup> we proposed a model describing the bacterial colony formation and its related bioluminescence. In this description, both phenomena are mediated by a long-range interaction which implements QS (QSa).

Here, modeling is improved to account for the presence of two different types of cooperators. By appropriately modifying the parameters of the model, it is possible to reproduce UC or DC behavior.

We analyze by numerical simulations the evolution of a mixed state of both these strains and examine the role of QSa in its stability over time. We show that it effectively acts as a regulator by mediating resource distribution based on UC, DC densities with the effect of increasing the typical survival time compared to that of pure states.

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## Biotechnologies in a genetic model organism to unveil biological processes underlying premature aging

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Aging is a gradual decline in cellular function and overall performance of an organism over time. As aging progresses, cellular damage accumulates, leading to diminished physical and mental capacities, as well as an increased susceptibility to diseases. Studies have shown that environmental pollutants can affect cellular markers of aging and contribute to the high incidence of age-related diseases. We utilized Drosophila melanogaster as a model organism to examine the effects of the environmental contaminant cadmium, which was introduced through the diet, on processes related to aging. The treated flies demonstrated a significant decline in learning and memory abilities over time, suggesting accelerated aging. Furthermore, specific molecular markers associated with aging, including transposable elements and genes related to memory and stress, were found to be deregulated in the brains of the flies. This indicates a premature loss of transcriptional and/or post-transcriptional regulation. These findings, along with the analysis of neuronal cell organization, provide a foundation for understanding how environmental stressors contribute to age-related degeneration.

### Evaluation of aquatic moss used as innovative biofilter for aquaponics

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The term aquaponics has been suggested for a system in which a RAS is combined with a hydroponic plant growth facility. Recirculating aquaculture systems (RAS) are land-based, closed-containment systems with water circulation. In RAS water is continuously cleaned and reused several times before being discharged.

In recent years this system has become more popular because of increasing scarcity of water resources as well as concerns over environmental pollution management. However, application of RAS is faced by several limitations, including high generation of nitrogen compounds that are toxic to fish. These compounds can be removed by processes that may be mechanical, physicochemical or biological. Among these, biological processes are more reliable, sustainable, economical and efficient. In this study we propose an innovative biofilter based on aquatic moss within an experimental aquaponics system. This material has been chosen thanks to his ability to absorb several pollutants, including nitrogen compounds, through his entire thallus. It also represents an effective mechanical filter for particles resuspended in water and can act as an optimal three-dimensional support for nitrifying bacteria, having a larger surface area than the solid supports normally used in biofilters. We showed its efficacy and evidenced that when the aquaponic system reaches saturation, the moss-based biofilter remains active becoming equivalent to a mature biofilter. Moreover, it resumes its specific activity with the decrease in fish concentration, since it metabolizes nitrogen compounds by regenerating itself. In conclusion, a moss-based biofilter shows a general superiority compared to a classic biofilter with inert media at relatively similar volumes.

### *Chaetomorpha linum* in the bioremediation of aquaculture waste: nutrient removal efficiency at the laboratory scale

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Aquaculture consists of the farming of aquatic organisms and today constitutes a very important economic sector of food production; therefore, it requires particular attention to the treatment of wastewater to avoid environmental damage and ensure sustainable management of natural resources. In aquaculture systems, nitrogen compounds are constantly produced, converted and consumed. The production of nitrogenous compounds by farmed fish creates an imbalance in the environment which can compromise water quality and the sustainability of economic activity. In this scenario macroalgae on account of their ability to remove the excess of nutrients from wastewater, can be used as a biofilter thus representing a valuable tool for achieving more sustainable aquaculture.

In this study, the concentration of nitrogenous compounds, i.e. ammonium ions, nitrites and nitrates in the waste collected in a maricolture plant (Maricoltura Mar Grande) located in the Mar Grande of Taranto (Ionian Sea) was determined. In addition, the variation of these nitrogenous compounds in the wastewater was investigated after the introduction of green seaweed *Chaetomorpha linum* in laboratory conditions. From the obtained results we highlighted the ability of this algal species to reduce the concentration of nitrogenous compounds. In particular, ammonium ions and nitrites were reduced at about a half by the action of *C. linum*. By contrast an increase of nitrates was observed as a consequence of the alga uptake of the other nitrogen forms. These results are noteworthy since high concentrations of nitrites and ammonium ions represent a hazard for fish and the marine life and thus suggest the employment of this algal species as a bioremediatior for aquaculture practices.

### Fabrication and characterization of PVA-electrospun tips for bacteriological swabs

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In this study, an innovative swab tip was produced using poly(vinyl-alcohol) (PVA) as bulk material and electrospinning for the processing method. PVA was selected as a possible material for sample collection as bacteriological swab tip because of its revealed efficiency in recovery and detection of exhaled bacteria. PVA mats were successfully obtained through electrospinning, a processing technique which can be easily scalable for intensive manufacturing. Since PVA has a hydrophilic nature and is soluble in water, a physical reaction by thermal crosslinking was chosen to increase its crystallinity, resulting in an improved stability of the material. PVA mats were then stable in aqueous solutions until 28 days, with suitable mechanical properties.

To obtain a prototype swab, in these preliminary *in vitro* tests, PVA mats were simply wrapped around PS sticks, to simulate a swab assembly, thus evaluating material performance in this panorama. In particular, PVA electrospun tip uptake and release capacities were investigated for different kinds of samples. Firstly, it was assessed its absorption capacities using water and 0.01 M PBS solution, then it was simulated a more realistic context with the detection of proteins, using BSA as a model protein, and the detection of bacteria, using *P. aeruginosa* PA01 and *S. aureus* SA-1 as Gram-negative and Gram-positive bacteria. In each test, the prototype showed great potential, with performances comparable to foam swabs currently commercially available and used worldwide.

These results represent a promising starting point for future investigations.

### Aquaculture waste bioremediation by the seaweed *Chaetomorpha linum*: Ca, CI, Na,K and pH measurements at the laboratory scale

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Aquaculture is growing worldwide and it is expected will more than double by 2050. However, aquaculture wastewater constitutes a serious environmental hazard in marine environment since it can affect changes in the physical-chemical characteristics of seawater, such as the reduction of dissolved oxygen and excess concentrations of nutrients, in turn causing alterations in both animal and plant communities. In this framework, in the present study the seaweed Chaetmomorpha linum was employed as biormendiator in order to reduce the phosphorous and nitrogen load due to aquaculture activity on account of our previous studies. The choice of algae to be utilized in aquaculture wastewater bioremediation requires careful discrimination since efficiency in nutrient removal may significantly change depending on the algal species In particular, here the concentration of Ca, Cl, Na,K and pH values were monitored in the waste collected in a maricolture plant (Maricoltura Mar Grande) located in the Mar Grande of Taranto (Inonian Sea) where of green seaweed Chaetomorpha linum was added in the laboratoryunderc ontrolled conditions (ore luc eore buio, Temparatura ). The vaule of pH in the waste varied from 7.9 to 8.9 by the action of C. linum leading to suggest that the algal species by the photosynthetic activity sequesters CO2 leading to an increase of pH. Na, CI, and k concentration were reduced at about two/three folds within the first 24 h by the action of C. linum on account of their employment in algal photosynthesis and osmosis. From the obtained results the ability of this seaweed to increase the concentration of calcium was also highlighted with a peak after 7 days. These results are noteworthy suggesting that the addition of C. linum in an aquaculture scenario could play several ecosystem services such as 1) the increase of seawater calcium concentration useful for other marine organisms for the construction of their shells and calcareous structures, 2) reduction of CO2 and consequently potential remediation of the recent marine environment acidification.

### Synthesis and cytotoxicity evaluation of six new cis-[Pt(NH3)2(Guo/dGuo)X] (X = CI, Br, I) platinum(II) complexes in three human cancer cell lines with varying cisplatin sensitivity

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Cancer still represents a serious threat to human health, due to the high pathogenicity, invasiveness and resistance to therapies. Cisplatin is the most widely used among metalbased chemotherapeutics and is used to treat a wide range of tumors. However, some tumor cells can develop resistance mechanisms over time, modifying the mechanisms that regulate apoptosis, thus making platinum-based ineffective. For this reason, the investigation of novel metal-based complexes has been an important focus in recent years, in order to synthesize new classes of anticancer agents with the final aim of exerting greater cytotoxicity and selectively against tumor cells. Among these, platinum(II) nucleoside compounds represent potential antimetabolites for antitumor therapy<sup>[1]</sup>. Complexes of the cis-[Pt(NH<sub>3</sub>)<sub>2</sub>(Am)Cl]<sup>+</sup> type (Am = heterocyclic amine based on pyridine, pyrimidine, purine, piperidine or saturated amine) have demonstrated high stability and solubility in aqueous media, and an interesting *in vitro* and *in vivo* antitumor potential against several tumors, including sarcoma and leukemia<sup>[2]</sup>. In this work we evaluate the cytotoxic activity of six complexes of the type cis-[Pt(NH<sub>3</sub>)<sub>2</sub>(Am)X]<sup>+</sup>, where Am = guanosine (Guo, **a**) or 2'-deoxyguanosine (d-Guo, b); X = CI (1), Br (2), I (3), on three cell lines with different sensitivity to cisplatin: HeLa (cervical adenocarcinoma), ZL-34 (pleural mesothelioma) and MCF-7 (breast adenocarcinoma). The cell viability was evaluated by SRB assay after treatment with Pt(II) complexes at increasing concentrations from 24 to 72 h. Cisplatin demonstrates to be more effective on all the tested cell lines, while comparing the complexes among themselves cis-[Pt(NH<sub>3</sub>)<sub>2</sub>(Guo)Br]<sup>+</sup> (2a) and cis-[Pt(NH<sub>3</sub>)<sub>2</sub>(dGuo)Br]<sup>+</sup> (2b) have higher cytotoxic activity against the tested cell lines with respect to the chlorido (1a, 1b) and iodido (3a, 3b) analogs, particularly on cervical adenocarcinoma (HeLa) cells. This work demonstrates that [Pt(NH<sub>3</sub>)<sub>2</sub>(Am)X]<sup>+</sup> complexes induce cancer cell death, and their activity can be modulated by the halogen substitution.



X = CI (1), Br (2), I (3)

**Figure 1:** Chemical structures of platinum(II) nucleoside monoadducts of the type (**a**) cis-[Pt(NH<sub>3</sub>)<sub>2</sub>(Guo)Cl + (**1a**, **2a**, **3a**) and (**b**) cis-[Pt(NH<sub>3</sub>)<sub>2</sub>(dGuo)Cl + (**1b**, **2b**, **3b**) complexes; X = Cl (**1**), Br (**2**) or l (**3**).

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## AFM-based chitosan nanopatterning using Pulse-Atomic Force Lithography technique

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Chitosan physical properties combined to its biocompatibility, make it a unique and environmentally-friendly resource for developing advanced materials for future technologies. In our research, we explored the potential of using chitosan films as substrates for Atomic Force Microscopy (AFM)-based lithography. We began by optimizing the fabrication protocol for spin-coating high molecular weight chitosan on silicon wafers functionalized with a silane. At first, we optimized the spin-coating high molecular weight chitosan on silicon wafers functionalized with a silane. Subsequently, we demonstrated the efficacy of the Pulse-Atomic Force Lithography (P-AFL) technique by successfully nanopatterning the chitosan substrates using NTEGRA AFM instrument equipped with an NSG30 tip.

P-AFL is a robust AFM-based mechanical lithography method that is easy to implement and offers nanometric precision in fabricating nanostructures. Specifically, we patterned sets of constant depth (Constant Pulse-AFL) and varying depth (Gradient Pulse-AFL) nanogrooves on a 100 nm chitosan film with high precision and reproducibility. In this framework, we successfully patterned nanochannels of 1  $\mu$ m in length, with depths ranging from about 2 nm to 20 nm and corresponding widths from about 47 nm to 109 nm. Moreover, we demonstrated the capability to modulate the applied normal force during the lithography process to obtain varying depth nanogrooves characterized by a smooth depth profile.

P-AFL on chitosan presents a compelling alternative to conventional lithography techniques, offering unprecedented details with a sustainable manufacturing process. These findings hold significant implications for the semiconductor industry, which is increasingly focused on environmentally-friendly manufacturing and sustainable processing methods.

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#### Molecular technologies applied to aquatic biodiversity assessment

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Molecular technologies, particularly high-throughput sequencing, have transformed the study of biodiversity over recent years using DNA traces or environmental DNA (eDNA). eDNA refers to genetic material obtained directly from environmental samples (such as soil, water, or air) without the need to physically capture or observe the organisms.

In this context, we analyzed environmental DNA (eDNA) to investigate the genetic and biological diversity of eukaryotic communities in Mediterranean lagoons. Using cytochrome oxidase I (COI) and ribosomal RNA 18S as marker genes, we explored the eukaryotic biodiversity present in these aquatic coastal environments. This high-throughput molecular surveying provides significant insights into the genetic diversity of eukaryotic species.

Our results demonstrate the efficacy of eDNA studies in elucidating the genetic and ecological structure of these communities, as well as identifying spatial variations in response to environmental variables within transitional aquatic ecosystems. This study highlights the importance of surveying genetic diversity as a foundational component for conservation strategies aimed at preserving natural ecosystems.

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