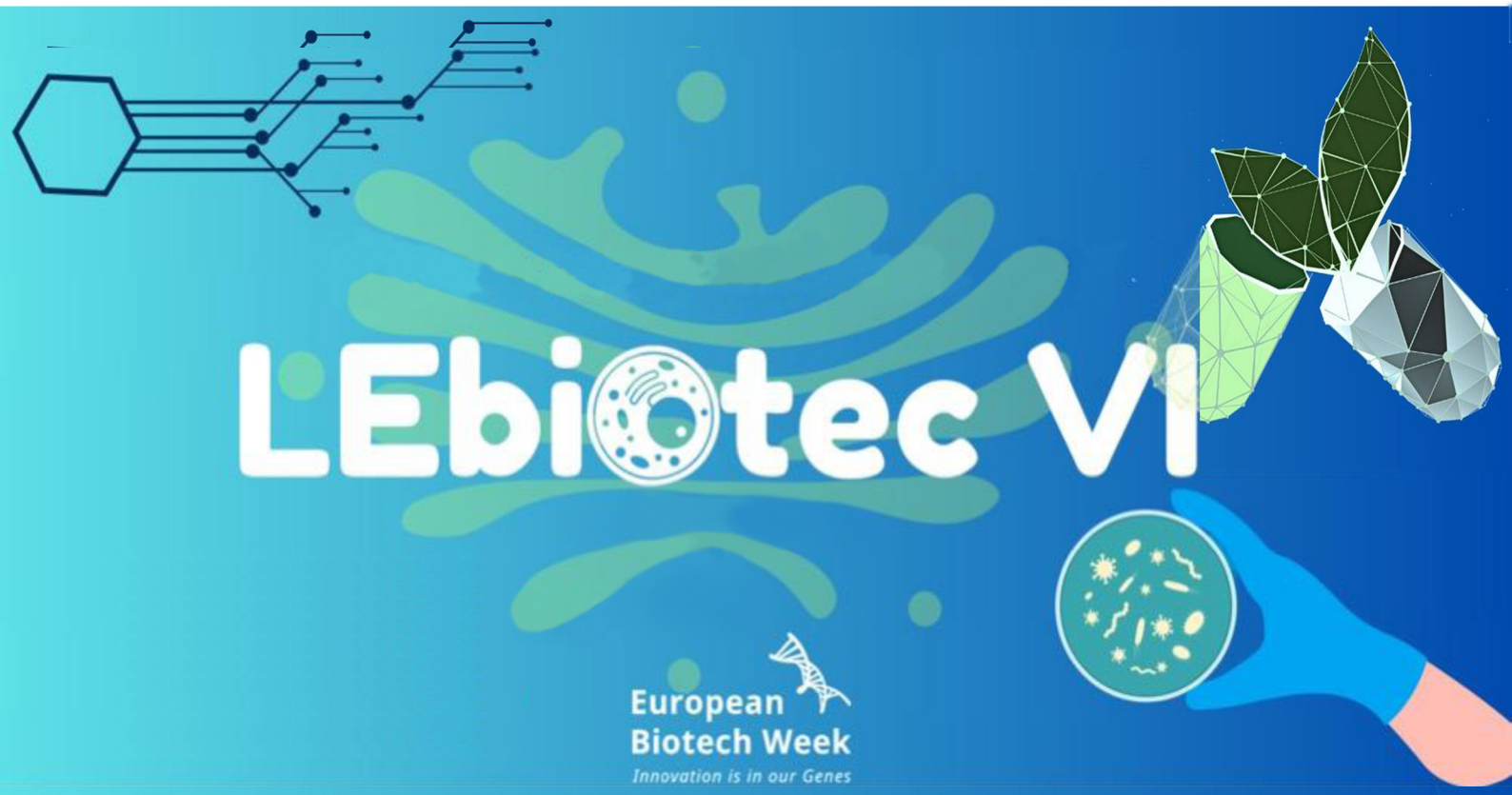

ABSTRACT BOOK

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Index

TALK

Biomimetic Nanoparticles for Tumor Targeting in Cancer Therapy

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Over the past few years, researchers have been exploring the use of nanoparticle-based drug delivery systems to overcome the limitations of traditional chemotherapy in cancer treatment. Nanoparticles (NPs) provide a platform for improved drug delivery to cancer by enhancing drug solubility, stability, and bioavailability.

In this frame we synthetized biomimetic nanoparticles composed by a polymeric core covered by cancer cell membrane, thus conferring to the assembled NPs the ability to entirely replicate the surface antigenic diversity of source cells selectively targeting cancer cells.

Resulting biomimetic NPs are physically characterized in morphology, size and correct coating with cancer cell membranes. The purity and retention of cancer cell membrane proteins on biomimetic NPs is assessed through biochemical analysis.

Also, cellular uptake of biomimetic NPs, and homotypic binding with different cell lines are assessed by flow cytometric analyses and Confocal Laser Scanning Microscopy imaging. These investigations confirm the higher internalization rates of biomimetic-NPs in their source cells, when compared to other cell lines, thus confirming the self-recognition capability typical of cancer. Resulting biomimetic NPs can be used to homotypically target cancer cells for biologically active molecules delivery thanks to a cancer-targeting strategy based on the intrinsic homotypic properties of cancer cell membranes.

Miniaturized microfluidic platforms (Lab-on-chip/Organ-on-chip) for medical diagnostics, monitoring and drug screening

Giusi Caragnano[1], Anas Munir[2], Alessandra Inguscio Noemi Petese[1], Silvia Rizzato[1], Annagrazia Monteduro[1], Michele Maffia [2], Giuseppe Maruccio[1]

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Microphysiological systems, organ-on-chip and multiorgans microdevices attracted considerable attention as novel tools for high-throughput and high-content research to achieve an improved understanding of diseases and to accelerate the drug development process towards more precise and personalized standards. An organ-on-chip is a miniaturized device that can mimic the functional unit of an organ and simulate human pathophysiology by also incorporating mechanical and chemical stimuli[1, 2]. Here we report the development of an Intestine-on-chip model, made in polydimethylsiloxane (PDMS), to recreate the intestinal epithelium-endothelium interface so as to have a tool for screening drugs used in the treatment of chronic gastrointestinal diseases and colorectal cancer [3]. Moreover, to take full advantage of capabilities of these microfluidic devices, they should be combined with efficient analytical methods. A recent trend is to make a device that integrates several functions that usually take place in the laboratory. These devices are named Lab-on-chip and offer great opportunities to enable continuous, automated data collection and in situ monitoring of functional indicators and biological responses, attracting great interest for medical diagnostics and drug screening. Such biochips were demonstrated to be suitable for ultrasensitive detection of biomarkers in flow immunoassays providing tools useful to achieve a diagnosis of tumours (or other diseases) and monitor their evolution by liquid biopsy approaches [4-7]. Electrochemical impedance spectroscopy, coupled with the chip, enables proliferation, viability, and migration assays to be performed on a cell population, thus making the platform suitable for conducting pharmacological studies and learning more about the disease.[8] [9-12]

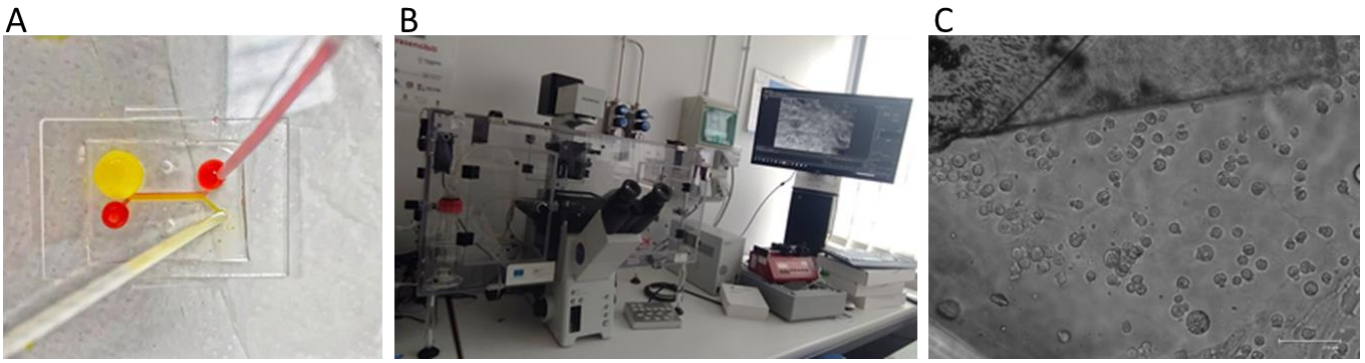

Fig.1. A) Foto del dispositivo sottoposto a test di flusso con Elve flow OB1MK3 con coloranti alimentari (rosso e giallo). B) Foto del sistema incubatore (Okolab)+ microscopio dove è stato tenuto in coltura per 3 giorni il dispositivo con all'interno cellule umane di adenocarcinoma al colon-retto (Caco-2). C) Immagine presa dal microscopio Evos Floid Invitrogen delle cellule Caco-2 appena seminate nel chip (scale bar:125 µm)

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Inquire the tumor microenvironment through *in vitro* 3d models with integrated sensing tools

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The tumour microenvironment (TME) defines the complex and dynamic components, cellular and non- cellular, that interact with cancer cells within a tumour. In their native environment, cancer cells are surrounded by stromal cells, the non-cancerous cell components of the TME, which includes cancer- associated fibroblast (CAFs), myofibroblast and various immune cells. This plethora of cells is embedded and structurally supported by the extracellular matrix (ECM), a network of proteins and carbohydrates, such as collagen, glycoproteins, and hyaluronic acid. The alteration or remodelling of the ECM is crucial for tumour cell behaviour, invasion and metastasis developing. Thus, the whole TME plays an important role in cancer progression and response to therapy.

Therefore, there is the crescent urgency to switch from traditional bi-dimensional cell cultures to three- dimensional *in vitro* models that better mimic the complexity of the *in vivo* environment, for understanding the intricacies of the TME and developing effective cancer therapies.

The development of innovative *in vitro* platforms for the investigation of co-cultures of cancer and stromal cells could be performed combining 3D biocompatible structures, such as electrospun fiber matrices, porous scaffolds, and hydrogels, with optical fluorescent ratiometric sensing tools for measuring, with high spatial and temporal resolution, the concentration of key biological analytes, such as oxygen and pH, within the TME.

The optimization of these 3D sensing platforms is paving the way for personalized medicine approaches, in which patient-derived cells are employed, allowing for individualized drug testing and cancer treatment optimization.

Diffuse large B cell lymphoma (DLBCL) in vitro model based on a newly synthetized chitosan/gelatin hydrogel

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Diffuse large B cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma, accounting for 30% of diagnoses worldwide. The advances in molecular biology techniques have been fundamental to achieve maximum understanding of DLBCL pathophysiology, but conventional 2D cell culture and mice models are not representative of the in vivo physiology. 2D cultures do not mimic growth profiles and cellular organization observed in vivo and lack the heterogeneity of tumor microenvironment (TME). Consequently, extracellular matrix (ECM) analogues can be designed to provide cells with a 3D structure and obtain a more faithful representation of the TME. In this work a chitosan/gelatin-based hydrogel has been developed to provide lymphoma cells with a 3D structure and obtain a more faithful representation of the TME. U2932 lymphoma cells have been encapsulated in the hydrogel, alone or in combination with WPMY-1 stromal cells, showing high viability by live/dead assay. Furthermore, co-culture studies showed the formation of cell spheroids after three days of culture, with respect to U2932 cells encapsulated alone. Based on these results, this hydrogel represents an excellent candidate for establishing a 3D in vitro model of DLBCL.

Reduction of biofouling on aquatic moss-based biofilters using Mediterranean crustaceans

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Aquatic mosses can be used as biofilters since they reduce heavy metals, nitrogenous compounds and nanoparticles in solution through absorption and uptake. They're also an effective mechanical filter for particles resuspended in water. However, over time, biofiltering efficiency can be compromised by the formation of a biofilm consisting of filamentous algae and cyanobacteria (biofouling). This phenomenon can obstruct the spaces between mosses' talli and reduce the water flow through the filter. Therefore, it would be appropriate to associate this with organisms capable of reducing biofouling by feeding on the contaminating microflora. In this study (Project fish RISE; PON 2014/20 ARS01_01053) was tested the behaviour of two Mediterranean aquatic crustaceans, i.e. the amphipod *Gammarus aequicauda* and the isopod *Lekanesphaera monodi*, by allowing them to feed, under laboratory conditions, on contaminated *Leptodictyum riparium*, a cosmopolitan species of moss common in Italy. Subsequently, the moss fragments used in the trial were observed under a confocal fluorescence microscope and, from the analysis of the acquired images, it was possible to deduce that the two species of crustaceans have a different feeding behaviour. In particular, it turned out that *Gammarus aequicauda* feeds on both the microflora and moss biomass, while *L. monodi* feeds mostly on the fouling microorganisms without damaging the moss, therefore this latter may represent a suitable organism for preserving moss efficiency as a biofilter.

Optical detection of human hemoglobin by a molecularly imprinted polymer prepared by a novel vapor-phase synthesis

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Molecularly imprinted polymers (MIPs) are synthetic materials with marked recognition capabilities like those of the natural receptors. However, compared to biological recognition elements such as enzymes, proteins and antibodies, MIPs are stable at harsh conditions, easy to synthesize, and inexpensive. Today these materials are used for a wide variety of purposes, from environmental to clinical/medical applications. Now, technological advancement leads to the need to couple these artificial receptors with miniaturized and complex systems. Application of MIPs to nanostructured materials is challenging due to diffusion-limited transport of monomers within the nanomaterial recesses, especially when the aspect ratio is >10. The room temperature vapor-phase synthesis of MIPs in nanostructured materials was explored. The vapor phase synthesis leverages a >1000-fold increase in the diffusion coefficient of monomers in vapor phase, compared to liquid phase, to relax diffusion-limited transport and enable the controlled synthesis of MIPs also in nanostructures with high aspect ratio. As proof-of-concept application, pyrrole is used as the functional monomer thanks to its large exploitation in MIP preparation; nanostructured porous silicon oxide ($nPSiO_2$) is chosen to assess the vapor-phase deposition of PPy-based MIP in nanostructures with aspect ratio >100; human hemoglobin (HHb) is selected as the target molecule for the preparation of a MIP-based $nPSiO_2$ optical sensor. High sensitivity and selectivity, low detection limit, high stability and reusability are achieved in label-free optical detection of HHb, also in human plasma and artificial serum. The proposed vapor-phase synthesis of MIPs is immediately transferable to other nanomaterials, transducers, and proteins.

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Innovative biotechnological filter based on aquatic moss to be applied in aquaculture

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Fish farming pollutes waters with high concentrations of nitrogenous compounds and therefore the use of plants for phytofiltration is one of the promising trends in environmental biotechnology to purify wastewater. Aquatic moss biomasses, due to the ability of the gametophyte to absorb pollutants through the whole of its surface, can act as live filtering material, furthermore they represent a possible 3D support for the classic nitrifying bacteria. In this study, in the frame of the project fish RISE (PON 2014/20 ARS01_01053), it was tested the variation of the amount of nitrogenous compounds such as nitrites, ammonium ions and urea by two species of moss, *Taxiphyllum barbieri* and *Leptodictyum riparium*, both in sterile and non-sterile conditions. These are able to metabolise nitrogenous compounds. Indeed, from the results obtained it is possible to confirm this ability, because the concentration of nitrogenous compounds decreases in time. The use of Nitrogen compounds related to different metabolic steps allow to discuss in more detail the action of the two compared species. The nature of pollutants was selected having in mind the needs of aquaponic biofiltration, but the applicability is wide. However, it is evident that aquatic mosses, thanks to numerous advantages, can be the basic component of biofilters for aquaponics, representing a new opportunity for the eco-sustainable recovery of water.

Saturated and monounsaturated fatty acids differently regulate lipid droplet metabolism, energetics, and autophagy in hepatic cells

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High-fat diets can induce the accumulation of hepatic lipid droplets (LDs), consisting mainly of triacylglycerols (TAG). Numerous studies have shown that LDs generated from mono- or polyunsaturated fatty acids have a protective role in the liver whereas saturated fatty acids induce hepatotoxicity. The different fatty acids showed different abilities to be incorporated into TAG. Therefore, we hypothesized a role for diacylglycerol acyltransferase (DAGT) 1 and DGAT2, enzymes of TAG synthesis, in fatty acid-induced hepatotoxicity. In the present study, we aimed to follow the metabolism of LDs induced by palmitic acid (PA), a saturated fatty acid, and oleic acid (OA), a monounsaturated fatty acid, and the action of these fatty acids on the activity and the expression of DGAT1 /2. Furthermore, we followed the effects of these PA and OA on liver cell energy and autophagy. For this, HuH7 liver cells were treated for 48 h with PA and OA, and confocal microscopy, western blot, and enzyme activity assay were performed. We found that PA, unlike OA, induces hepatotoxicity and cell death. Furthermore, PA induced less TAG accumulation than OA and was more oxidized in mitochondria. PA treatment induced increased endoplasmic reticulum stress, block of autophagy, and reduction in DGAT1 expression, while OA did not affect both DGAT1 and DAGT2 expression. Inhibition of DGAT1 in OA-treated cells induced the formation of LDs like those of PA-treated cells and hepatotoxicity. These results indicate that DGAT1 may play a key role in the different lipotoxicity induced by saturated and monounsaturated fatty acids in the liver.

Shrimp processing waste as a source of antioxidant molecules for the pharma industry: a focus on green extraction strategies

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The recovery of waste biomass is a key point in the transition to a circular economy, favoring the reduction of environmental and economic impacts associated with its disposal and, at the same time, the procurement of biomolecules and biomaterials of industrial interest. Astaxanthin and chitin represent examples of valuable products occurring in shrimp shells wastes. To make the extraction process of these bioactive compounds more sustainable, the replacement of traditional solvents by hydrophobic natural deep eutectic solvents (NADES) was investigated. Specifically a NADES obtained by mixing menthol (ME) and acetic acid (AA) in a molar ratio of 1:1 was employed. The AXT extraction method proved to be fast and green, being complete in a short biomass-NADES contact time and requiring very low energy input. Ethanol, an bio-based solvent, was also found to be a good option for the extraction of AXT from shrimp shells. The AXT yields obtained by *Aristaeomorpha foliacea* wastes ranged around 350-370 µg/g with fresh powder. The Trolox equivalent antioxidant capacity (TEAC) of ME:AA and ethanol extracts was investigated, resulting higher than the one expected on the basis of AXT concentration alone, likely due to the co-extraction of additional antioxidants compounds. These results highlighted the potential of ME:AA and ethanol as ecological and efficient solvents for the recovery of bioactive molecules and confirm the strong antioxidant activity of shrimp shell extracts, which might find application in the pharmaceutical and nutraceutical field.

Establishment of an in vitro gastrointestinal barrier model to study nano and microplastics pollution

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Microplastics and nanoplastics are fragments of less than 5 mm in size emerged as ubiquitous environmental contaminants. These minute plastic particles result from the breakdown of larger plastic items and are used in various consumer products. As they find their way into ecosystems, including marine environments and terrestrial systems, they have raised concerns about their potential impact on both environmental and human health.

One significant route of human exposure to microplastics and nanoplastics is through the consumption of contaminated food and water. The gastrointestinal system plays a crucial role in mediating the interaction between these plastic particles and the human body. Understanding the potential risks associated with microplastic and nanoplastic ingestion necessitates a comprehensive assessment of their effects on the intestinal barrier, which acts as the body's first line of defence against foreign substances.

The aim of this work is to set up an *in vitro* gastrointestinal barrier model based on the co-culture of three cell lines, Caco-2, HT29 and Raji B, as a valuable tool for evaluating the interactions between microplastics/nanoplastics and the gastrointestinal system. The developed 3D model was then used for a preliminary study analysing the internalization of polystyrene micro/nanospheres with different sizes (200 nm and 40 nm). In both cases, internalization was observed mainly within the cells; minimally they remained trapped on the cell surface, probably due to the presence of mucus. Further studies will be necessary to better understand the internalization mechanism and the cytotoxic or genotoxic effects that the spheres can induce on the intestinal barrier.

Influence of the targeted brain structures on spatial behavior of fiber photometry signals

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Neuroscience studies aim at a more comprehensive understanding of the brain. The gold standard technique for investigating neural activity is electrophysiology, but it lacks cellular specificity. This can be overcome by taking advantage of genetically encoded light-sensitive proteins to target specific cell subpopulations and by the development of implantable interfaces to optically interrogate neural circuitry. This was followed by research activity to deal with brain opacity due to the scattering of photons in brain tissue, thus limiting the optical access at depth. Neuroscientists therefore focused on the development of implantable devices to deliver and collect light from deep into the tissue, achieving both cell specificity and spatial resolution. Among the available optical neural interfaces, flat-cleaved optical fibers were exploited for optical monitoring of functional fluorescence, a technique called fiber photometry (FP). However, monitoring physiological phenomena through FP requires accurate quantification of differences in photon propagation in different brain regions. In this framework, we estimated how the collection properties of implantable optical devices are affected by different anatomical structures of the mouse brain, estimating the light collection volumes of flat optical fibers into fixed mouse brain slices. We analyzed this in three different brain areas, concluding that their anatomical and cellular peculiarities determine the amount and the depth from which photons can be retrieved. We also present the implementation of a custom optical setup for exploiting optoelectrical neural interfaces based on tapered optical fiber for simultaneous FP and electrophysiology, resulting in a novel multifunctional tool to interrogate brain circuits at depth.

NSC-34 cells as a model to study extracellular vesicles release-based cell-to-cell communication in amyotrophic lateral sclerosis

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The NSC-34 cell line is a widely used model for studying the pathogenetic mechanisms that characterize the forms of amyotrophic lateral sclerosis (ALS). The main feature of SLA is the functional damage of alpha motoneurons due to the accumulation of misfolded proteins, including SOD1, FUS, and TDP43. In the last years, *in vivo* experiments and clinical studies suggest the role of neuroinflammation in the pathophysiology of the disease, indicating the alteration of cell-to-cell communication an actual area of research in ALS. In particular, the involvement of peripheral immune cells seems to be very interesting. Among the various modalities of intercellular communication, extracellular vesicles (EVs) released by cells arouse increasing interest in the scientific community.

Here, we explore if NSC-34 cells are a suitable model for studying the EVs-mediated neuroinflammation occurrence in ALS. EVs obtained from the motoneuron-like cell line NSC-34 transfected with different SOD1 mutations (G93A, A4V, G85R, G37R) were characterized by transmission electron microscopy, cytofluorimetry, and western blot. The EVs were isolated in small and large vesicles fractions and were used to culture Raw 246.7 murine macrophages and their response in terms of polarization in the M1 or M2 phenotype was evaluated by analysis of mRNA levels of pro- and anti-inflammatory cytokines, suggesting that the EVs from mSOD1 NSC-34 induce in Raw 264.7 macrophages the switch to mixed M1 and M2 subpopulations.

Overall results suggest the suitability of mSOD1 NSC-34 as a model to study EVs-based inflammatory modulation relevant to modulating neuroinflammation in ALS patients.

Biotechnological approach to the evaluation of the short-term stress response in plant cells

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Abiotic stress factors, like drought, salinity, temperature and toxic elements, can limit plantsgrowth and productivity. Several publications have investigated physiological and cellular effects derived from substantial changes in gene expression or triggered by drastic physical changes, but mechanisms inducing immediate endomembrane remodeling remain mostly unknown.

In this work, looking for new targets for genetic improvement, we isolated and transformed tobacco protoplasts with a set of endomembrane fluorescent markers that preferably label structure related to the conventional or unconventional secretion pathway, and we evaluatedthe effects of some abiotic stresses (cold, drought, salinity, and cadmium) on the endomembrane system in a short period of time.

The endomembrane system was unaffected by stresses caused by cold, 4°C, or drought (generated with the application of mannitol). When salt stress was applied, we observed a greater involvement of the ER in the formation of structure related to the unconventional secretion pathway: incremental doses of sodium chloride showed an increase in structure not only related to the unconventional pathway, but also of hybrid structure in which the marker of the unconventional secretion pathway co-localized with the marker of the conventional secretion pathway. When cadmium stress was applied, we observed a generalslowdown in both conventional and non-conventional secretion pathway.

It is unclear if these changes are immediate or the result of genetic control, but knowing these mechanisms will have important implications for the improvement of crops resilience in the context of climate change.

Advances in biosensing: from electrochemical impedance spectroscopy (EIS) to Localized Surface Plasmon Resonance (LSPR), surface acoustic waves (SAW) and beyond

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In recent years, the development of innovative and versatile sensing technologies found application in different fields such as medicine, agriculture, food safety, environmental and industrial monitoring. A wide range of strategies were employed in literature. In this respect, we will report and compare different read-out approaches that we exploit in our laboratories in response to specific needs for several biological applications.

Electrochemical impedance spectroscopy (EIS) allows the development of multipurpose biochips suitable for (1) monitoring cell viability, cytotoxicity, migration and proliferation as well as drug-induced cell behaviour *[1, 3]*, (2) the ultrasensitive detection of biomarkers for cancer diagnosis in serum samples, toxins/allergen, pathogens and contaminants in food and the environment *[4-6]*. Recently, such impedance chips have been modified for detection of plant diseases such as Grapevine leafroll-associated virus 3 *[5]* and gas sensing *[7]*.

In the case of small molecules, more complex and sensitive read-out schemes can be preferable. For this purpose, we also investigate alternative strategies based on (1) surface acoustic waves (SAW *[6]*), (2) Localized Surface Plasmon Resonance (LSPR) *[8]*, (3) Split ring resonators *[9]* and (4) magnetoresistive sensors *[10]*.

Results from the Lab on Chip (LoC) platform are in good agreement with the output of standard assays. We demonstrated that the miniaturized platform presents several advantages in terms of reagents consumption, operator time and assay costs, portability, scalability and automated sample handling.

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Epicardial adipose tissue (EAT) and pericardial adipose tissue (PAT) as cell models to assess patient responsiveness to therapeutics

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Background. The abundance and inflammatory stigmata of epicardial(EAT) and pericardial adipose tissue(PAT) is associated with higher carotid intima-media thickness, suggesting a role of EAT and PAT in the development of atherosclerosis. Curbing EAT and PAT dysmetabolism and inflammation may represent a novel therapeutic target to prevent coronary atherosclerosis.

Purpose. Development of a feasible and efficient method for isolation of adipocytes from human PAT and evaluation of adipocyte response to therapeutics and micronutrients

Methods. PAT was collected from coronary patients undergoing surgery for coronary stenosis and from patients undergoing aortic or mitral valve surgery and immediately enzymatically processed. Isolated adipocytes were morphologically and molecularly characterized. Cell responsivity was evaluated by exposure to inflammatory stimuli or to docosahexaenoic acid(DHA), a well-known cardio-protective fatty acid.

Results We obtained pure cultures of adipocytes that can be sub-cultured for several days without losing viability and retaining the ability to respond to stimuli. Basal expression of inflammatory genes in adipose cells was higher in coronary patients than in aortic and mitral surgery patients. Exposure of adipocytes to TNFα significantly induced the expression of MCP-1 and IL-6, ($p < 0.05$), while downregulated the expression of UCP-2 and PPARγ ($p < 0.05$). On the other hand, the exposure of adipocytes to DHA resulted in a downregulation of MCP-1 and IL-6 expression ($p < 0.05$) and in the upregulation of UCP-1,-2 and PPARγ ($p < 0.05$).

Conclusion(s). Our data propose a new efficient method for isolating adipocytes from PAT and for using them as a bio-reactor to test differences in different cardiovascular conditions.

Le biotecnologie microbiche al servizio dell'economia circolare: strategie per la valorizzazione di sottoprodotti agroalimentari

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La perdita e lo spreco dei prodotti agro-alimentari insistono sull'economia globale per circa 900 miliardi di dollari all'anno. Ora più che mai questi due aspetti non sono solo una questione sociale e ambientale, ma rappresentano anche una significativa perdita economica sia per i paesi sviluppati che per quelli in via di sviluppo.

Negli ormai necessari approcci di economia circolare, le risorse organiche derivanti dai sottoprodotti dell'industria agro-alimentare possono essere recuperate come ulteriori fonti di composti bioattivi utili (nutraceutici e cosmeceutici, nuovi principi attivi naturali), come ingredienti per l'industria alimentare, materie prime per l'industria cosmetica e/o restituite in modo sicuro al suolo sotto forma di fertilizzante organico.

Il processo di fermentazione è un metodo efficace per stabilizzare e valorizzare sottoprodotti agroindustriali, mediante l'impiego di microrganismi selezionati e delle loro capacità e potenzialità metaboliche e di interazione con le matrici vegetali. Le biotecnologie microbiche sono state applicate con successo in un nuovo metodo di trattamento delle acque di vegetazione derivanti dall'estrazione dell'olio di oliva. I risultati ottenuti pongono le basi per un nuovo approccio sostenibile ed economico per favorire la trasformazione di rifiuti inquinanti e costosi da smaltire in una nuova fonte di acqua per la fertilizzazione e l'irrigazione per i Paesi del Mediterraneo che soffrono di scarsità idrica, soprattutto a causa dei gravi effetti del cambiamento climatico.

Il pretrattamento microbico è stato inoltre esplorato come una potenziale strategia per ottenere composti ad elevato valore aggiunto da sottoprodotti di diverse filiere agroalimentari come le bucce di cipolla, le foglie di scarto della cicoria e del radicchio, i residui di potatura dell'olivo e della vite. Impiegati come substrato di fermentazione, da questi sottoprodotti è possibile potenziare la resa di estrazione di composti fenolici e ed eventualmente aumentare l'attività antiossidante. Questi primi risultati rappresentano il primo passo nello sviluppo di un processo di bio-raffineria per produrre prodotti ad elevato valore aggiunto da scarti o residui di lavorazione.

Sintesi su misura e funzionalizzazione della biotina di nanosistemi di silice bicolore e loro potenziale per applicazioni biomediche.

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Le nanoparticelle di silice sono uno dei materiali più utilizzati per la sua biocompatibilità, facilità di sintesi e modifica di superficie. Dove molecole fluorescenti vengono aggiunte all'interno o sulla superficie della particella per il monitoraggio e il controllo all'interno degli organismi.

In questo lavoro, abbiamo proposto un metodo Stöber a due cicli per la sintesi su misura di nanoparticelle di ossido di silice fluorescente core-shell (SiO_2) utilizzando due fluorofori commerciali: verde nel nucleo e rosso nel guscio. Entrambi i fluorofori possono essere visualizzati da diverse lunghezze d'onda di eccitazione in diversi intervalli di emissione, per ottenere una migliore comprensione delle nanoparticelle in sistemi cellulari complessi.

Per ottenere dispersioni stabili e biocompatibili abbiamo ottimizzato l'uso della biotinaa nono solo come disperdente, stabilizzante, ma anche parte fondamentale per la terapia del cancro. Diverse dimensioni sono state analizzate e caratterizzate come segue: i) dimensione e morfologia, ii) composizione, iii) dispersione e stabilità, iv) proprietà di fluorescenza e v) interazione nei sistemi vivi. Con questo, sono state identificate molteplici potenziali applicazioni biomediche come: imagining, targeting, drug delivery e come strumento di calibrazione. Aprendo un mondo di possibilità per gli strumenti teranostici e diagnostici.

Harnessing the Potential of Genome Editing in Wheat

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Wheat production is facing a challenge from climate change. Rising temperature is one of the effects of climate change that represents a significant threat leading to a 3-8% decrease in the global average production of major crops for every 1°C increase in temperature.

To overcome this challenge, a fundamental role is played by climate-resilient crops. An interesting mechanism employed by plants to improve stress tolerance is the production of cuticular wax, a protective layer covering the surface of most plant organs.

One focus of our research is to provide knowledge about the role played by cuticular waxes under drought and heat stresses in durum wheat.

To pursue our goal, we are studying three different regulatory mechanisms through CRISPR/Cas9 technology. The selected genes include a transcription factor belongingto the AP2 family, associated with the negative regulation of wax biosynthesis in Arabidopsis; an E3 ubiquitin ligase that interacts with a positive regulator of wax biosynthetic genes; and a Gγ protein involved in calcium signalling perception, associated with a QTL for wax content and thermotolerance in rice.

A second goal of our research concerns the establishment of wheat cell suspension cultures of three bread wheat starch mutants (and the wild type) previously generated through TILLING, mutated in key genes involved in starch biosynthesis. Thesecultures will constitute a valuable resource to study the relationship between starch composition and key cellular processes of the plant. Furthermore, it will be used as starting materials to test the efficiency of the CRISPR- based transformation vectors.

Oleoylethanolamide (OEA) restores skeletal muscle insulin signaling and mitochondrial energetics in rats fed on a high-fat diet

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Skeletal muscle plays a fundamental role in glucose homeostasis by internalizing large quantities of ingested glucose, under the action of insulin. High-fat diets (HFDs) can increase muscle lipid content and deregulate glucose homeostasis. Oleoylethanolamide (OEA), an endocannabinoid-like compound, has been shown to have several beneficial effects against HFD-induced metabolic imbalances. We investigate the effect of OEA on HFD-induced rat muscle dysmetabolism. Male Wistar rats were divided into four groups: fed a low-fat diet (control); fed the control diet and received intraperitoneal (i.p) injection of OEA (LO) for 2 weeks; fed a high-fat diet (HFD) (HV); and fed the HFD and received i.p injection of OEA for 2 weeks (HO). Muscle lipid content, Western blot analysis, and energy parameters were analyzed in all experimental groups. We found that HV had triacylglycerol accumulation compared to both the LV and LO groups. Furthermore, a greater expression, in HV compared to LV and LO, of the phosphorylated form of proteins involved in insulin pathways, was measured. Administration of OEA to HFD-fed rats reduced lipids and restored insulin signaling. HV rats had increased expression of complexes II, III, and IV of the respiratory chain, but no change in the expression of complexes I and V. Furthermore, carnitine palmitoyltransferase-1 activity and expression were increased in HV compared to both LV and LO rats. The OEA reported the expression of all analyzed proteins to control values. OEA improves muscle insulin signaling by reducing lipid accumulation and restoring mitochondrial functions.

POSTER

Bio-production of natural phytostimulants and their application under abiotic stress conditions

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Developing circularity processes in every production chain is an important objective of the new economy and in plants propagation the use of waste and by-products is particularly interesting.

Temporary Immersion Bioreactors (TIBs) technology is used for mass propagation in different plant species being a controlled in vitro system that allows to obtain genetically similar and pathogens-free material with a higher yield, a high quality production and a reduced impact on the environment. During the propagation in TIBs, the liquid culture medium can be enriched with metabolites produced by the plant and that makes it a useful waste product for other purposes, such as use in agriculture as a plant biostimulant.

The aim of this research was to evaluate the phytostimulant effect of water and culture media from the propagation in temporary immersion bioreactors of aquatic mosses whose biomass, once increased, has other biotechnological uses. Initially, *in vitro* assays were carried out on tobacco model plants under normal conditions and under abiotic stress observing how, especially in salt stress, the response of plant, in terms of growth, was better, even compared to the non-stressed control. Successively, a phenomic study was carried out at High-throughput plant phenotyping platform placed at the ALSIA Centro Ricerche Metapontum Agrobios s.r.l. where, these by-products of moss micropropagation, were tested on tomato to study plant growth, performance, and composition based on multi-spectrum, high-throughput image analysis to detect morphometric and physiological parameters in addition to traditional measurement methods.

Multifaced carbon nanoparticles from spent coffee grounds for photo-induced and electrochemical applications

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The electronic and optical features so far observed for carbon-nanostructures encourage the investigation on these innovative, functional, bio-compatible and eco-sustainable materials, focusing the attention on the development of green synthesis procedures, in the frame of circular economy, starting from waste biomass.[1] In particular, for their wide potential photoinduced application in different fields, from biology to technology, it's useful tuning the photochemical and photophysical features of carbon nanoparticles (CNPs) and this is possible thanks to CNPs easiness of processing with high precision, nanometric size and the possibility to be functionalized through sustainable procedures. Further, protocols with low environmental and energy impact can be designed exploiting waste materials as carbon source. In this contribution, indeed, various bio-waste and by-products, such as coffee grounds and coffee silver-skins, were used as carbon sources. [2] CNPs were thus obtained by means of simple, cheap and ecological hydrothermal synthesis procedures according to an oxidation process. The obtained CNPs were characterized by various spectroscopic techniques including, UV-Visible absorption fluorescence, FT-IR, Raman and XRF spectroscopy. CNPs morphology, instead, was investigated by means of Atomic Force Microscopy. The synthesized CNPs have been investigating as electro-catalysts for water oxidation processes and as photosensitizers for photodynamic therapy applications.[3]

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Recovering inorganic biomaterials from seafood industry wastes: properties and applications of soft calcite extracted from mussel shells

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Global fisheries and aquaculture production reached an all-time record of 214 million tonnes (MT) in 2020, and they will play an increasingly important role in providing food and nutrition in our future. The world's consumption of aquatic foods will continue to rise with the world's population, but growth must be sustainable. Aquatic animal production is forecast to grow another 14% by 2030 and of the total production molluscs comprised 13%, about 17 MT every year. That is to say discarded shells with high disposal costs and considerable environmental impacts. Here we focus on mussel shells waste as a valuable raw material to be biorefined, to obtain $CaCO_3$-based products with an established market or even innovative materials for advanced applications. *Mytilus galloprovincialis* sp. shells are a renewable source of biogenic $CaCO_3$, currently obtained by mineral extraction from rocks, containing up to 99.9% of calcite and aragonite. Recovering shells will relieve strain on landfills and represents a sustainable strategy to implement the blue economy by obtaining high value-added products. We are investigating the intriguing properties of soft calcite, an inorganic biomaterial with a high specific surface, as confirmed by morphological analyses. We are currently improving its preparation method to make the process more sustainable and scalable and we are testing a potential application of calcite as substitute of other high impact and non-renewable materials for the removal of aqueous contaminants. Specifically, the results relevant to the adsorption of a new emerging pollutant (Tetracycline) will be presented.

Protein Kinase C activation regulates NDRG1 expression

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N-myc downstream regulated gene 1 (NDRG1) is a member of the NDRG family of intracellular proteins, and plays a central role in a wide range of biological processes including stress response, differentiation, and maintenance of the myelin sheath. The overexpression of NDRG1 is an indicator of poor prognosis in various pathological conditions. Here, we found that NDRG1 is an independent prognostic marker of poor outcome in breast cancer (BC). The relationship with an aggressive phenotype was underlined by the survival analysis, where Kaplan-Meier curves showed a worse clinical outcome in the subgroup of Triple Negative BC (TNBC) with high NDRG1 expression. In vitro, CRISPR-based inactivation of NDRG1 allowed us to demonstrate that this protein is required for breast cancer cell invasion, without affecting viability. NDRG1 expression is regulated by a variety of molecular mechanisms, including transcriptional and post-translational control. We observed that different acute stress conditions converge on protein kinase C (PKC) activation driving enhanced NDRG1 expression through a signaling pathway that involves ROCK/AMPK/Akt kinases. This newly discovered mechanism was specific for

NDRG1 as the expression of other NDRG members was not affected. Together, our results suggest that pathophysiological PKC-mediated activation of NDRG1 may be a response mechanism to metabolic stress and anticancer agents.

Extracellular vesicles derived from Citrus limon, Punica granatum, and Actinidia deliciosa **modulate inflammation in Caco-2 cells**

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In recent years there has been an explosion of interest in plant extracellular vesicles (P-EVs) both to understand the role that they naturally play in plants and also for their therapeutic potential against several diseases. Like mammal EVs, P-EVs contain plant-specific small molecules with functional roles and therapeutic potential. While therapeutic interest in P-EVs began with the identification of antioxidant properties, many studies are suggesting a wide therapeutic potential, including the possibility of exploiting them as drug delivery vectors due to their relatively low production cost, good tolerability, and the absence of zoonotic or human viruses which may be present in mammalian EVs. Among the beneficial effects, the impact of P-EVs on gut inflammation is not yet well understood.

In this study, we evaluated the capability of *Citrus limon*, *Punica granatum*, and *Actinidia deliciosa***-**derived EVs in modulating inflammation and oxidation in Caco-2 cells, a human colon epithelial cancer cell line used as a model of human intestinal absorption of drugs and other compounds. Cells were treated with LPS and TNF-α, or H_2O_2 to induce inflammation or oxidation respectively. The anti-inflammatory effect was evaluated by analyzing cytokines gene expression with RT-qPCR. P-EVs exert both an antioxidant and anti-inflammatory activity upon internalization in Caco-2 cells visualized by confocal microscopy, suggesting the capability of P-EVs to modulate inflammation and their potential beneficial effect on intestinal mucosa.

Acute toxicity of nanoplastics on *Artemia franciscana*: study of the effects of polyethylene terephthalate (PET) nanoplastics on the swimming behavior

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Anthropogenic activities result in the release of pollutants into the marine environment, which represents a worldwide threat to the health of marine organisms and in turn and endanger for biodiversity. Among contaminants of emerging concern micro- and nanoplastics (MPs/NPs) have become a priority issue in recent years due to their presence and accumulation in all studied ecosystems. The present work aims to study the effects of polyethylene terephthalate (PET) NPs on the motility of mesozooplankton, using *Artemia franciscana* as a model organism. This species is a mesozooplankton organism widely used in ecotoxicological studies. In the last decade, *Artemia Franciscana* has started to gain attention as a biological model suitable for nanoecotoxicity testing. For the purpose of the study, polyethylene terephthalate (PET) environmentally relevant model nanoplastics, similar to those found in the marine environment, produced by means of a fast top-down approach based on mechanical fragmentation, were used. Behavior analysis of the organisms exposed for 24 and 48h to PET NPs was performed using a cell phone camera employing image recognition protocols for automated analysis, allowing real-time integration of behavioral recordings with measurements of physiological outcomes during acute exposure of *Artemia franciscana* to nano-sized PET. The short-term exposure to PET nanoparticles induced significant alterations, consisting in changes of trajectories and acceleration. The obtained results highlight the potential impact that PET NP litter can exert on marine mesozooplankton. The used methodological approache represent a new direction

that could miniaturize and revolutionize research in aquatic ecotoxicology to study the effects of nanoplastics in field biomonitoring applications.

Lab-on-chip for phytopathogen monitoring

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Recently, with globalization and climate change, in Europe there have been several epidemic outbreaks of phytopathogens such as bacteria, viruses and nematodes, considered a serious threat to agriculture. *Xylella fastidiosa* subsp. *Pauca* is considered one of the most dangerous and expected to have an impact of 1.9-5.2 billion euros in Italy [1]: it has spread very rapidly in the Salento peninsula, attacking the olive trees, destroying the landscape and causing a crisis in olive oil production [2] [3]. Equally, *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine fanleaf virus* (GFLV) affect grapevines at leaf level, compromising their survival and causing, worldwide, huge crop losses. Border controls and conventional diagnostic techniques are not sufficient for containment; thus, reliable diagnostic approaches are needed. Lab-on-a-Chip (LOC) technologies represent a promising strategy to enable rapid, simple, sensitive, versatile monitoring [2].

In this respect, our research group recently optimized a LOC based on impedance spectroscopy for the detection of GLRaV-3 and GFLV through a sensing module consisting of electrodes functionalized with specific antibodies [4]. Compared to a traditional serological method (ELISA), the results, confirmed our LOC as an innovative technology for on-field monitoring. Recently, SAW transducers were also employed for this purpose, exhibiting superior performance [5].

Finally, as a further approach, we are working on a miniaturized qPCR system for an on-field detection. In this case, 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 of all process steps and detect *X. fastidiosa* by a laser and a detector integrated in the sample miniaturized system.

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Extraction and Valorization of Antioxidant Polyphenols from Olive Leaves Using Environmentally Friendly Solvents

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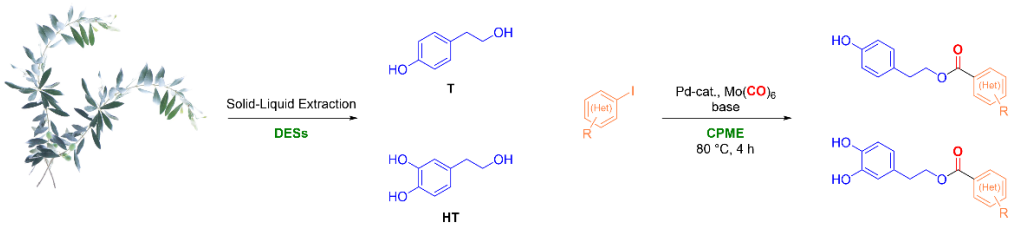
Tyrosol (T) and hydroxytyrosol (HT) are olive-derived phytochemical polyphenols with a great biological activity related to their biological properties.[1] In particular, HT exhibits numerous pharmacological activities and benefits for human health such as antioxidant, anti-inflammatory, anti-tumor, anti-viral, anti-bacterial and anti-fungal activities.[2] Traditionally the extractions of these polyphenolic compounds have been carried out with Soxhlet techniques, using water or hydroalcoholic solutions as the solvent. However, the high temperatures reached in the process affect the thermolabile molecules of HT and T. Furthermore, due to the high affinity between water and polyphenols, the following purification step becomes complex. A valid alternative to classical extraction solvents was represented by Deep Eutectic Solvents (DESs), a promising class of eco-friendly extraction media composed by at least two constituents, which mixed in suitable stoichiometric ratios, generate a eutectic mixture liquid at room temperature.[3]

In this communication, we present an eco-friendly solid-liquid extraction of T and HT from olive leaf as wastes, mediated by DESs as solvents. The use of DES favors a very selective and environmentally friendly extraction, preserving the stability of the polyphenols. Moreover, to valorize the polyphenols extracted, they were subjected to a "green" alkoxycarbonylation reaction, using $Mo(CO)_6$ as a solid and safe to handled CO source.[4,5] The methodology was carried out in cyclopenthyl methyl ether (CPME) as a bio-derived reaction media (Scheme 1). The antioxidant properties of the novel synthetized ester derivatives were also assessed.



Scheme 1. Ecofriendly extraction of tyrosol (T) and hydroxytyrosol (HT) from olive leaves with DESs and their "green" alkoxycarbonylation in the bio-derived CPME.

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Ruolo del controllo qualità del reticolo nella risposta allo stress da metalli pesanti in *Arabidopsis thaliana*

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I metalli pesanti sono naturalmente presenti nella crosta terrestre, ma con l'avvio dell'industrializzazione la loro concentrazione nell'ambiente è aumentata a tal punto da compromettere la qualità della vita degli esseri viventi a livello globale.

I meccanismi di assorbimento, accumulo e detossificazione evoluti dalle piante per sopravvivere alla presenza di matrici contaminate da metalli, sono ben documentati in letteratura, ma poca attenzione è stata dedicata agli effetti dei metalli sullo stress del Reticolo Endoplasmatico (ER). Una nostra recente pubblicazione riporta che l'attenuazione della via Unfolded Protein Response (UPR) riduce la percezione dello stress del ER aumentando la tolleranza al cadmio in piante di *Arabidopsis thaliana* (1).

Il pathway UPR è strettamente connesso al Controllo Qualità del Reticolo Endoplasmatico (ERQC), macchinario che garantisce il corretto ripiegamento delle proteine in modo tale che solo quelle correttamente ripiegate possono raggiungere la loro localizzazione cellulare. L'alfa-glucosidasi II dell'ER (α-GII) è uno degli enzimi chiave del controllo qualità, poiché ammette i clienti delle glicoproteine ripiegate nell'ERQC e li rilascia da esso (2).

Abbiamo investigato per la prima volta gli effetti dello stress cronico da cadmio su un mutante della α-GII di *A. thaliana*. Sorprendentemente, analisi fenotipiche e molecolari hanno rivelato che tale mutante mostra una maggiore tolleranza al cadmio rispetto alle piante Wt, indicando che ERQC è coinvolto nella risposta allo stress. Comprendere i meccanismi molecolari alla base della tolleranza potrebbe permettere di generare piante in grado di tollerare una maggiore quantità di metalli da utilizzare per il fitorimedio con un impatto significativo sull'ambiente.

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microRNA come biomarcatori di dolore muscolo scheletrico nei runners: il protocollo dello studio MiMuS

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INTRODUZIONE. I miRNA sono coinvolti nella generazione e progressione del dolore muscolo scheletrico, condizione che causa un significativo onere clinico, economico e sociale. Nei runners, la presenza di dolore muscolo scheletrico correlato allo stato infiammatorio o al danno tissutale in corso può avere effetti anche sulla capacità di allenamento e performance. È stato avviato uno studio per valutare l'espressione di miRNA associati al dolore in runners con (casi) e senza (controlli) dolore muscolo scheletrico; osservare come variano i miRNA in seguito a un intervento kinesiologico volto a ridurre il dolore nel gruppo dei casi; valutare eventuali correlazioni tra miRNA, citochine, stress e caratteristiche individuali e comportamentali; caratterizzare la funzione dei miRNA mediante analisi *in silico*.

METODI. Il protocollo prevede la rilevazione dei miRNA nel sangue e nella saliva dei runners arruolati all'inizio, contestualmente alla somministrazione di un questionario per valutare le caratteristiche individuali e comportamentali, dopo l'intervento kinesiologico e a 3 mesi di follow-up.

RISULTATI. L'analisi della letteratura ha evidenziato che miR-124-3p, -146a-5p, -150-5p, -155-5p sono coinvolti nella patogenesi del dolore muscolo scheletrico e nell'infiammazione, e miR-133b e -206 nella risposta al danno muscolare, oltre ad essere espressi nel tessuto muscolo scheletrico.

CONCLUSIONI. Da un punto di vista di salute pubblica, l'identificazione di potenziali biomarcatori correlati al dolore potrebbe essere utile per prevenire, mediante idonei interventi, traumi, infortuni e algie croniche.

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