Abstract Book

PhD Day (November 14, 2024)

PhD Course in Biomolecular and Pharmaceutical Sciences

Cycles: **XXXVII – XXXVIII- XXXIX**

Coordinator: **Prof.ssa Assunta Pandolfi**

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PhD Course in Biomolecular and Pharmaceutical Sciences XXXVII Cycle

"miRNA-mediated alterations of inflammatory pathways in Multiple Sclerosis"

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Introduction: Multiple sclerosis (MS) is a neurodegenerative disorder marked by peripheral immune dysregulation, prompting the infiltration of activated immune cells into the central nervous system. This leads to localized inflammation, multifocal demyelination, and axonal degeneration. Cytokines, pivotal in immune response modulation, fuel the inflammatory cascade, exacerbating disease progression and severity. MicroRNAs (miRNAs), important post-transcriptional regulators of gene expression, have increasingly been recognized for their role in the pathogenesis of MS. As a result, they are emerging as promising therapeutic targets and potential biomarkers for the diagnosis and treatment of MS.
Aim: To elucidate miRNAs role in MS pathophysiology, the expression profile of miRNAs involved in cytokine pathways was investigated in the peripheral blood mononuclear cells (PBMCs) and serum of relapsing-remitting MS (RRMS) patients compared to healthy controls (HCs). This approach aimed to uncover disease-related molecular networks that could serve as potential biomarkers.
Methods: Real-Time quantitative PCR was employed to analyze the expression of a panel of miRNAs (the Human miFinder Focus Panel, Qiagen) associated with MS and the cytokine system, as identified through GeneCards annotation. The analysis was conducted on PBMCs and serum samples from three treatment-naïve RRMS patients and three HCs. Dysregulated miRNAs were identified utilizing statistical methodologies, while bioinformatic tools were applied to infer functional miRNA-target interaction networks. Six candidate miRNAs were subsequently assessed during a validation phase on a broader subject cohort, following the same procedures used in the initial screening phase.
Results: From the screening phase, differentially expressed miRNAs were identified in RRMS subjects compared to HCs across both PBMCs and serum samples. Six candidate miRNAs underwent further bioinformatic analysis, which confirmed their involvement in immune-related pathways and revealed overlapping targets among multiple miRNAs. In the validation phase, significant expression changes were confirmed for miR-125b-5p (downregulated in PBMCs and upregulated in serum), miR-20a-5p (upregulated in serum), and miR-223-3p (downregulated in PBMCs). SMAD4, a key signal transducer in the TGF-β pathway, emerged as the only common target gene for these three miRNAs.
Conclusion and perspectives: Analysis of the expression profiles of miRNAs panel revealed the dysregulation of a specific subset, shedding light on the molecular mechanisms driving the pathology. Following the exploratory screening phase, the validation stage confirmed the dysregulation of three miRNAs in RRMS patient *vs* HCs, all converging on the regulation of the transcription factor SMAD4, the central signal transducer of the TGF-β pathway. Ongoing experiments aim to explore differences in the expression and production of inflammatory downstream targets (IL-10, IFN-γ, TNF-α), which are directly or indirectly regulated by TGF-β/SMAD4 signaling. Confirming the alteration of miRNA patterns and their targets would enhance the understanding of MS and support their potential use as molecular biomarkers for the disease.

"Protein Replacement Therapy: production and biochemical characterization of recombinant SIL1 protein"

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Recessive genetic diseases arise from mutations or deletions in genes, resulting in loss of function or absence of the encoded protein [1]. Marinesco-Sjögren syndrome (MSS) is a rare autosomal recessive disorder which presents with cerebellar ataxia, myopathy and cataracts [5]. It is primarily caused by mutations in the SIL1 gene, which encodes a nucleotide exchange factor for BiP, a key endoplasmic reticulum (ER) chaperone [6]. SIL1 deficiency results in the accumulation of misfolded proteins, leading to cellular stress and ultimately cell death. Gene therapy and recombinant protein treatments aim to correct the functional deficits associated with recessive genetic diseases [2]. The present study aims to develop a protein replacement therapy for MSS using cell-penetrating peptides (CPPs) fused to the SIL1 protein.
To this end, we assessed the expression of SIL1 lacking the initial 31 N-terminal amino acids fused to various CPPs. These constructs were cloned into the pGEX6P1 vector, resulting in the production of recombinant proteins with an N-terminal GST tag. Subsequently, we optimized the expression in BL21(DE3) bacterial cells and purification of the selected recombinant SIL1 construct, fused to the transactivator of transcription (TAT) peptide, through affinity chromatography. We provided evidence of correct folding, thermal stability and tetrameric structure of the protein by circular dichroism and dynamic light scattering. To obtain the three-dimensional structure of the purified protein, we used protein X-ray crystallography. The purified protein was subjected to specific conditions (pH, temperature, salt concentration) which led to the formation of protein crystals. Crystals exposed to an X-ray beam formed a diffraction pattern that allows three-dimensional positions of each SIL1TAT atom. Future studies will investigate the chimera toxicity, cellular uptake, tissue distribution and therapeutic efficacy in woozy mouse, model of MSS. This research lays the foundation for a new therapeutic approach for MSS and potentially for other genetic disorders caused by protein deficiencies.

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"Facile synthesis of Pt-Cu-Fe nanoparticles anchored on reduced graphene oxide for the Hydrogen Evolution Reaction"

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Nowadays, the hydrogen evolution reaction (HER) plays a critical role in the economical generation of pure hydrogen fuel. Platinum (Pt)-based catalysts can effectively improve the HER by reducing energy activation and substantially increase the reaction rate. Although platinum-based materials are regarded as the state-of-the-art electro-catalysts for HER, high cost and quantity scarcity hamper their scale-up utilization in industrial deployment.
Owing to its novel properties, such as high electrical conductivity and large specific surface area, graphene has been found as a suitable support material for the electrocatalyst design. Therefore, the exploration of PtM alloy catalysts anchored on reduced grapahene oxide with low Pt loading, high HER activity and stability has become the focus of HER research[1].
This study is focused on understanding the electronic structure change and its effects on the electrocatalytic performance[2] when monometallic and trimetallic nanoparticles are electrochemically reduced on reduced GO through chronoamperometry.
First the electrochemical surface area of the bare glassy carbon electrode and the glassy carbon electrode modified with drop-casted graphene oxide was evaluated through the Randles-Sevcik equation. Then, the nucleation of the metallic particles is observed through cyclic voltammetry on the modified glassy carbon electrode.

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"Recovery of bioactive compounds from leaves as by-products of olive oil production"

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The cultivation of olive trees and the production of olive oil generates massive amounts of wastes. For this reason, the valorisation of by-products deriving from *Olea europea* L. (Fam. Oleaceae), has been a topic of great interest in recent years [1]. Leaves, the most abundant waste, are already widely known as remedies for infections, hypertension, arrhythmia, intestinal spasms, cancer, and many others and thus their extract is used as main ingredient of several pharmaceutical products. Indeed, they are rich in health-promoting functional components, of which the secoiridoid glycoside oleuropein is one of the most abundant, together with its aglycones hydroxytyrosol and elenolic acid, the latter less biologically studied until now [2].

To this regard, a microwave assisted procedure was performed to obtain an aqueous extract with a high content in oleuropein. Subsequentially the active compound was selectively isolated through solid phase adsorption (SPA) with the use of lamellar solids, powerful tools to enhance the chemical stability of the molecule, and surely facilitating its use in olive-leaf based phytopreparations. Finally, an eco-friendly, readily available, and reusable catalyst like H_2SO_4 supported on silica was applied for the hydrolysis of oleuropein into its two main components. An extract enriched in the monoaldehydic dihydropyran form of elenolic acid was obtained by an acid-base work-up and this was later on characterized by GC-MS. A semipreparative scale HPLC method was also validated for the final isolation of elenolic acid, which was finally characterized through NMR and HRMS.

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"Innovative species-specific formulations for the balance of the skin microbiota"

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The skin microbiota plays a crucial role in maintaining cutaneous homeostasis and acts as a barrier against pathogenic microorganisms. *Staphylococcus epidermidis* is a key commensal bacterium contributing to skin health, while *Staphylococcus aureus* is a pathogenic species often related with dysbiosis and frequently associated with antimicrobial resistance, complicating treatment strategies. This PhD project explores innovative, eco-sustainable antimicrobial approaches using natural extracts derived from food waste, in detail pomegranate peel extract (PPE) and almond hull extract (AHE), and green technologies including plasma-activated water (PAW).
PPE demonstrated remarkable antimicrobial and anti-adhesive activity against skin *S. aureus* biofilm formation, while promoting *S. epidermidis* growth, highlighting its selective specie-specific action and effectiveness for topical applications. AHE showed efficacy in inhibiting *S. aureus* and *Escherichia coli* in formation and mature biofilms, with low cytotoxicity on skin cells, further supporting its suitability for topical use. PAW, an eco-friendly technology, successfully reduced *S. aureus* biofilm leaving unaltered the *S. epidermidis* amount in mono-species biofilm, offering a chemical-free solution for pathogen control.
These findings suggest that natural extracts and green technologies can represents a novel strategy for innovative formulations for managing skin dysbiosis, aligning with the approaches of One Health and eco-sustainability. Future studies will investigate the synergistic potential of combining these approaches to enhance antimicrobial effectiveness and develop next-generation skincare products.

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Acknowledgments
PhD fellowship (code n. DOT1353593) in the framework of PON R&I 2014/2020, Action IV.5-" PhD on green issues", funded by the Ministry of University and Research (MUR), Italy, FSE-REACT-EU.

"Therapeutic Effects of Resolvin D1 on Lung Inflammation and Exercise Limitations in Cystic Fibrosis: Preclinical Insights"

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Background: Cystic fibrosis (CF) is a genetic disorder characterized by chronic lung inflammation, mucus buildup, and exercise intolerance. Despite advancements in CFTR modulators, patients continue to suffer from significant respiratory issues due to persistent mucus obstruction and inflammation.

Aims: This study aimed to investigate whether Resolvin D1 (RvD1), a specialized pro-resolving lipid mediator derived from omega-3 fatty acids, can alleviate lung inflammation, improve airway function, and enhance exercise capacity in CF models. The research seeks to establish RvD1 as a potential adjunct therapy for managing CF-related symptoms.

Methods: The effects of RvD1 were tested on human airway cells exposed to CF mucus and in transgenic mice overexpressing the epithelial sodium channel (βENaC), which mimics CF lung pathology. RvD1's impact on inflammation was assessed through leukocyte response, RNA sequencing to identify molecular pathways, and exercise tests to measure physical endurance.

Results: RvD1 significantly reduced inflammation in airway cells by modulating gene expression linked to inflammatory pathways and stress responses. In βENaC mice, RvD1 decreased the number of neutrophils and other inflammatory cells in the lungs, improved clearance of Pseudomonas aeruginosa infection, and enhanced physical activity levels by lowering energy expenditure during exercise. The treatment also corrected metabolic imbalances associated with CF.

Conclusion: The findings suggest that RvD1 can effectively reduce lung inflammation and improve exercise tolerance in CF, providing a basis for its potential therapeutic use to manage chronic muco-obstructive lung conditions. Further studies are needed to confirm these effects in clinical settings

"Protective Effects of Aged Black Garlic Extract (Abge) On Prostate Cancer"

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Academic Tutor: Prof. Luigi Brunetti

Chronic inflammation is a well-established risk factor for various cancers, including prostate cancer (PCa). This study investigates the potential protective effects of aged black garlic extract (ABGE) against inflammation-induced prostate damage and its impact on prostate cancer cell lines. Using an ex vivo model of inflammation induced by Escherichia coli lipopolysaccharide (LPS) in prostate tissue from C57BL/6 male mice, we assessed the anti-inflammatory properties of ABGE by measuring the gene expression levels of key pro-inflammatory biomarkers (COX-2, NF-κB, TNF-α, and IL-6).

Furthermore, the therapeutic potential of ABGE on prostate cancer cells was evaluated through in vitro functional assays, including colony formation, tumorsphere formation, cell migration, and phosphorylation arrays targeting key signaling pathways (MAPK, AKT, JAK/STAT, and TGF-β). ABGE exhibited significant anti-inflammatory and antioxidant effects, likely due to its polyphenolic compounds, particularly catechin and gallic acid.

In the ex vivo model, ABGE significantly reduced the expression of pro-inflammatory genes (COX-2, NF-κB, TNF-α, and IL-6). In vitro, ABGE suppressed cell proliferation, colony and tumorsphere formation, and cell migration in prostate cancer cells. These findings suggest that ABGE may serve as a promising therapeutic agent with both anti-inflammatory and anti-cancer properties, warranting further research to explore its role in managing inflammation and prostate cancer.

"Valorization of grape pomace extracts against cranberry, elderberry, rose hip berry, goji berry and raisin extracts: Phytochemical profile and in vitro biological activity"

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Grape pomace, composed of grape peels and seeds, represents the main solid waste in the winemaking process. It is a good source of polyphenols, secondary metabolites in plants showing a wide range of biological effects. In this work [1], we investigated the potential advantages of using grape pomace from Montepulciano d' Abruzzo grapes to produce nutraceuticals comparing its extracts with those of commercially available berries and fruits (cranberry, elderberry, rose hip, goji berries and raisins) in terms of antioxidant activity and enzyme inhibition activity.
Three extraction techniques have been applied: a conventional decoction, an ultrasound-assisted extraction and a microwave-assisted extraction. Preliminary colorimetric assays were performed on each dried extract for the determination of the total phenolic and flavonoid content. The highest phenolic and flavonoid content was determined in grape pomace extracts. The phytochemical profile was assessed by HPLC-ESI-Q-TOF-MS (Figure 1). Grape pomace extracts accounted for the most abundant number of characterized compounds, a total of 67. The antioxidant activity of each extract was determined by DPPH, ABTS, CUPRAC, FRAP, metal chelating ability and phosphomolybdenum assays. The best antioxidant activity in DPPH, ABTS, CUPRAC, FRAP and phosphomolybdenum assays was detected for grape pomace extracts in accordance with the total phenolic content. Goji and elderberry extracts showed greater metal chelating abilities than the other extracts. This activity could be related to non-phenolic chelators such as peptides or polysaccharides. Finally, enzyme inhibition assays were performed against cholinesterase, glucosidase, amylase and tyrosinase. Grape pomace extracts showed the best tyrosinase inhibition activity which can be useful in case of hyperpigmentation disorders. In summary, given the high antioxidant activity and the good tyrosinase inhibition, grape pomace could be used as an alternative to other commercially available fruit and berries to produce nutraceuticals, hence contributing to the circular economy by the valorisation of by-wastes products in the wine industry.

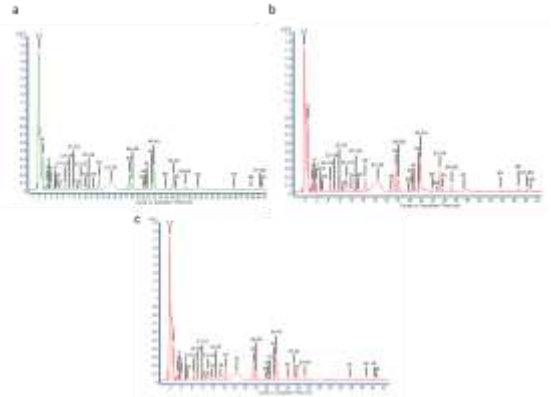

Figure 1. Base peak chromatograms of Grape pomace decoction (a), ultrasound-assisted extract (b) and microwave-assisted extract (c); HPLC-ESI-Q-TOF-MS in negative ion mode.

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(ASSENTE)

"Validation of Aseptic Process of [18f] Fluodeoxyglucose"

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Nuclear medicine is a branch of medicine that uses radiopharmaceuticals for its purposes. The use of radiopharmaceuticals can be an important and effective approach especially in oncology. A radiopharmaceutical consists of a targeting moiety and a radionuclide. Radio nuclides decay has different emission of particles or electromagnetic radiation (positrons, gamma rays, etc.). These emissions are important for the selection of a radionuclide for imaging or therapy in nuclear medicine. [^{18}F] Fluodeoxyglucose ([^{18}F] FDG) is a positron emitter that is used in PET for imaging. The positron, after having traveled a short distance from the point where it was emitted, encounters an electron which is part of the surrounding biological matter, giving rise to the phenomenon of annihilation.

The aim of this work focused on the development and validation of an aseptic process for the production of [^{18}F] FDG sterile injectable solution, with AIC proprietary to Itel telecommunications s.r.l.

The facility now produces [^{18}F] FDG with terminal sterilization. Compared to the product with terminal sterilization having an activity of 250 MBq/ml, the new equivalent specialty of [^{18}F]FDG produced in asepsis will present an activity of 1 GBq/ml allowing to obtain a sterile and apyrogenic finished product with higher yields compared to the current process; this will allow us to supply a greater number of Nuclear Medicine UOCs, as long as they are present within a kilometer radius compatible with the duration of the radiopharmaceutical (approximately 10 hours).

A Risk Assessment was written, to evaluate the need to carry out new validation protocols relating to analytical methods (chemical and microbiological) for the release of the radiopharmaceutical.

The final step of this work was to carry out the validation of the entire production process through the execution of a Process Validation protocol in which at least 3 batches of product were carried out for each synthesis module used.

A stability study was performed on the same batches used for the process validation protocol to confirm that the quality of the product is satisfactory for the entire shelf life proposed within its packaging. The shelf life of the pharmaceutical product was defined as 12 hours after the date and time of production (t0) at 25°C and 40°C. All validation batches produced on the site were analytically compliant with the required specifications.

Therefore, it is possible to conclude that the production process of Fluodeoxyglucose (^{18}F) ITELPHARMA 1 GBq/mL in asepsis is robust, reproducible, reliable and compliant with high quality standard.

Using these validation data, a dossier CTD was prepared for the product, and it was submitted to the National Regulatory Authority (AIFA-Italian Medicines Agency), in order to obtain suitable AIC (Authorisation for Marketing).

PhD Course in Biomolecular and Pharmaceutical Sciences XXXVIII Cycle

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Industrial Tutor: Prof. Vincenzo Dimiccoli

(ASSENTE)

"Role Of Human Dermal Fibroblasts in Impaired Angiogenesis Associated with Type 2 Diabetes"

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Co-tutor: Dr. Caterina Pipino

Human dermal fibroblasts (HDF) are the most abundant cells in the dermis. HDF produce and organize extracellular matrix (ECM), regulate skin homeostasis and wound healing by interacting with cells located in the dermis like endothelial cells (ECs). Of note, HDF produce cytokines and growth factors (GF) like vascular endothelial GF (VEGF) and fibroblast GF (FGF) that promote and regulate ECs behavior and angiogenesis. Moreover, HDF produce ECM proteins like fibronectin and collagen I, impacting tubules formation. On the other hand, ECs can influence HDF migration, GF release and ECM deposition. In type 2 diabetic (T2D) patients wound healing is compromised, which frequently results in chronic ulcers formation. HDF isolated from T2D patients show reduced ECM production as well as decreased proliferation and migration, collectively contributing to delayed healing. In addition, fibroblast dysregulation, which results in reduced angiogenic factors release and ECM production/degradation imbalance, negatively influence angiogenesis. Nevertheless, the specific mechanisms that interplay between HDF and ECs in the skin of T2D patients are not completely understood.

The aim of the project is to better understand the role of diabetic HDF, isolated from a T2D patient, in the mechanism underlying reduced angiogenesis and impaired ECs migration in diabetic conditions, with a focus on identifying the potential pathways involved.

Firstly, differences between healthy and T2D HDF (N_ and diabHDF, respectively) were investigated by analyzing cell metabolic activity, DNA content and gene expression of collagen 1 (*COL1*), Mothers against decapentaplegic homolog 3 (*SMAD3)*, *VEGFa* and chemokine (C-C motif) ligand 2 (*CCL2)*.

To investigate diabHDF role in ECs angiogenesis related mechanisms an indirect co-culture system (transwell) was employed. For this, human umbilical vein ECs isolated from control (C-HUVEC) and gestational diabetic women (GD-HUVEC) were used. In particular, the latter showing a pro-inflammatory phenotype is a suitable *in vitro* model to recapitulate diabetes induced dysfunction and to explore diabHDF role. C- or GD-HUVEC were cultured in the well plate, while N_ or diabHDF were cultured in the transwell in a ratio of 1:2. After 6 days in co-culture, scratch assay and Matrigel tube formation assay on C- and GD-HUVEC were performed.

Of note, diabHDF showed significantly reduced cell viability and DNA amount compared to N_HDF over time. Moreover, diabHDF showed lower *COL1* and increased *VEGFa* and *CCL2* gene expression compared to N_HDF. No differences were detected in *SMAD3* gene expression. HUVEC co-incubated for 6 days with diabHDF showed a reduced cell migration as well as a reduced number of master segments, junctions, meshes and branching length in the tube formation assay compared to basal condition and HUVEC incubated with N_HDF.

These preliminary data indicate that diabHDF reproduce some features of T2D induced dysregulation and seem to exert negative effects on the process of angiogenesis when co-incubated with C- and GD-HUVEC,

suggesting the involvement of specific diabHDF driven pathways in this regard. Future experiments will be performed to further investigate the potential involvement of specific pathways that control HUVEC angiogenesis in response to diabHDF.
These data were produced during my research period abroad (February-July 2024) at the Laboratory for Biointerfaces, Empa (Swiss Federal Laboratories for Materials Science and Technology) St. Gallen (Switzerland), under the supervision of Dr. Markus Rottmar and his team.

PhD student: Alessia Cosi
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(ASSENTE)

"Evidence of hydrogen spillover on chemically reduced graphene oxide in copper-catalysed methanol synthesis via CO2-Fischer-Tropsch"

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PhD student: Stefano Di Giacomo
Academic Tutor: Prof. Antonella Fontana

Developing sustainable carbon-neutral fuel pathways is crucial for addressing global climate challenges. This research explores the synthesis of methanol through the CO2-Fischer-Tropsch (FT) reaction, focusing on the use of copper-based catalysts supported by reduced graphene oxide (rGO). The work leverages the unique properties of rGO, such as its defect-rich lattice and sp2 structure, to enhance the efficiency of methanol synthesis via hydrogen spillover. Unlike conventional catalysts, rGO facilitates the activation and migration of hydrogen species, enabling effective CO2 reduction.
Key findings include temperature-dependent variations in methanol selectivity, attributed to changes in the catalytic surface and the loss of CO2 binding defects at higher temperatures. The study demonstrates that methanol formation is optimized at lower temperatures, where oxygen-containing defects on rGO enhance CO2 interaction and support a dual-site reaction mechanism. Furthermore, cyclic temperature adjustments reveal a potential "self-healing" property of the rGO surface, which influences catalytic performance over time.

This investigation underscores the potential of graphene-based materials in stabilizing metal nanoparticles and advancing catalyst design. This work offers new insights into sustainable methanol synthesis technologies by improving hydrogen spillover and reducing catalyst deactivation. Such innovations align with the European Green Deal's emphasis on carbon capture and utilization, paving the way for e-fuel production and advancing renewable energy solutions.

This study is part of the XXXVIII PhD cycle in Biomolecular and Pharmaceutical Sciences at the Università degli Studi "G. d' Annunzio" and is supported by Carbotech Srl under the NextGenerationEU initiative.

Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani

"Selective extraction of active compounds from phytoextracts adsorbed on LDHs"

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Industrial Tutor: Dr. Roberto Spogli

Solid phase extraction (SPE) is a technique based on van der Waals interactions, hydrogen bonding, dipole-dipole forces, and cation-anion interactions that is used to isolate a single compound or a class from a complex system as it can be in the case of natural extracts. In order to obtain an efficient and selective system it is necessary to study the interaction of different absorptive solids with the molecules of interest and the extract. Layered double hydroxides (LDHs) are lamellar inorganic solids of general formula $[M(II)_{1-x}M(III)_x(OH)_2]^{x+}A^{n-}_{x/n} \cdot m H_2O$ that have been proven to be efficient in the selective extraction of gentiopicroside and amarogentin from *gentian lutea* and of emodin and chrysophanol from *polygonum cuspidatum*. The adsorption process of these molecules on various LDHs has been studied testing different materials (LDHs and silica) with different functionalizations in order to better understand the effect of the anion and of the matrix, wether the interaction takes place on the surface or in the interlayer region and the effect of the surface area. LDHs were characterised via XRPD, SEM, FT-IR and BET analysis before and after the adsorption process.

The results obtained showed that both the intercalated anion and the inorganic matrix are important parameters in order to obtain higher pecentages of adsorption and that the interaction between the target molecules and the solids takes place via weak forces and on the surface of the solid.

"Purification of food dyes through solid matrices: Tartrazine and Brilliant Blue FCF by lamellar solids and microspheres"

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Synthetic dyes are used in preference to natural dyes in various industrial fields to color plastics, textiles, food and beverages, because of their higher stability, better tinting capacity and lower cost. On the other hand, their role has been demonstrated in the development of several pathologies due to a prolonged dietary intake, and because of this limit concentrations have been set for their usage.
Developing techniques for their identification and quantification is highly required, especially in matrices in which these dyes are co-existent. The present poster is about the assessment of a method of simultaneous identification of synthetic dyes Tartrazine (TZ) and Brilliant Blue FCF (BB) in green-colored artificial mixtures and food and beverage matrices (liqueur, green apple flavored candies, dried pomelo peels, "icing green" food coloring agent and soft drink). A selective extraction of TZ and BB was carried out by a panel of high yield adsorption solids (layered double hydroxides, zirconium-based lamellar solids, inorganic oxides and hydroxides and phyllosilicates) and the yields of extraction has been obtained through HPLC-DAD. The results of the screening both in artificial mixture of dyes and in food and beverages matrices proved that some materials (MgO, MgAl-DBS and Florisil) showed high selectivity and high extractive yields towards just one dye (TZ for MgO; BB for MgAl-DBS and Florisil) so these were assessed as the most promising material of the panel. Additionally, for these materials it has been demonstrated a dose-dependent increase of adsorption capacity. Their behavior was explained by their chemical structure and ability to produce ionic bonds, hydrogen bonds, acid-base interactions, Van der Waals interactions. At least, a novel approach has been experimented using chitosan microspheres (CH) to absorb TZ and assessing a protocol that allows a best result in terms of absorption yield (5 mg of CH - 10 ppm TTZ). Concluding, it was assessed a method for the selective removal of synthetic dyes from a mixture of two in food and beverage matrices. This finding may represent an initial step to develop novel methodologies for the separation and detection of food and beverages coloring agents in complex matrices, diminishing the risk of possible interferences between dye chemicals.

"EV20/Omomyc: A Novel Dual MYC/HER3 Targeting Immunoconjugate"

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MYC is one of the most important therapeutic targets in human cancer. Many attempts have been made to develop small molecules that could be used to curb its activity in patients, but most failed to identify a suitable direct inhibitor. After years of preclinical characterization, a tissue-penetrating peptide MYC inhibitor, called Omomyc, has been recently successfully used in a Phase I dose escalation study in late-stage, all-comers solid tumor patients. The study showed drug safety and positive signs of clinical activity, prompting the beginning of a new Phase Ib combination study currently ongoing in metastatic pancreatic adenocarcinoma patients. In this manuscript, we have explored the possibility to improve Omomyc targeting to specific cancer subtypes by linking it to a therapeutic antibody. The new immunoconjugate, called EV20/Omomyc, was developed by linking a humanized anti-HER3 antibody, named EV20, to Omomyc using a bifunctional linker. EV20/Omomyc shows antigen-dependent penetrating activity and therapeutic efficacy in a metastatic model of neuroblastoma. This study suggests that directing Omomyc into specific cell types using antibodies recognizing tumor antigens could improve its therapeutic activity in specific indications, like in the pediatric setting.

"Selective umbelliferon-based hCA IX and XII inhibitors as anti-proliferative agents: in vitro and in silico investigations"

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Carbonic Anhydrases (CAs, EC 4.2.1.1) are ubiquitous metalloenzymes that catalyze the interconversion of carbon dioxide (CO_2) and water (H_2O) to bicarbonate (HCO_3^-) and protons (H^+).[1] The continuous division and growth of cancer cells prevent access to oxygen through blood vessels in solid and metastatic tumors. Low pH levels can influence different physio-pathological mechanisms, for example extracellular and intracellular acidosis menace viability of cells. Furthermore, cancer cells can adapt to pH changes by controlling the synthesis of pH-regulating factors. Among them, CAs IX and XII are overexpressed in hypoxic cancerous tissues and influence necrosis around the tumor, controlling pH.[2]
Coumarin scaffold has been shown as CA inhibitor, bacause it can occlude the entrance of the active site and anchor to the zinc-bound water molecule/hydroxide ion followed by the hydrolysis of lactone ring. In this context, we report a library of coumarin-based molecules, selective on human CA IX and XII.
The coumarin scaffold was added to a terminal lipophilic tail, composed by vanillin, isovanillin, ethylvanillin or *o*-vanillin (vanilloid moiety), through an alkyl (2-6 methylene functions) or a triazole linker (Figure 1), following the drug design tail-approach.[3]
All the synthesized derivatives showed micromolar K_i values on the two cancer-correlated isoforms, *in silico* studies guided us to the comprehension of the interaction between the coumarin derivatives and the enzyme. Moreover we have tested the most potent derivatives on adenocarcinomic human alveolar basal epithelial cells (A549 cells).

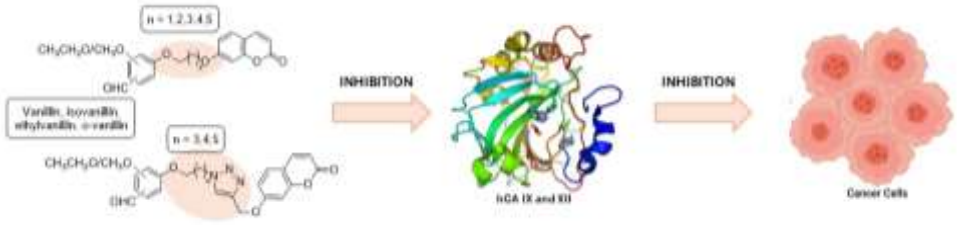

Figure 1. Overview of the library of coumarin-based vanilloid derivatives able to inhibit the activity of human Carbonic Anhydrase (hCA) IX an XII, as potenial anti-proliferative agents.

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"***Evaluation of cellular signaling pathways regulating wound healing properties induced by snail slime extracted by Cherasco method"***

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Snail slime (SS), sometimes referred as snail mucus, is a complex viscoelastic fluid secreted by gastropod mollusks such as Helix aspersa, which has garnered significant scientific interest due to its unique composition and potential applications. Primarily composed of water, it contains a diverse array of biologically active compounds including glycoproteins, proteoglycans, hyaluronic acid, allantoin, glycolic acid (GA), and antimicrobial peptides. This natural substance offers a potent blend of moisturizing, exfoliating, and healing properties; indeed, the beneficial components of SS work synergistically to promote skin regeneration, boost collagen production, and provide powerful anti-inflammatory and antioxidant effects. The current study aimed to evaluate the role of SS in regulating molecular mechanisms involved in cell recovery processes. In particular, we sought to compare the impact of SS to that of one of its main components, represented by GA, on Keratinocytes (HaCat) and Endothelial (EA. hy926) cell lineages by analyzing pathways involved in inflammatory response, cell migration, and wound healing processes. SS was administered to keratinocytes and endothelial cells, then, cell viability through MTT test, cytotoxicity by LDH assay, gene and protein expression through Real-Time PCR and western blot, and wound healing through scratch assay, were measured. The obtained results show that SS is well tolerated by keratinocytes, differently from GA. The latter, in fact, appears more cytotoxic and able to induce inflammation. Results evidence that SS administration recruits the PI3K/Akt pathway, thus regulating the NF-κB transcription factor and COX2 gene expression. This SS-regulated signaling pathway induces brief inflammation, recognized as an essential and initial passage in wound healing processes. Moreover, SS demonstrated to induce Matrix Metalloproteinases 2 and 9 upregulation in HaCat cells, necessary for cell migration and debris clearance and a basepoint for new blood vessel formation; scratch wound healing assay, performed on endothelial cells, further confirms this point. In conclusion, SS is a potent agent in regulating cellular signaling pathways and opens up future avenues for its potential use in wound healing.

This abstract was produced during attendance at the PhD program in Biomolecular and Pharmaceutical Sciences at the "G. d'Annunzio" University of Chieti-Pescara, Cycle XXXVIII, with the support of a scholarship financed by the Ministerial Decree no. 351 of 9th April 2022, based on the National Recovery and Resilience Plan (PNRR) - funded by the European Union – Next Generation EU - Mission 4 "Education and Research", Component 1 "Enhancement of the offer of educational services: from nurseries to universities" - Investment 3.4 "Advanced teaching and university skills".

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(ASSENTE)

"Clinical variables affecting propionyl carnitine metabolism: towards a better comprehension of newborn screening results"

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Background: Newborn screening (NBS) is a public health preventive medicine program with the aim of early detection of different inborn errors of metabolism (IEMs) to prevent adverse outcomes. Italian NBS program is actually screening for 40 IEMs on dried blood spot (DBS) samples. The application of second-tier tests (2-TT) can minimize false positives. Currently, in our laboratory we have NBS data from the first and second-level tests carried out after alteration of propionyl-carnitine metabolism of approximately 37.000 newborns screened in Abruzzo region between 2019 and 2023; 12% of the total samples required the execution of 2-TT.
Methods: NBS was performed using a flow injection-tandem mass spectrometry analysis (FIA-MS/MS) for the detection of 36 IEMs. DBS were extracted with the NeoBase2 Nonderivatized MSMS kit (PerkinElmer) and analyzed with FIA platform RenataDX – Screening System (Waters Corporation). We considered as primary biomarkers propionylcarnitine (C3), C16 (palmitoyl carnitine) Met, the C3/acetylcarnitine (C3/C2), C3/C16, andC3/Met ratios. For 2-TT two DBS disks were extracted for the simultaneous determination of methylmalonic acid, methylcitric acid and homocysteine by LC-MS/MS analysis with Xevo TQ-S microTriple Quadrupole Mass Spectrometry. Statistical analysis was carried out with the T-test calculator (GraphPad) investigating ~3000 samples which presented an altered profile at the first level. Clinical data and physiological conditions were taken into consideration.
Results: Some of the observed conditions reported significant values on the T-test, especially in relation to pharmacological treatments, weight and nutrition. Our data confirm that the metabolic profile is influenced not only by the genome, but also by environmental factor, drugs, diet, and a variety of clinical variables.
Discussion: These results, albeit preliminary, lay the foundations for creating customized cut-offs to reduce the number of false positives at the first level.

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"Surface modification of solid lipid nanoparticles with peptide fragments for the brain delivery"

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Lipid formulations, such as solid lipid nanoparticles (SLNs), are gaining considerable attention worldwide as drug delivery systems for various clinical applications. Over the years, SLNs, due to their unique features such as small size and advantageous physicochemical properties, have emerged as suitable drug carriers, delivering drugs effectively and in a site-specific manner [1]. Additionally, SLNs properties can be improved by enhancing their ability to penetrate body barriers by introducing suitable functionalization on the particle surface. Various modified lipids are now being used to covalently conjugate ligands, such as peptides, for investigating active targeting, as a promising strategy that allows nanoparticles to be directed to specific sites, enabling the transport and delivery of drugs. These features make SLNs an ideal drug delivery system with several key features for treating neurodegenerative disorders (NDs) [2]. Indeed, although many drug delivery approaches have been explored for the treatment of NDs, ultimate success has not yet been achieved due to challenges such as a lack of target specificity and low bioavailability. These drawbacks are mainly related to the presence of the blood-brain barrier (BBB), which complicates the pharmacotherapy for CNS disorders, impeding the drugs to enter the brain. Designing surface-modified SLNs with molecules recognized by receptors/transporters, overexpressed in the BBB, as well as cell-specific receptors in brain tissue, offers a potential solution to these limitations [3].
Starting from this evidence, the aim of this work was the development of SLNs grafted with 20 aminoacids of leptin (Lep_{20}) intended for CNS delivery. Lep is a natural ligand capable of crossing the BBB by receptor-mediated transport [4].
The conjugate SA-PEG-Lep20 was synthesized by a single-step reaction through Michael addition and the conjugation efficacy was assessed through NMR spectroscopy. The NMR spectra revealed that the Mal signal at 7 ppm completely disappeared in the spectrum of the desired conjugate compound (SA-PEG-Lep_{20}) due to the reduction of maleimide double bond. Lep_{20}-SLNs and blank SLNs were then prepared using the emulsification and solvent evaporation method and the characterization was performed via Dynamic light scattering (DLS), Atomic Force Microscopy (AFM), and Raman Spectroscopy. Cellular permeation study was carried out across Caco-2 cell line. DLS analysis revealed increased dimension for Lep_{20}-SLNs, compared to blank SLNs, as well variation in ζ-potential. These findings may suggest variation on SLNs surface due to the presence of the peptide. AFM images displayed spherical morphology and dimensions that confirm the DLS results. The higher roughness of Lep_{20}-SLNs (1.138 ± 0.184 nm) compared to blank SLNs (0.963 ±0.227 nm) suggests the effective presence of Lep_{20} on the SLNs surface. These results were further confirmed by Raman spectra revealing a significant reduction in the ratio of the peak intensity at 2890 cm^{-1} out of 2930 cm^{-1}, attributable to an increase in the intermolecular interactions, related to the conjugation. Lep_{20}-SLNs and blank SLNs displayed both high Caco-2 permeability confirming that the scaffold represents a useful tool to improve the crossing of drugs through biological membranes.

In conclusion, the surface-modified Lep_{20}-SLNs formulation has been successfully developed and thoroughly characterized. Lep_{20}-SLNs, which exhibit improved permeability, could represent a promising
option for selective CNS delivery. Future research will focus on loading Lep_{20}-SLNs with active compounds for the treatment of NDs.

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"*Understanding The Male Factors Affecting Intrauterine Insemination Outcomes: A Retrospective Descriptive Study*"

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Background: Among assisted reproductive techniques (ART), intrauterine insemination (IUI) is considered a first-line procedure for infertile couples. It is a cost-effective, non-invasive therapy indicated in cases of unexplained infertility, moderate male factor, functionally normal tubes, cervical dysfunction, anovulation, minimal endometriosis, immune causes, vaginismus, and erectile dysfunction. This study aimed to determine which potential prognostic factors could predict the success rate of IUI and therefore clinical pregnancy. Particular attention was given to semen parameters having a key role in the IUI technique. Female and male age, female BMI, IUI cycles number, infertility factors, endometrial thickness on the day of IUI, smoking habits of both sexes, and mainly pre-washed sperm quality were the variables analysed.

Methods: The present retrospective descriptive study was conducted by considering 721 IUI cycles with women aged ≤38, ranging from the first to the third attempt, which were performed from 2018 to 2023 in the Assisted Reproduction Centre of the "Gaetano Bernabeo" Hospital in Ortona (Italy). Regarding the analysis of semen parameters, we have considered both WHO guidelines 2010 and 2021 because there are different benchmarks among the years evaluated for the present study. From 2018 to 2020 we have adopted the reference values of WHO 2010 (sperm concentration ≥15 million/ml, sperm motility ≥32%, and sperm morphology >4%). From 2021 to 2023, however, we have considered the updated WHO guidelines of 2021 (sperm concentration ≥16 million/ml, sperm motility ≥30%, and sperm morphology >4%). T-test and chi-square test were performed to compare quantitative and qualitative variables, adopting a significance level of $p<0.05$

Results: The present study showed that the pregnancy rate following IUI treatment was 13.85% (100/721 cycles) and revealed a significant association between women's age and pregnancy outcomes ($p= 0.03$). Moreover, the current study demonstrated that the highest success rate was achieved after the first IUI attempt ($p= 0.04$), despite the literature states that there is a greater probability of success after several IUI tries. For what concern the analysis of semen parameters, sperm concentration (2018-2020) and sperm morphology (2018-2023) were found to be statistically significant. An unexpected finding was a non-significant association between sperm motility and pregnancy rate in fact, almost all pregnancies were achieved despite asthenospermic seminal fluids.

Conclusion: Although our study identified some statistically significant data for clinical pregnancies in IUI treatments, we were unable to find one specifically significant data indicating the success of IUI, especially regarding semen parameters. In this context, it would be interesting to perform further studies, particularly related to semen, to identify one or more parameters that may have an impact on IUI effectiveness.

Finanziato dall'Unione europea NextGenerationEU
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"Broad-spectrum rescue of mutant GPR179 secretion"

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Protein folding occurs in the Endoplasmic Reticulum (ER), where a quality control system ensures that only correctly folded proteins are released, while retaining the misfolded ones. However, in individuals with mutations in genes encoding secreted proteins, the ER Quality Control (ERQC) system can become overactive, retaining proteins that may still have residual function. These "responsive mutants" could potentially work if secreted, but the ERQC either retains them or degrades them, leading to severe diseases.
This project, funded by the Telethon Foundation, focuses on exploring the role of UGGT (UDP-glucose glucosyltransferase), that re-glucosylates misfolded proteins, preventing their secretion and ensuring proper folding. The research aims to investigate whether reducing the stringency of this quality control can restore the function of defective proteins. Specifically, the study targets TDark genes, genes with largely unknown functions, that meet three key criteria: being membrane glycoproteins, targeted by UGGT, and associated with rare hereditary diseases when mutated. Bioinformatics analysis and molecular modeling allowed to identify 10 key glycoproteins involved in various genetic disorders. Among them, we focused on the G-protein coupled receptor 179 (GPR179) that is crucial for signal transduction in depolarizing bipolar cells of the retina, and it is located at the postsynaptic membrane of ON-bipolar cells' dendritic tips, playing a key role in vision. Mutations at the GPR179 gene are associated with a rare genetic disease known as Congenital stationary night blindness (CSNB).
We observed different subcellular localization patterns of WT GPR179 in human cells. A centrosome-like localization of GPR179 was never observed before, thereby requiring experimental confirmation by immunofluorescence co-staining with known markers of the cellular centrosome (e.g. Arl13b and acetylated tubulin). We plan to evaluate whether the absence of UGGT1 or UGGT2 isoforms is sufficient to restore the secretion of the mutated GPR179 in the same subcellular structures observed for the wt molecule. The long-term goal of the project is to assess whether pharmacological inhibition of UGGT could represent a promising therapeutic strategy for a widespread rescue of the secretion of mutant glycoproteins in order to alleviate a diseased phenotype.

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"Study of the potential antitumor effects of OxxySlab on bladder cancer cell lines".

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Background: Bladder cancer (BC) is among the top ten cancers affecting men, and the incidence of BC is higher in men than in women. In 75% of cases, bladder cancer is diagnosed as confined to the mucosa (non-muscle invasive disease "NMIB"). In the 25–30% patients, BC has already invaded deeper layers of the bladder wall (MIBC—muscle-invasive disease) or formed metastasis. The main treatment for patients with NMIBC is transurethral resection (TURBT), while in patients with MIBC radical removal of the bladder (radical cystectomy RC) occurs. Typically, TURBT is complemented by intravesical instillations of Bacillus Calmette-Guérin (BCG) to prevent the recurrence and progression of BC. The incidence of metastatic bladder cancer increases with age and is more common in older people. The most common cause of BC death is associated with metastases. Since current therapeutic strategies have proven unsatisfactory, the investigation of alternative approaches such as those based on the use of a multi-strain probiotic formulation could provide promising therapeutic options. Probiotics may be useful as an adjuvant therapy to reduce the recurrence rate or increase the disease-free period after surgery. Furthermore, in vitro and in vivo studies have shown beneficial antitumor effects of selected probiotic strains, but until now, researchers are unable to establish the mechanisms by which they inhibited tumor growth.

Aim: In this study, we aimed to evaluate the antitumor effects of an OxxySlab lysate, a multi-strain probiotic formulation, on human BC cell lines at different stages of malignancy.

Materials and Methods: Human bladder carcinoma, muscle invasive (T24 and 5637) and non-muscle invasive (RT4), cell lines were exposed to different concentrations of probiotic lysate. The proliferation rate and cell number were measured using the IncuCyte®Live Cell Imager system and the trypan blue dye exclusion assay, respectively. Clonogenic potential and migration ability were also assessed.

Results: Exposure to probiotic lysate significantly reduced cell proliferation in T24, 5637 and RT4 cells. Migration was evaluated only on one muscle invasive BC cell line (T24) and one non-muscle invasive BC cell line (RT4) and a reduction in migration was observed in both cell lines. Furthermore, probiotic lysate treatment strongly decreased the clonogenic potential especially in muscle-invasive cell lines. Non-muscle invasive RT4 cells appeared less sensitive to lysate exposure, due to their more differentiated and superficial state.

Conclusion: Overall, these results highlight the ability of the probiotic formulation to impact key proliferative, migratory, and clonogenic properties of tumoral cells. Based on this data, it will be interesting to further evaluate the molecular mechanisms underlying OxxySlab's inhibitory effects on cellular growth. Experiments using 3D cell models have already been planned.

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"Sustainable Activation of Polyphenols from Pomegranate Waste: Developing Innovative Cosmetic Formulations"

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The valorization of agro-industrial by-products, such as pomegranate waste, offers environmental and economic benefits by reducing waste and recovering valuable bioactive compounds like phenolic acids and flavonoids [1]. Pomegranate peels and seeds are rich in antioxidants, with compounds such as ellagic acid promoting skin health by inhibiting tyrosinase and exhibiting anti-inflammatory effects making them beneficial for cosmetic applications [2]. Flavonoids, particularly aglycones like quercetin, demonstrate superior antioxidant and therapeutic activities compared to their glycosylated forms [3]. Recent advancements in fermentation techniques have shown promise in converting glycosides to more active aglycones, improving their absorption [4]. This process aligns with the increasing consumer demand for natural ingredients in cosmetic products, highlighting the potential of fermented plant extracts in cosmetic applications, especially for anti-aging and skin health [5]. This work develops a sustainable extraction procedure based on enzymatic sources to bio-convert glycosylated molecules into their aglyconic forms, enhancing biocompatibility, bioavailability, and skin penetration.
Ultrasonication and enzymatic fermentation were combined to obtain the final extract reached in bioactive molecules. The pomegranate peel, after being dispersed in appropriate solvent, was sonicated using Ultrasound-Assisted Extraction (UAE) and then, the extracts obtained were incubated with an enzymatic complex and kept at 37 °C in the incubator for 14 days.
All samples were then subjected to sonication, centrifugation, and subsequent analyses at two different time points, before the start of the fermentation process (G0) and after 14 days (G14) to monitor the conversion of glycosylated molecules in their aglycone form by High-Performance Liquid Chromatography (HPLC- DAD). The results allowed the identification and monitoring of the loss of signal over time of the loss of signal related to glycosylated molecules, cyanidin 3-glucoside and pelargonidin 3-glucoside, used as standards and present in peel P. Granatum extracts. The antioxidant capacity (DPPH, ABTS, FRAP assays) and total phenol content (Folin-Ciocalteu assay) were evaluated, and we detected an increase in the total phenol content in the fermented extracts at G14, differently than the unfermented ones at G0. The antioxidant capacity was higher in G14 fermented samples than in unfermented one G0. Cell metabolic activity and cytotoxicity on Human Gingival Fibroblasts (HGFs) were investigated and the results revealed that all tested sample doses appeared perfectly tolerable by HGF cells, as the cellularly vitality rate always exceeds 85% and it was also detected a statistical reduction in cell toxicity when treated with samples of fermented extracts. A facial emulsion containing a fermented extract was developed, characterized, and tested for eficacy in vivo on a sample of volunteers. The following skin conditions were evaluated: superficial and deep skin hydration, trans epidermal water loss (TEWL), wrinkles, redness, and texture. The cosmetic treatment resulted in a reduction of wrinkle and redness indices and an improvement of the skin texture uniformity. It was also noted an increase in both surface and deep skin hydration and a reduction in TEWL.
These promising results open new opportunities to develop functional products derived from fruit peels, contributing to the sustainable recovery of valuable food resources.

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"Study of the effects of a high fat diet on transcriptomic and epigenetic signatures in sperm obtained from obese men undergoing IVF"

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INTRODUCTION:
Obesity due to a HFD can have a negative impact on the reproductive potential, mainly due to altered semen parameters such as impaired sperm concentration, motility, morphology and DNA fragmentation. From a molecular point of view, there are several causal effects linked to obesity and low fertility: oxidative stress or ROS overproduction due to obesity has been shown to trigger epigenetic responses, such as abnormal sperm DNA methylation, histone modifications and modulation of small non-coding RNAs, such as miRNAs, associated with reduced male fertility and altered spermatogenesis in an In Vitro Fertilization (IVF) context.

AIM:
The aim of the project is to study the effect of the environment in terms of an excessive intake of non-esterified fatty acids (NEFAs) due to a high fat diet (HFD) lifestyle, on transcriptomic and epigenetic profiles in male reproductive cells of obese men compared to non-obese men undergoing protocols for Assisted Reproductive Technology (ART) purposes.

RESULTS:
Promoter methylation pyrosequencing analysis was carried out for 2 genes, *FTO* and *PEG1/MEST* in spermatozoa of 15 obese compared to 15 normal weight patients. 4 CpG sites were analysed for each gene. No statistically significant difference was evidenced between the mean methylation percentages of all 4 FTO promoter sites in the spermatozoa of the obese patients compared to the normal weight ones. No statistically significant difference was evidenced between the mean methylation percentages of all 4 PEG1/MEST sites in the spermatozoa of the obese patients compared to the normal weight ones. PEG1/MEST CpG 1 resulted significantly (p-value $< 0,05$) hypermethylated in the spermatozoa of obese patients compared to normal weight patients.

CONCLUSION AND PERSPECTIVES:
This project's data combined could provide valuable insight into the pathogenic relationship between nutritional metabolic diseases and infertility through omics and gene target investigations of potential molecular biomarkers that could be useful predictors of sperm competency, ultimately improving the efficiency of ART protocols by personalized treatments. The next experiments will investigate the methylation pattern of the H19 and IGF2 genes, important for embryonic development and imprinting, as well as the transcriptomic signature in spermatozoa of patients affected by obesity.

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"Novel Peptides s Agonists/Antagonists of FPR2 for the Treatment of Ulcerative Colitis"

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Academic Tutor: Prof. Adriano Mollica

The inflammatory bowel disease (IBD), Chron's disease and ulcerative colitis are among the idiopathic colorectal inflammatory disorders. As in other inflammatory processes, neutrophils migrate into inflamed tissue in response to chemotactic signals, stimulating various antimicrobial functions including ROS production, phagocytosis, and degranulation. The interruption of this process or the excessive recruitment of neutrophils can cause a dysregulation of the inflammatory process which hinders its resolution.
The Formyl Peptide Receptors (FPRs) are human G protein-coupled receptors located on neutrophils capable of binding specialized pro-resolving mediators and participating in the resolution process. The human FPR family constitutes FPR1, FPR2/ALX and FPR3.
The synthetic hexapeptide WKYMVm, an agonist of the FPR1 and FPR 2 receptors, showed a therapeutic effect against ulcerative colitis by blocking the decrease in body weight, colon shortening and mucosal destruction induced by DSS (in a model mice). In contrast, the FPR2-receptor was inhibited by the WRWWWW peptide, indicating the crucial role of this receptor in inflammatory pathology.
The aim of this study is to design and synthesize WKYMVm agonist/antagonist analogues, testing their binding capacity to the FPR2 receptor and their anti-inflammatory activity at different concentrations in vitro. Future development comprises their encapsulation in different liposomal formulations, investigation of their absorption and possible anti-inflammatory effects in a 2D CaCo2/HT29 and CaCo2/HT29-MTX culture model used in Crohn's disease study.
The novel synthesized peptides may represent useful therapeutic agents against chronic intestinal inflammation.

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"Targeting platelet hyperactivation in acute pulmonary inflammation in cystic fibrosis"

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Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CFTR gene. While CFTR modulators have significantly improved protein folding and function, chronic lung inflammation and recurrent infections remain the leading causes of mortality in CF patients. Platelets (PLTs) have emerged as key contributors to CF-related inflammation, exhibiting a hyperactive, pro-inflammatory phenotype that perpetuates chronic inflammation and hinders resolution. Despite this, the role of PLTs in CF is poorly understood, and treatments targeting PLT activation are lacking.

Thus, innovative approaches to diminish PLT activation could be useful to counter regulate CF inflammation, bacterial infection, and lung damage in CF. To this end, we tested the potential of resolvins (RvD), key proresolving lipid mediators (SPMs) that drive the resolution phase of inflammation, in targeting PLT hyperactivation and restoring an effective pro-resolution program. To address this, 2 specific aims were pursued:

1. Understanding PLTs' role in dysfunctional acute immune response in lung inflammation of CF disease by screening RvD bio-actions;
2. Testing the selected RvD ex vivo and in vivo as anti-PLT agents.

Initial findings indicate that platelet TxB2 levels, a marker of lung damage, remain unaltered in CF patients despite prolonged CFTR modulator therapy, suggesting that restoring CFTR function alone is insufficient to normalize platelet activity. Testing SPMs such as RvD3 and RvE1 revealed their ability to selectively reduce TxB2 levels in CF platelets activated by inflammatory stimuli, such as Pseudomonas aeruginosa, but not by hemostatic agents.

In vivo experiments in CFTR-deficient mice infected with P. aeruginosa demonstrated that RvD3 administration reduced platelet activation, bacterial load, and neutrophil infiltration in the lungs, as confirmed by histological analysis. These findings highlight RvD3 as a potential therapeutic agent for modulating the pro-inflammatory phenotype of CF platelets and promoting resolution of inflammation.

These findings collectively highlight that platelet activation plays a pivotal role in CF lung pathology, emphasizing the potential of RvD3 as a potent and effective therapeutic strategy to attenuate inflammation by promoting resolution.

Future research will focus on elucidating the effects of RvD3 on platelet-neutrophil interactions and exploring how shifting these interactions toward a pro-resolving state may enhance bacterial clearance. Furthermore, investigations will aim to provide mechanistic insights into the impact of RvD3 on these processes.

"A Green approach for The Extraction of Grape Pomace Oil: In Vitro and In Vivo Biological Evaluation"

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In the last years we have witnessed a gradual but slow transition from a linear economy model to a circular economy model, an economic system that promotes the development of a sustainable society. This new model leads to replacing the linear economy, reducing waste production, increasing the life cycle of a product so that it can be reused as much as possible[1]. In the past, grape pomace were used for the production of alcoholic beverages, today also as a soil improver, biogas production and used in combustion processes to produce energy[2]. In the nutraceutical sector, scientific studies show how grape pomace contains compounds with a powerful biological value and beneficial effects on the body. In particular polyphenols, a group of natural organic compounds present in many foods and which have numerous beneficial properties, including antioxidant and anti-inflammatory activity[3]. The aim of the work is to extract bioactive compounds in grape pomace, food waste product, using two different types of extractive solvents: green solvent (DES) and conventional organic solvent (n-Hexane) to obtain two extracts of grape pomace in oily composition: C1 and C2 respectively. Following an HPLC analyses for the phytochemical profile, biological in vitro and in vivo evaluation. The yield obtained through the extraction process is: 10% RE for sample C2 and 5,8% RE for sample C1. The analysis of the sample extracted by conventional technique shows a complex chromatographic profile rich in substances at 270 nm, confirming the excellent capacity of n-hexane as an extracting solvent for grape pomace oil, as compared to chromatographic profile of DES. Data show how C1 has a higher total phenolic and flavonoid content than C2, respectively: 18.65 mg GAE/g and 0.72 mg RE/g compared to 15.62 mg GAE/g and 0.13 mg RE/g. These results also explain how C1 has a higher antioxidant power than C2 in the tests: CUPRAC, FRAP, Phosphomolybdenum and chelating metals. Instead, C2 has a greater inhibitory enzymatic activity towards enzymes such as: glucosidase and butyrylcholinesterase. While C1 has a greater inhibitory activity towards tyrosinase and amylase. In biological assays, mice were pretreated with a dose of extract (100 μg) s.c. into the mice hind paw, 15 min before Zymosan s.c. injection in the same way and were evaluated the edema formation and hyperlagesic effects, before and 4 h after the Zymosan administration. The edema formation was significantly inhibited by C2 extract and C1 extract resulted ineffective. After Zymosan administration in the mice paw, a hyperalgesic effects was recorded as a reduction in nociceptive threshold to thermal stimuli. Extracts induced a reversion of the hyperalgesia induced by Zymosan, with the following order to magnitude: C2>C1. Then a Formalin test was conducted: administration of extracts at the dose of 100 μg
did not change the animals' behavioral response induced by Formalin in the early phase. Extracts induced a significant reduction in the licking time induced by the aldehyde in the late phase of the test, with the same order of magnitude as observed in the hyperalgesia experiments namely C2>C1. In conclusion, grape pomace oil extracted by DES, is rich in phenols and flavonoids, with strong antioxidant and enzyme inhibitory properties, partly superior to those obtained with n-hexane solvent. Both extracts show anti-nociceptive activity in in vivo assays. Future developments will focus

on improving the extraction efficiency of DES. Furthermore, it will be necessary to define the safety profile of the extract in order to outline a potential nutraceutical product.

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"Bioactive compounds (postbiotics) from waste by-products of microbial fermentation process, in fermented medium, as new functional ingredients "

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Recent advances in functional foods have highlighted the potential of bioactive compounds derived from microbial fermentation by-products. This study focuses on the characterization and optimization of post-biotics produced by Lactobacillus plantarum SGL 07 from industrial fermentation broths. Traditionally viewed as waste, these broths are utilized as a source of valuable post-biotic ingredients for applications in human, veterinary, and environmental health. The research demonstrates that targeted nutrient supplementation during fermentation can significantly enhance the production of bioactive compounds such as 3-phenyllactic acid (PhLA) and indole-3-lactic acid (ILA), which possess antimicrobial and anti-inflammatory properties. The findings suggest promising applications of these post-biotics in functional foods and propose future avenues for scaling production and broadening their therapeutic effects.

"The combination of hyaluronic acids and collagen boosts human Achilles tendon-derived cell escape from inflammation and matrix remodeling in vitro"

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In vitro preclinical studies showed how high and low molecular weight hyaluronic acids (HMWHA and LMWHA, respectively) contribute to tendon healing thanks to their mechanical support and anti-inflammatory properties. Considering the role of collagen in tendon and extracellular matrix composition, this study examines whether a potential synergistic effect of collagen and hyaluronic acids might be exploited to improve the treatment of tendinopathies. The anti-inflammatory activity was evaluated on human Achilles tendon-derived cells conditioned with the LPS-stimulated macrophage-derived medium (24 and 72 h) and on a co-culture model of tenocytes and inflamed macrophages (24 h). The *in vitro* collagen/HAs ratio was developed based on the formulation of a commercial food supplement named TendoGenIAL™. The modulation of markers related to the extracellular matrix (ECM) remodeling such as collagen type I was investigated to elicit the regenerative pathway. Data show that the combination of HMWHA and collagen reduces inflammation in macrophages by downregulating CD14. Additionally, this combination enhances the modulation of extracellular matrix proteins involved in tissue remodeling, such as CD44. Both high and low molecular weight hyaluronic acid with collagen counteract PGE_2 secretion in inflamed tenocytes. HMWHA is the best treatment in enhancing collagen type 1 secretion from tenocytes. Our data confirm the anti-inflammatory activity of both hyaluronic acids and collagen, highlighting a synergistic effect. Similarly, the association promotes tenocytes' proliferation and thus ECM remodeling.

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"Analysis of the physiology, dormancy and correlation to the antimicrobial resistance/tolerance in Helicobacter pylori; interaction with the commensal microbiota in the oro-gastric-intestinal tract. Innovative sustainable therapeutical formulation to reduce the antibiotic consumption in a One Health approach"

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Helicobacter pylori is a Gram-negative, microaerophilic bacterium that colonizes the gastric mucosa and is associated with various gastric diseases, including gastritis, peptic ulcers and gastric cancer[1]. The emergence of antibiotic resistance in *H. pylori* poses a significant obstacle to its infection eradication. The project aims to explore different therapeutic approaches to counteract this bacterium. The attention was focused on Resveratrol (RSV), a natural compound with anti-inflammatory and antimicrobial properties, which has shown promise as an alternative therapeutical agent but to enhance its bioavailability and activity, structural optimization is necessary due to its limited solubility in biological systems.[2]

Our investigation has focused on the possible explanations for the antibacterial activity, *fla*A and *mot*A gene expressions, and *H. pylori* membrane permeability/fluidity changes after treatment with RSV-derivatives. It was observed that treatment with the best RSV-derivatives had an inhibitory effect on *H. pylori*, with a reduction in bacterial viability, modulation of gene expression and changes in cell membrane fluidity and permeability.

These findings indicate that RSV-derivatives have an anti-*H. pylori* effect, which could be interesting candidates for innovative therapeutic approaches.

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This work was produced while attending the PhD programme in Biomolecular and Pharmaceutical Sciences at the University of "G. d'Annunzio" Chieti-Pescara, Chieti, Cycle XXXIX, with the support of a scholarship financed by the Ministerial Decree no. 118 of 2nd March 2023, based on the NRRP - funded by the European Union – Next Generation EU - Mission 4 "Education and Research", Component 1 "Enhancement of the offer of educational services: from nurseries to universities" - Investment 3.4 "Advanced teaching and university skills" and Investment 4.1 "Extension of the number of research doctorates and innovative doctorates for public administration and cultural heritage"

"Modulation of UGGT in the ER:a potential approach to address ciliopathies associated with TCTN3 mutation"

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Background: An important fraction of the proteome (mostly secreted glycoproteins) is directed to the Endoplasmic Reticulum (ER) for proper maturation. Here, the newly synthetized protein chain is guided to the secretory pathway by the ER lectin chaperon system. Proteins that are considered not correctly folded are sent back to reglucosylation by the UDP-glucose: glycoprotein glucosyltransferase 1 (UGGT1) and UDP-glucose: glycoprotein glucosyltransferase 2 (UGGT2), acting as a key gatekeeper in the folding pathway of the protein. It has been observed that individuals affected by congenital rare diseases carry missense mutations in genes encoding secreted glycoproteins that, despite being defective, can exhibit residual activity ("responsive mutants").
In this study, careful research and analysis led to the identification of TCTN3 as a possible UGGT 1/2 client. TCTN3 is a Tdark glycoprotein expressed by the tectonic family member-3 gene and localized in the transition zone complex (TZC). It has been hypothesized that TCTN3 plays a fundamental role in the ciliogenesis of the primary cilium (PC). Demonstrating its importance in the formation of PC, is that subjects affected by TCTN3 mutations suffer from serious forms of ciliopathies, such as Joubert syndrome. Secretion of TCTN3, in individuals with a mutation that partially affects protein functionality, can be rescued by a modulation of UGGT system, potentially restoring the normal phenotype.
To analyze this, we subcloned TCTN3 WT and its pathological variant in a mammalian expression vector, as fused to a green fluorescent protein. Then, we expressed these variant TCTN3 in mammalian cells, such as HEK-293, HeLa, MCF7 and HT29, and carried out systematic analysis of their respective localization by confocal microscopy. Of note, we were able to observe a sub-cellular localization of the wt molecule in proximity of the primary cilium. On the other hand, this pattern is largely lost when the pathological variant is expressed, and a diffuse cytoplasmic localization is observed. These findings support our hypothesis that acting on UGGT1/2 could help rescuing the localization, and possibly the activity, of mutant TCTN3 in the affected individuals.

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(ASSENTE)

"***Development of innovative closed systems (Isolator Technology) for the GMP manipulation of tissues of interest in regenerative medicine"***

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Regenerative Medicine includes a range of innovative approaches focused on replacing and repairing cells, tissues, and organs damaged by disease, trauma, or aging [(1)]. This field primarily employs two key strategies: stem cell-based therapies and the implantation of scaffolds or biomaterials, often pre-integrated with cells or growth factors. However, the most significant limitations are the lack of standardized protocols that adhere to Good Manufacturing Practices (GMP), and the requirement for complex management frameworks such as specialized "clean rooms"[(2)]. In this context, a compact closed system, known as isolator technology, designed to process human-derived cells and tissues under strictly sterile conditions (ISO5) [(3)], can be highly advantageous. In fact, this system reduces logistical, operational, and economic demands. The project's aim is to design a multifunctional isolator for the GMP manipulation of bone and cartilage tissue. Hence, a study involving osteoporotic elderly patients undergoing orthopedic surgery was carried out to obtain primary human osteoblast cell culture starting from bone fragments. Osteoblastic markers and mineral matrix deposition were evaluated by flow cytometry and Alzarin red Assay respectively. The results show the optimization of the procedure to permit the manipulation under Multifunctional Isolator, according to GMP, of human bone fragments to obtain osteoblast. Further studies will be focused on the design and development of Multifunctional Isolator prototype at Comecer S.p.A company.

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