IJAE

Italian Journal of Anatomy and Embryology

Official Organ of the Italian Society
of Anatomy and Histology

79° Congresso
della Società Italiana di Anatomia e Istologia
L'Aquila, 10-12 settembre 2026

79 TH Meeting
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Vol. 130
N. 1
2026
Supplement
ISSN 1122-6714

FIRENZE
UNIVERSITY
PRESS

IJAE
Italian Journal of Anatomy and Embryology
Official Organ of the Italian Society of Anatomy and Histology

Founded by Giulio Chiarugi in 1901

Journal e-mail: ijae@unifi.it – Web site: https://www.fupress.com/ijae

Firenze University Press
via Cittadella, 7
I-50144 Firenze, Italy
E-mail: journals@fupress.com
Available online at
https://www.fupress.com/ijae

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Italian Journal of Anatomy and Embryology
https://www.fupress.com/ijae
ISSN 1122-6714 (print) | ISSN 2038-5129 (online)

Direttore responsabile: **Ferdinando Paternostro, University of Firenze, Italy**
Editor in Chief: **Francesco Fazi, Sapienza University of Roma, Italy**

Published by Firenze University Press

Firenze University Press
Università degli Studi di Firenze
via Cittadella, 7, 50144 Firenze, Italy
www.fupress.com

Invited Lectures

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Visualizing the human body without a single cut

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The discovery of X-rays by Wilhelm Röntgen in 1895 marked the first time anatomists could visualize the interior of the human body without the use of surgical instruments. The introduction of the first clinical CT scan in 1971 represented another major milestone in medical imaging and is widely regarded as one of the greatest advances since the discovery of X-rays. By 2005, the development of four-dimensional (4D) CT technology enabled dynamic imaging of beating hearts, blood flow, kidney function, and respiratory motion, while also providing highly accurate anatomical measurements. This presentation reviews the evolution of computed tomography (CT) and other innovative imaging technologies that have transformed the way anatomists visualize, analyze, and manipulate anatomical structures. Topics include 2D, 3D, and 4D imaging, as well as macro-milling and micro-milling techniques for anatomical research and education. We will also demonstrate a range of visualization protocols, quantitative measurement tools, and surgical simulation applications. The presentation concludes with a brief introduction to synchrotron imaging and its applications in ultra-high-resolution anatomical and anthropological research. The primary goal is to demonstrate how these technologies have been integrated into our work, to share the methods and resources we have developed with the broader anatomical community, and to introduce several innovative educational courses that have successfully incorporated these advanced imaging techniques.

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https://med.stanford.edu/content/sm/anatomy-library.html/

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From the Roman city of Amiternum to the foundation of L'Aquila: archaeology, landscape and urban resilience through the centuries

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The history of L'Aquila represents one of the most significant examples of continuity and transformation of the urban landscape in central Italy. The city has its roots in the ancient Roman town of Amiternum, which experienced a gradual political and economic decline between Late Antiquity and the Early Middle Ages, accompanied by the reorganization of settlement patterns across the surrounding territory. Within this context, the medieval castle system developed, becoming the principal framework for territorial control and the organization of local communities.

During the thirteenth century these communities promoted the foundation of L'Aquila, an almost unique example of a medieval city established through the union of numerous surrounding castles, each contributing to the political, social and urban identity of the new municipality. Throughout the following centuries, the city underwent profound transformations caused by political, economic and seismic events, while demonstrating an extraordinary capacity for resilience that continuously reshaped its urban fabric without interrupting its historical continuity.

Drawing upon the results of recent archaeological investigations carried out at Amiternum, Ocre, Rocca Calascio, Preturo and other sites in the L'Aquila territory, this lecture will provide a concise overview of the evolution of the territory from the Roman period to the present day, highlighting archaeology as a key instrument for reconstructing collective memory and understanding long-term processes of urban and territorial transformation.

Keywords: Amiternum, L'Aquila, Medieval archaeology, castles, urban history, resilience.

Plenary Lectures

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Morpho-Functional Features of the Liver: From Structural Organisation to the Omics Sciences

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The liver is an amphicrine gland that, on the one hand, synthesizes and secretes bile, which is essential for lipid digestion and absorption, and, on the other, releases into the bloodstream the metabolites required for cellular homeostasis by transforming substances absorbed from the subdiaphragmatic digestive tract, spleen and pancreas into molecules indispensable for physiological metabolism. This dual function is made possible by the highly specialized microvascular organization of the liver, which receives blood from both the portal vein and the hepatic artery.

Contrary to the long-standing paradigm accepted for centuries, studies performed using scanning electron microscopy (SEM) of vascular corrosion casts have demonstrated that the terminal branches of the hepatic artery do not empty directly into the hepatic sinusoids. Instead, they give rise to the peribiliary and periportal capillary plexuses, from which small venules originate and subsequently drain into the hepatic sinusoids, indicating that the sinusoidal circulation is supplied exclusively by venous blood.

This highly specialized microvascular architecture is profoundly disrupted in liver cirrhosis, where regenerative nodules develop and exhibit, upon fractal analysis, reduced structural complexity and diminished efficiency of metabolic exchange, ultimately leading to the loss of the physiological porto-central zonation of the hepatic parenchyma.

Morphological and functional studies of cholangiocytes have led to the identification of two distinct cholangiocyte subpopulations— i.e small cholangiocytes and large cholangiocytes—which differ in their morphology, location, functions and neuroendocrine properties. These studies have also demonstrated the expression of estrogen receptors and vascular endothelial growth factor receptors, both of which play a pivotal role in regulating cholangiocyte proliferation and adaptive responses during biliary injury.

Particular attention has also been devoted to the hepatic stem/progenitor cell compartment, comprising bipotential hepatic progenitor cells (HPCs) located within the Canals of Hering and multipotent stem cells residing in the peribiliary glands, which retain the capacity to differentiate into hepatocytes, cholangiocytes, and endocrine pancreatic cells. Under physiological conditions, hepatic homeostasis and cellular turnover are maintained by mature parenchymal cells, whereas activation of the stem/progenitor compartment occurs only in response to severe acute liver injury, such as fulminant hepatitis, or chronic liver diseases of prolonged duration, including cirrhosis and chronic cholangiopathies. More recent investigations have further demonstrated the involvement of HPCs in the pathogenesis of highly prevalent disorders such as metabolic dysfunction-associated steatotic liver disease (MASLD).

Finally, studies investigating the neoplastic transformation of cholangiocytes and the pathogenesis of cholangiocarcinoma (CCA) through an integrated multimodal approach—including quantitative morphological assesment, computed tomography (CT), radiomics, and spatial transcriptomics—have enabled the identification of distinct morphologic and clinicopathological CCA subtypes. These subtypes are characterized by different degrees of biological aggressiveness, molecular profile, clinical outcomes, prognostic profiles, and potential therapeutic vulnerabilities, thereby providing the basis for increasingly personalized diagnostic and therapeutic strategies.

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Osteoclasts, the Hematopoietic Stem Cell Niche, and Gene Therapy: New Perspectives in Pathological Bone Remodeling

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Osteopetrosis comprises a heterogeneous group of inherited disorders characterized by impaired osteoclast function1,2,3. TCIRG1-related infantile malignant osteopetrosis is the most common form of autosomal recessive osteopetrosis (ARO), accounting for approximately 58% of cases. It belongs to the group of osteoclast-rich osteopetroses, in which normal or increased numbers of osteoclasts are unable to resorb bone owing to defects in the osteoclast-specific subunit of the vacuolar proton pump encoded by *TCIRG1*. Patients typically present during the first months of life with severe bone marrow failure, hepatosplenomegaly, anemia, thrombocytopenia, and optic nerve compression. The identification of *TCIRG1* mutations represented a major turning point in the understanding and management of osteopetrosis, revealing the molecular and cellular heterogeneity of the disease and enabling genotype-driven therapeutic approaches. Hematopoietic stem cell transplantation remains the only curative treatment and, since its first successful application in the early 1980s, has established the hematopoietic origin of the disease and paved the way for the development of innovative therapies, including gene therapy4,5.Interestingly, the progressive reduction of bone marrow space and extensive marrow fibrosis that characterize the disease promote the mobilization of large numbers of hematopoietic stem and progenitor cells (HSPCs) into the peripheral blood, particularly in young patients with severe disease. These spontaneously circulating HSPCs display a composition more closely resembling pediatric bone marrow than healthy peripheral blood, being enriched in primitive progenitors and containing bone marrow-restricted populations. Here, we define the molecular and functional landscape of these circulating HSPCs and demonstrate that they can be efficiently genetically engineered and expanded ex vivo to generate a clinically relevant autologous cell product for gene therapy. These findings provide the biological rationale for exploiting circulating HSPCs as a readily accessible source of stem cells and support the development of innovative gene therapy approaches for TCIRG1-related osteopetrosis.

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Abstract

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Cytoskeletal dynamics inhibition as a strategy to improve KRAS inhibitor response in PDAC

Annalisa Pia Abbinantefina[1], Claudia Tito[1], Michela Coiro[1], Dionino Marco Giangrande[2], Maurilio Sampaolesi[1,3], Valerio Fulci[2], Silvia Masciarelli[1], Gianni Colotti[4,] Francesco Fazi[5]

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Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of pancreatic cancer cases and remains a leading cause of cancer-related mortality, with a 5-year survival rate of approximately 13%. One of the PDAC hallmark is the presence of oncogenic KRAS mutations, with $KRAS^{G12D}$ being the most prevalent allele (~45% of cases) [1]. Although MRTX1133 is a highly potent and selective $KRAS^{G12D}$ inhibitor in preclinical models [2], several studies have shown that KRAS inhibition rapidly triggers compensatory signaling pathways that restore downstream oncogenic signaling, thereby limiting its long-term therapeutic efficacy [3]. Interestingly, our preliminary microscopic analyses in $KRAS^{G12D}$ PDAC cell lines revealed that MRTX1133 treatment induces cytoskeletal remodeling, according with the well-established role of KRAS in regulating signaling pathways controlling actin cytoskeleton organization, including RHO signaling. Based on these data, we aimed to investigate if targeting ROCK, an effector of the RAS/Rho pathway, could enhance therapeutic sensitivity of PDAC cells line to treatment with MRTX1133. We showed that the combination of MRTX1133 and ROCK inhibitor Y-27632 significantly decreased cell proliferation. Additionally, KRAS-mutant PDAC cells rely on DRP1-driven mitochondrial fission to sustain OXPHOS and redox balance, while ROCK acts as a central regulator of mitochondrial dynamics by promoting DRP1 activation and actin-dependent recruitment, thereby linking RhoA/RAS signaling to metabolic alteration and redox imbalance. [4,5] Consistently, MitoTracker analyses revealed that the dual inhibition of RAS and ROCK shifted mitochondrial morphology from a fragmented network toward elongated and fused structures. Functionally, this morphological change is associated with a marked, time-dependent ROS accumulation and a significant reduction in NRF2 expression, indicating a collapse of antioxidant defenses and redox homeostasis. Accordingly, proteomic analyses revealed a compensatory metabolic rewiring toward fatty acid oxidation under combined treatment. Overall, our findings identify ROCK-dependent cytoskeletal remodeling as a central regulator of mitochondrial stress adaptation in MRTX1133-treated $KRAS^{G12D}$ PDAC cell line and demonstrate that cytoskeletal–mitochondrial regulation is a critical determinant of sensitivity to KRAS inhibition, supporting ROCK co-targeting as a mechanistically realistic therapeutic strategy.

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Keywords: PDAC, Combination therapy, Cytoskeletal remodeling, Mitochondrial dynamics, Redox homeostasis.

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In vitro analysis of breast cancer associated fibroblasts phenotypic plasticity

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Breast cancer-associated fibroblasts (BCAFs) are major stromal components of tumor microenvironment (TME) and contribute to tumor progression through interactions with cancer cells [1, 2, 3]. This study investigated the phenotypic plasticity of primary BCAFs in response to different culture conditions and its impact on breast cancer (BC) cell growth in vitro. In our previous study, we demonstrated the phenotypic plasticity of primary human BCAFs (ML BCAFs) in vitro [4]. Specifically, ML BCAFs cultured as spheroids and subsequently reverted to adherent growth (Rev BCAFs) after 216 h of three-dimensional (3D) culture displayed an inactivated phenotype. This phenotype was characterized by reduced α-smooth muscle actin (α-SMA) expression and a shift from a pro-tumorigenic state to one less supportive of BC cell growth in vitro [4]. Building on these findings, we performed a more comprehensive characterization of ML BCAFs, Rev BCAFs, and their corresponding immortalized cell lines generated by SV40-mediated immortalization (ML BCAFs-SV40 and Rev BCAFs-SV40). To this end, we analyzed transcriptomic and secretomic profiles using RNA sequencing coupled with Gene Set Enrichment Analysis (GSEA) and ELISA-based assessment of conditioned media, respectively. Transcriptomic analysis revealed downregulation of genes associated with TGF-β and KRAS signaling in Rev BCAFs and Rev BCAFs-SV40 compared with ML BCAFs and ML BCAFs-SV40, respectively. Conversely, genes involved in reactive oxygen species-related pathways were upregulated in Rev BCAFs and Rev BCAFs-SV40. ELISA analysis revealed significant differences in the secretion of several cytokines and growth factors between conditioned media from Rev BCAFs/Rev BCAFs-SV40 and ML BCAFs/ML BCAFs-SV40. These transcriptomic and secretomic changes were associated with a reduced pro-tumorigenic effect in vitro of Rev BCAFs and Rev BCAFs-SV40 compared with their ML counterparts. Collectively, these findings indicate that BCAFs are not irreversibly committed to a pro-tumorigenic phenotype but can revert to a state that is less supportive of BC cell growth in vitro. Furthermore, identifying genes, signaling pathways, and secreted factors involved in BCAF phenotypic plasticity may contribute to the development of novel therapeutic strategies targeting the tumor stroma.

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Keywords: breast cancer, tumor microenvironment, breast cancer associated fibroblasts.

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The spatial heterogeneity of histopathological and ultrastructural features in rotator cuff lesion-associated long head biceps tendinopathy

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Despite extensive investigation, the morphological alterations underlying tendinopathy remain poorly characterized[1,2,3]. This study aimed to investigate the spatial distribution of histopathological and ultrastructural alterations in long head of the biceps tendon (LHBT) tendinopathy associated with rotator cuff (RC) tears, and to correlate these findings with clinical parameters. Twenty-three pathological LHBT samples were collected from patients undergoing arthroscopic surgery for concomitant RC tear repair. Each sample was divided into two regions: proximal, corresponding to the intra-articular portion, and distal, corresponding to the extra-articular portion. Histopathological alterations were assessed using parameters included in the semi-quantitative Bonar score: tenocyte morphological changes on haematoxylin and eosin staining, collagen bundle disorganization on Masson's trichrome staining, and increased ground substance deposition on Alcian blue staining. Ultrastructural assessment of collagen fibrils was performed by electron microscopy in two representative samples with different Bonar scores. The study identified a compartment-specific pattern of degeneration, with significant differences between proximal and distal tendon regions in total Bonar score ($p<0.001$), tenocyte cellularity ($p<0.001$), and matrix organization ($p<0.01$). Among the evaluated parameters, the tenocyte cellular response showed the strongest association with RC lesion severity ($p<0.05$). Ultrastructural observations confirmed different degrees of collagen fibril disorganization and ground substance deposition between tendon regions with distinct histopathological degeneration grades. In particular, tendons showing higher histopathological degeneration displayed a more heterogeneous and disorganized collagen fibril diameter distribution, supporting the presence of ongoing tendinopathic remodeling. Overall, these findings suggest that LHBT tendinopathy associated with RC tears is characterized by spatially distinct biological processes of failed repair, adaptation, and degeneration, with degenerative changes predominantly localized in the intra-articular portion of the tendon. The combined use of histological and ultrastructural approaches may therefore provide a more comprehensive assessment of region-specific degenerative patterns in tendinopathy.

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Keywords: tendinopathy, rotator cuff, ECM.

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Reversing the Cascade: NCX2 Inhibition Safeguards Axons Against Oxaliplatin-Induced Neurotoxicity

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Oxaliplatin (OHP) remains a primary chemotherapeutic agent for colorectal cancer, yet its clinical utility is severely restricted by OHP-induced peripheral neurotoxicity (OIPN). This condition manifests in two distinct syndromes: acute, transient axonal hyperexcitability and chronic, persistent axonal damage (AxD). We hypothesized a mechanistic link between these, positing that OHP causes voltage-gated sodium channel (NaV) dysfunction thus elevating intracellular Na^+, which forces the Sodium-Calcium Exchanger (NCX) into reverse mode, driving toxic Ca^{2+} accumulation and subsequent AxD. To test this, we utilized primary mouse dorsal root ganglia (DRG) cultures and 3D live-cell imaging to evaluate NCX's role in neurodegeneration. Sustained OHP exposure (7.5 uM for 48 hours) induced severe neurotoxicity, marked by pronounced neurite fragmentation, autophagic stress, and necroptosis. To intercept this pathway, we evaluated both pharmacological inhibition using the selective NCX inhibitor SEA0400 (1uM for 3 hours pretreatment) and genetic knockdown via siRNAs (5nM for 24 hours) targeting the highly expressed NCX2 isoform. Notably, both pharmacological blockade with SEA0400 and specific siRNA-mediated downregulation of NCX2 strongly mitigated these pathological alterations, effectively preserving neurite integrity and preventing activation of cell death pathways. Our findings demonstrate that the NCX family, particularly the NCX2 isoform, acts as a critical mediator of OHP-induced axonal degeneration. Consequently, targeting NCX2 offers a promising therapeutic strategy to prevent OIPN, with potential broader applicability to other neuropathic conditions.

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Keywords: oxaliplatin neuropathy, oxaliplatin neurotoxicity, axonal damage, NCX2, peripheral neuropathy.

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Development of a new three-dimensional Bone Marrow construct as Integrated Platforms for Regenerative Medicine Applications

Francesca Arciprete[1], Viola Velardi[1], Joseph Lovecchio[2], Antonio Menini[3], Clotilde Castaldo[3], Franca di Meglio [3], Giovanna Della Porta[4], Vincenzo Piemonte[5], Maria Zingariello[1]

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The bone marrow (BM) is a unique microenvironment, able to promote and support hematopoiesis through the coordinated activity of endosteal and vascular niches. Within this complex and heterogenous system, cellular interactions, extracellular matrix cues, biochemical gradients, and mechanical stimuli, regulate hematopoietic stem and progenitor cell fate [1]. Reproducing the dynamic complexity of the BM microenvironment *in vitro* remains a major challenge [2]. Recent advances in biomaterials and nanotechnology [3] offer new opportunities to engineer modular platforms capable of mimicking key features of the BM niche. However, most existing approaches focus on isolated components of the system and lack the level of integration required for functional maturation. Here, we present preliminary experimental results on a three-dimensional (3D) construct designed to replicate the key features of the endosteal bone marrow niche, engineered through polymeric hydrogel scaffold functionalized with decellularized trabecular bone and growth factor–loaded PLGA nanoscarriers (NCs). Specifically, decellularization and preservation of native extracellular matrix composition on decellularized and decalcified trabecular bone samples were morphologically validated. Rhodamine positive PLGA NCs were loaded with bone morphogenic growth factors type 2, enabling localized and sustained delivery of bioactive cues. A polymeric hydrogel was used to integrate the two components. To obtain a 3D BM endosteal niche, the resulting platform was thus cultured with MC3T3-E1 cells under conventional conditions to evaluate cell adhesion, spatial distribution and short-term viability. A perfusion bioreactor was used to mimic the specific biological conditions environment. Cell proliferation and adhesion are observed up to 15 days of in vitro culture. Serial sections of 30μm thickness were analyzed to verify that the engineered microenvironment supports cell survival and early differentiation of cultured cells and β-Catenin expression was used to validate the cell engraftment. These findings provide a proof-of-concept for the use of this approach as a modular building block for more complex 3D BM tissue engineered constructs, supporting the development of promising platforms that bridge fundamental hematopoietic biology and regenerative clinical applications.

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Keywords: bone marrow Niche, hematopoiesis, 3D Scaffolds, grow factor delivery.

IJAE
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Targeting Muscle Dysfunction in Myotonic Dystrophy Type 1 Through Combined Modulation of Myogenesis and Membrane Excitability by Resveratrol and Low-Dose Diazoxide

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Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy and is characterized by progressive skeletal muscle weakness, myotonia, impaired muscle regeneration, and multisystem involvement [1]. Despite significant advances in understanding the molecular mechanisms underlying DM1, effective therapies capable of improving muscle function remain limited. Emerging evidence suggests that both defective myogenesis and altered membrane excitability contribute substantially to disease progression [2, 3], highlighting the need for therapeutic strategies targeting multiple pathogenic mechanisms simultaneously.

Based on this rationale, therapeutic strategies simultaneously targeting membrane excitability and impaired myogenesis may offer greater benefit than approaches acting on a single pathogenic mechanism. Diazoxide, a potassium channel opener already approved for other clinical indications [4], has been proposed to stabilize muscle membrane excitability and reduce myotonia, whereas resveratrol, a natural polyphenolic compound with pleiotropic biological activities [5], was investigated for its ability to modulate muscle differentiation and calcium homeostasis.

Preliminary in vitro studies demonstrated that resveratrol promotes skeletal muscle differentiation by enhancing myotube formation, increasing the expression of key myogenic markers (MyoD, myogenin, and myosin heavy chain), and improving myoblast fusion and maturation, as evidenced by higher myotube and fusion indices. To investigate the mechanisms underlying these effects, calcium homeostasis was evaluated in differentiating myotubes. Electrophysiological recordings and calcium imaging revealed that resveratrol significantly reduced L-type calcium currents and intracellular calcium transients. These findings suggest that resveratrol modulates calcium-dependent pathways involved in myogenesis and membrane excitability, thereby supporting the formation of more mature and functionally stable muscle fibers.

Building on these preliminary findings, future studies will extend the investigation to patient-derived cellular models of DM1 to evaluate the combined effects of resveratrol and diazoxide on muscle differentiation, calcium homeostasis, membrane excitability, and disease-associated molecular alterations. The translational potential of this approach will subsequently be evaluated in preclinical animal models and, ultimately, in early-phase clinical studies aimed at exploring its safety and therapeutic efficacy in individuals affected by DM1.

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Keywords: Myotonic dystrophy type 1, Skeletal muscle differentiation, Resveratrolo, Diazoxide, Translational medicine.

IJAE
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Angelo Ruffini: neuroanatomist of embryos and receptors

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Angelo Ruffini (1864–1929) was an Italian physician, anatomist, and embryologist best known for discovering the mechanoreceptors now called Ruffini corpuscles and for his studies on embryological development. After gradu-ating from the University of Bologna, he pursued research and teaching in histology, embryology, and anatomy while also working as a hospital director to support his family. Ruffini collaborated with leading scientists of his time, in-cluding Sir Charles Sherrington, and received important scientific recognition. His research significantly advanced the understanding of sensory nerve endings, neuromuscular spindles, and embryonic gastrulation. In his 1925 treatise Fis-iogenia (1), he described embryological development and introduced the concept of stichotropismus. He also developed innovative histological staining and microscopy techniques. Ruffini became famous for his contribution to the first description of small, encapsulated nerve endings by use of gold chloride staining. These mechanoreceptors are now known as Ruffini corpuscles. Some histological slides that Ruffini used to describe with great accuracy both the corpuscles as well as other findings were in possession of Isabella Galliani, a professor at the University of Bologna, who passed away in 2019. Galliani gave the slides to one of us (PG), and we have been able to analyse Ruffini's orig-inal histological work from 1892. Although not widely recognized outside Italy during his lifetime, Ruffini's contribu-tions remain fundamental to neuroanatomy, neuroscience, and developmental physiology (2).

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Keywords: Angelo Ruffini, Ruffini Corpuscles, Gold Chloride Staining.

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Caffeic Acid Phenethyl Ester (CAPE) Derivatives as Promising Anticancer Agents

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Background

Caffeic acid phenethyl ester (CAPE) is a bioactive compound known for its anticancer properties [1]. Structural modifications of CAPE may further enhance its biological activity. In this study, a series of CAPE derivatives were synthesized [2] and evaluated for their antiproliferative and stability-related effects in prostate (PC3) and breast (MCF7) cancer cell lines.

Methods

Cell viability was assessed using crystal violet assays in 2D cultures after 24 and 48 h exposure to twelve synthesized derivatives (1 a-f and 2 a-f). Caffeic acid (CA) and CAPE were used as reference compounds. The most active molecules (1a and 1d) were further analyzed for their effects on clonogenic survival, cell migration, apoptosis, and cell cycle distribution. In addition, the selected compounds were evaluated in 3D spheroid models of PC3 cells.

Results

CAPE and several derivatives exhibited significant antiproliferative effects, with compounds 1a and 1d showing the highest efficacy in both PC3 and MCF7 cell lines. Clonogenic assay revealed a complete inhibition of colony formation following treatment with CAPE, 1a, and 1d in both cell lines. Among the tested compounds, CAPE showed the strongest inhibitory effect on cell migration after 48 h, followed by 1a whereas 1d displayed a weaker effect. Apoptosis analysis revealed a significant induction of apoptotic cell death, while cell cycle analysis revealed a reduction in the G0/G1 phase and a significant accumulation of cells in the S and G2/M phases. Notably, in 3D spheroids a different cellular response compared to 2D cultures was found.

Conclusions

These findings demonstrate that CAPE derivatives, particularly 1a and 1d, exert strong antiproliferative effects by inhibiting colony formation, reducing cell migration, inducing apoptosis, and altering cell cycle progression in cancer cells. The observed differences between 2D and 3D models highlight the importance of tumor architectural complexity in modulating drug response. Overall, these derivatives represent promising candidates for anticancer development; however, their application in 3D models may require optimized formulation or delivery strategies.

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Keywords: CAPE derivatives, anticancer activity, prostate cancer, breast cancer, drug response.

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A bioinformatic and fuzzy logic modelling of the desmin/myo-inositol interactome for bone anabolism and its translational relevance.

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Objective: To investigate the protein-protein interaction network or interactome of the cytoskeletal filament desmin (DES) during osteogenesis by multipotent mesenchymal stromal cells (MSCs) (1), and the role of the insulin-mimetic myo-inositol (MYO) in this signalling (2, 3). Mat Meth: A knowledge-guided, *in silico* DES-MYO interactome was obtained using the STRING v12 and STITCH v5 libraries, and topologically analyzed with the Gephi software. Consistency between this interactomic knowledge and data-based interactions of DES with cytoskeletal proteins/osteogenic enzymes/transcription factors±MYO was evaluated through AI-based, fuzzy logic principles. *In vitro* studies were pursued using embryonic NIH 3T3 cells (courtesy of Prof. Lucio Cocco), and human primary MSCs. DES and β-actin were identified by immunocytochemistry (IC) and/or western blotting (WB) using rabbit polyclonal and mouse monoclonal Abs (IC 1:50-1:800; WB 1:1000-1:5000), Fast Red-Alkaline Phosphatase (AP), ABC/DAB, chemiluminescence, and WB densitometry. Osteogenesis (2.5-100x10^3cc/monolayer) was analyzed between 48h-28 days, MYO administered in a 320-640 μM range, and mineralization evaluated by calcium spectrophotometry. An *in vivo* paraplegic patient with tibial osteomyelitis was treated with MYO (3 gr/day x 3 months), and bone growth evaluated by grey-level densitometry on X-ray films. Correlation between DES and β-actin, and differences in bone density (lesioned tibia vs talus, calcaneus, navicular) were analyzed by Pearson's r coefficient and one-way ANOVA, respectively (significance if $p < 0.05$). Results: *In silico* analysis showed that AKT and β-actin are "hubs" for the cytoskeletal signals downstream of DES (Vim, Vinc, Cofilin, Hspb1, Rhoa). These signals travel to RUNX2 and AP, through GSK3β. In turn, GSK3β is a "bottleneck" towards the YAP/TAZ-SMAD osteogenic network. Predicted AKT stimulation by MYO was supported by *in vitro* evidence of reciprocal fluctuations in DES and β-actin during basal and MYO-enhanced mineralization, and *in vivo* increased bone density in a disused and lesioned tibia. Conclusions: DES represents a new and early signal in bone anabolism. Enhancement of reciprocal fluctuations of DES and β-actin by MYO suggests that, though it bypasses the DES signal, its response involves the same cytoskeletal pathway. Bone regeneration in the tibia of an immobilized patient raises the possibility that MYO triggers mechanotransduction in the absence of mechanical forces, as during disuse bone loss for fracture, spine paralysis, and microgravity. Grant MYOCAL IRCCS-IOR 2026.

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Keywords: desmin, β-actin, myo-inositol, osteogenesis.

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Decoding Monocyte Immune Signatures in COVID-19: A Single-Cell Multi-Omics Comparison of Natural Infection and Vaccination

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Monocytes are key regulators of innate immunity and play a central role in the inflammatory response to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). However, a comprehensive characterization of monocyte heterogeneity across different COVID-19 immunological conditions remains limited. This study aimed to investigate monocyte phenotypic and transcriptional profiles in SARS-CoV-2-naïve, convalescent, and vaccinated individuals using a targeted single-cell multi-omics approach [1].

Peripheral blood samples were collected from nine adult subjects divided into 3 study groups: (1) non-infected subjects (*Naïve group*, n = 3), (2) post COVID-19 convalescent subjects (*Healed group*, n = 3) and (3) patients that were vaccinated against SARS-CoV-2 (*Vaccine group*, n = 3). Monocytes were enriched by magnetic separation and analysed using the BD Rhapsody™ platform, integrating targeted single-cell RNA sequencing and AbSeq surface proteomics. Unsupervised clustering, differential gene/protein expression analysis, and pathway enrichment analyses were performed.

Three monocyte clusters were initially identified, two clusters corresponding to classical monocytes, while one representing non-classical monocytes. Vaccinated subjects exhibited a significant reduction in non-classical monocytes compared with naïve individuals (8.9% vs. 16.7%; $p < 0.0001$), although considerable inter-individual variability was observed. Differential expression analyses identified 38, 56, and 45 genes in the *Healed vs. Naïve*, *Vaccine vs. Naïve*, and *Vaccine vs. Healed* comparisons, respectively. Transcriptomic profiles remained highly concordant between classical and non-classical subsets, suggesting that global differences were not driven by subset abundance. Stratification according to antibody responses identified 40 differentially expressed genes and enriched biological pathways related to immune activation and intracellular organelle organization, highlighting molecular signatures associated with protective immunity.

This study provides a high-resolution characterization of monocyte heterogeneity across distinct COVID-19 immune states. The integration of transcriptomic and proteomic single-cell data identifies antibody-associated monocyte signatures and reveals considerable donor-specific variability, offering novel insights into innate immune mechanisms underlying natural infection and vaccine-induced immunity.

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Keywords: Monocytes, COVID-19, single-cell multi-omics, SARS-CoV-2 vaccination, innate immunity.

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Engineering the Osteochondral Interface: Bio-Hybrid Scaffolds Tailored for Cartilage and Subchondral Bone Regeneration

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Osteochondral defects associated with osteoarthritis represent a major clinical challenge due to the poor intrinsic regenerative capacity of the osteochondral unit [1]. Tissue engineering offers a promising strategy to restore joint function through the use of biomimetic scaffolds capable of supporting coordinated regeneration of cartilage and subchondral bone [2]. In this context, this study aimed to develop and characterize novel bio-hybrid scaffolds based on oxidized polyvinyl alcohol (OxPVA) combined with two clinically established biological matrices: the collagen membrane Bio-Gide® and the deproteinized bovine bone mineral Bio-Oss®. Two scaffold configurations were fabricated: a bilayer structure consisting of an OxPVA support layered with the collagen membrane (OxPVA/Col_bilayer), and a blend structure incorporating bone granules directly into the OxPVA hydrogel (OxPVA/Bone_blend). Scaffolds were cross-linked via freeze-thawing cycles and characterized for ultrastructure (scanning electron microscopy, histology), mechanical properties (compression tests), permeability (FRAP), and in vitro biocompatibility using HM1-SV40 mesenchymal stromal cells. Results showed that OxPVA/Col_bilayer scaffolds exhibited a highly porous, fibrillar collagen surface that significantly enhanced cell adhesion and proliferation. After 7 days, cells formed a confluent monolayer with extensive cytoplasmic extensions. In contrast, OxPVA/Bone_blend scaffolds displayed a denser microstructure with reduced porosity; cells remained viable but preferentially formed spheroidal aggregates around exposed bone granules. These differences are ascribable to the distinct accessibility of bioactive cues due to the specific configuration. Importantly, all scaffold formulations were non-cytotoxic, preserving cell viability above the 70% ISO threshold.

Mechanical testing demonstrated that bilayer scaffolds possess lower compressive stiffness, suitable for cartilage-mimicking applications, while blend scaffolds show significantly higher stiffness, appropriate for subchondral bone support. FRAP analysis indicated improved molecular diffusivity in all bio-hybrid constructs compared to OxPVA alone, suggesting that the integration of biological matrices into the hydrogel not only enhances polymer bioactivity but also positively modulates scaffold permeability.

In conclusion, the scaffold architecture and spatial distribution of bioactive components critically regulate structural, mechanical, and biological performance, supporting the potential of OxPVA-based bio-hybrid scaffolds for stratified osteochondral tissue regeneration.

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Keywords: Bio-hybrid scaffolds, oxidized PVA, natural matrices, osteochondral regeneration, tissue engineering, osteoarthritis.

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Granins as Neuroprotective Agents in Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta, associated with the accumulation of misfolded α-synuclein aggregates [1]. Granins are a class of acidic secretory proteins associated with dense-core secretory vesicles, expressed in neuronal cells where they play key roles in the biosynthesis of neurotransmitters [2]. Since they have been suggested to act as molecules that stabilize neuronal stress, in this study we aim to investigate the potential neuroprotective role of granins using an established *in vitro* model of PD. Dopaminergic-like SH-SY5Y cells were exposed to rotenone toxicity to reproduce the pathological features of PD, including the aggregation of α-synuclein. To evaluate the potential neuroprotective activity, one granin, selected based on a previous screening, was administered to cells 24 hours prior to rotenone exposure. In the presence and absence of granin treatment, cell viability was assessed using the Alamar blue assay, while quantitative polymerase chain reaction (qPCR) was performed to identify changes in PD-related, inflammatory, and stress markers. Pre-treatment with the selected granin significantly attenuated rotenone-induced cytotoxicity, preserving neuronal morphology and resulting in a ~25% increase in cell viability compared with cells treated exclusively with rotenone (p = 0.0001). At the molecular level, qPCR analyses revealed that rotenone exposure significantly increased the expression of α-synuclein (p = 0.0001), nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) (p = 0.0001), and nuclear factor kappa B (NF-κB) (p = 0.0011), while downregulating tyrosine hydroxylase (TH) expression (p = 0.0063). Pre-treatment with the selected granin counteracted these alterations, limiting α-synuclein accumulation (p = 0.0001) and the induction of oxidative stress and inflammatory markers (NOX4 and NF-κB, p = 0.0001 and p = 0.0012, respectively) while preserving TH expression levels (p = 0.0032). Overall, these findings suggest a neuroprotective role of the selected granin in PD models, highlighting its therapeutic potential to modulate disease-related pathways. Further studies will be conducted to explore its translational relevance in *in vivo* animal models.

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Keywords: Parkinson's Disease (PD), SH-SY5Y cells, rotenone, granins, α-synuclein, neuroprotection.

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Curcuma caesia Counteracts Doxorubicin-Induced Cardiac Injury by Reducing Oxidative Stress and Apoptotic Signaling

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The remarkable improvement in cancer survival achieved over recent decades has highlighted the importance of managing the long-term adverse effects of anticancer therapies. Among these, cardiovascular toxicity has emerged as a major cause of morbidity and mortality in cancer survivors[1]. Doxorubicin (DOX), one of the most effective and widely used anthracycline chemotherapeutic agents, is associated with dose-dependent cardiotoxicity characterized by oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis, which may ultimately lead to irreversible cardiac damage and heart failure[2].

Growing interest has focused on natural bioactive compounds as potential cardioprotective agents capable of mitigating chemotherapy-induced toxicity. Curcuma caesia Roxb. (black turmeric) is a medicinal plant rich in antioxidant and anti-inflammatory phytochemicals, although its protective role against DOX-induced cardiotoxicity remains poorly investigated[3,4].

In the present study, we investigated the protective effects of Curcuma caesia extract against DOX-induced toxicity in H9c2 rat cardiomyoblasts. Exposure to DOX markedly increased oxidative stress and apoptotic cell death, confirming its detrimental effects on cardiac cells. Pre-treatment with Curcuma caesia significantly attenuated these alterations, reducing intracellular oxidative stress levels and limiting apoptosis. These protective effects were demonstrated through confocal microscopy analyses and confirmed by Western blot evaluation of proteins involved in oxidative stress response and apoptotic pathways.

Overall, our findings suggest that Curcuma caesia exerts a cardioprotective action against DOX-induced cellular damage and may represent a promising natural strategy to counteract chemotherapy-associated cardiotoxicity.

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Keywords: Doxorubicin, Curcuma Caesia, Nutraceutical compound, apoptotic pathways, H9c2.

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Blue Turmeric (Curcuma caesia Roxb.): A Promising Source of Bioaccessible Phenolics for Joint Health

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Osteoarthritis (OA) is the most common chronic joint disorder and a leading cause of pain and disability worldwide. The disease is characterized by the progressive degradation of articular cartilage, a process strongly associated with persistent inflammation and oxidative stress within the joint microenvironment. In particular, pro-inflammatory cytokines such as IL-1β contribute to cartilage damage by promoting the production of inflammatory mediators, impairing cellular homeostasis, and disrupting antioxidant defense mechanisms in chondrocytes.

In the search for novel dietary strategies and natural compounds capable of counteracting these pathological processes, increasing attention has been directed toward plant-derived bioactive molecules. Blue turmeric (Curcuma caesia Roxb.), a rare and still poorly investigated species belonging to the Zingiberaceae family, represents a promising source of phenolic compounds with potential antioxidant and anti-inflammatory properties. However, information regarding its phytochemical composition, the bioaccessibility of its compounds after digestion, and its effects on joint cells remains limited.

The present study aimed to characterize the phenolic profile of a blue turmeric rhizome extract (BTE), evaluate the stability and bioaccessibility of its bioactive compounds following simulated gastrointestinal digestion, and investigate the biological activity of both the native extract and its digested fraction (dBTE) in an in vitro model of osteoarthritis based on IL-1β-stimulated human chondrocytes (C-28/I2).

HPLC-MS/MS analysis revealed a complex phytochemical profile dominated by phenolic acids and flavan-3-ols. Although gastrointestinal digestion led to a partial reduction of some compounds, a relevant fraction of the phenolic constituents remained detectable, suggesting a satisfactory degree of bioaccessibility. Biological assays demonstrated that both BTE and dBTE effectively protected chondrocytes from IL-1β-induced cellular damage. Specifically, treatment with the extracts improved cell viability, preserved mitochondrial function, and counteracted the IL-1β-mediated suppression of Nrf2, a key regulator of the cellular antioxidant response. Furthermore, BTE significantly reduced the release of the pro-inflammatory cytokine TNF-α and helped maintain normal cell morphology under inflammatory conditions.

Overall, these findings indicate that the phenolic compounds present in blue turmeric remain at least partially available after gastrointestinal digestion and retain their biological activity. The observed antioxidant and anti-inflammatory effects support the potential role of blue turmeric as a functional food ingredient or nutraceutical candidate for the maintenance of joint health and the prevention of osteoarthritis-related cartilage degeneration.

Keywords: Blue turmeric, *in vitro* digestion, bioaccessibility, inflammation, osteoarthritis, novel food.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Tissue-Specific Ultrastructural Changes in Fabry Disease: A Comparative Study of Affected Organs

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Fabry disease (FD) is an X-linked lysosomal storage disorder caused by α-galactosidase A deficiency, which leads to progressive intracellular accumulation of globotriaosylceramide (GB3) across multiple organ systems [1]. This deposition drives structural damage that preferentially involves the cardiovascular, gastrointestinal, and reproductive systems, although the extent and pattern of ultrastructural injury differ considerably from one tissue to another, shaping disease progression and clinical presentation [2,3]. To characterize these tissue-specific changes, we examined myocardial, intestinal, and testicular biopsies from FD patients by transmission electron microscopy (TEM) and light microscopy (LM), complemented by morphometric assessment to quantify GB3 content and its effect on cellular and subcellular architecture [3]. TEM demonstrated widespread of GB3 deposition in cardiomyocytes, smooth muscle cells, endothelial cells, and pericytes, where it formed the characteristic myelin-like inclusion bodies typical of the disease. In the myocardium, cardiomyocytes showed extensive cytoplasmic inclusion bodies accompanied by displacement of organelles, whereas enterocytes accumulated GB3 chiefly within lysosomal compartments, with consequent alteration of cellular morphology. In testicular tissue, GB3 was concentrated in vascular and interstitial cells, while Sertoli and Leydig cells remained largely spared; we additionally noted thickening of the basement membrane and degeneration of spermatogonia, pointing to an indirect effect of vascular damage on spermatogenesis. Taken together, these findings indicate that the ultrastructural consequences of FD are organ-dependent, with cardiomyocytes displaying prominent cytoplasmic disruption, enterocytes showing lysosomal inclusions, and testicular cells exhibiting predominantly vascular-related alterations. This tissue-specific pattern of GB3 accumulation and cellular injury helps explain the heterogeneous clinical manifestations of the disease. Finally, defining these organ-specific ultrastructural changes may provide a morphological basis for evaluating how therapeutic intervention acts across the differently affected tissues, and for monitoring its effects over the course of the disease.

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Keywords: Fabry disease, globotriaosylceramide, transmission electron microscopy, ultrastructural changes, lysosomal storage disorder.

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Intestinal Wall Modifications in an Animal Model of Hypertension: Possible Protective Interventions

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Hypertension is among the most common chronic diseases worldwide and represents a major risk factor for cardiovascular complications. Although several factors contribute to its development, including poor dietary habits, excessive salt intake, smoking, substance abuse, and physical inactivity, the mechanisms underlying its onset and progression are not yet fully understood [1]. In recent years, increasing attention has been directed toward the role of the gastrointestinal tract in cardiovascular disorders. Experimental and clinical evidence indicate that hypertension is frequently associated with alterations in intestinal homeostasis, including dysbiosis, impaired epithelial barrier function, and changes in mucosal secretion [2,3]. These alterations may not simply be a consequence of elevated blood pressure but could actively contribute to disease progression through gut–cardiovascular interactions. In the present study, we investigated the effects of hypertension on intestinal wall architecture and function using the Spontaneously Hyper-tensive Rat (SHR), a well-established model of essential hypertension. We also evaluated whether treatment with (+)-thioctic acid (TIO), a compound with a strong antioxidant activity, administered alone or in combination with the cholinergic precursor choline alphoscerate (α-GPC), could counteract hypertension-associated intestinal alterations. As expected, SHR animals displayed significantly higher systolic and diastolic blood pressure than normotensive Wistar–Kyoto (WKY) rats. Histological analyses revealed an alteration of the colonic wall with an increase in submucosal collagen deposition and a reduced luminal area of submucosal arterioles. These structural changes were accompanied by functional impairment of the intestinal mucosa. Secretion of acidic mucopolysaccharides (stained with Alcian Blue, pH 2.5), glycoproteins (Periodic Acid–Schiff staining), and the intestinal mucin-2 was significantly decreased in SHR rats compared with WKY controls, suggesting a compromised mucus barrier. Hypertension also affected epithelial integrity with a reduction in the expression of the tight-junction proteins zonula occludens-1 and claudin-5, indicating an increase in intestinal permeability in hypertensive animals. This condition is linked to hyperstimulation of the activity of enteric glial cells, which are involved in maintaining intestinal homeostasis. The data showed that markers such as glial fibrillary acidic protein and ionized calcium-binding adaptor molecule-1 appear upregulated in hypertensive animals. Consistent with these findings, the expression of the pro-inflammatory mediators interleukin-1β and tumour necrosis factor-α was increased in SHR rats, supporting the presence of a pro-inflammatory intestinal microenvironment associated with hypertension. Treated groups showed a reduction of collagen accumulation, an improvement of mucous production, partial restoration of epithelial barrier integrity, and lower expression of inflammatory markers compared with untreated SHR rats, suggesting that the TIO, either alone or combined with α-GPC, mitigated several of the alterations observed in hypertensive animals. Taken together, these findings highlight the impact of hypertension on intestinal homeostasis, affecting vascular remodelling, mucosal function, barrier integrity, and local inflammatory responses. The observed protective effects of the tested treatments further support the involvement of the gut in hypertension pathophysiology and suggest that preserving intestinal homeostasis may represent a complementary strategy for limiting disease-associated damage. Further studies are needed to clarify the mechanisms linking intestinal dysfunction and blood pressure regulation and to determine the therapeutic potential of targeting the gut–cardiovascular axis.

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Keywords: Hypertension, gut–cardiovascular axis, intestinal homeostasis, antioxidant compound, cholinergic precursor.

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Morphological Evidence of Protective Effects of Acorn Extract on High-Fat Diet Metabolic Disfunctions in Sprague-Dawley Rats

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Obesity is a chronic multifactorial disease characterized by excessive adipose tissue accumulation and is a major risk factor for metabolic syndrome, type 2 diabetes, and cardiovascular disease [1]. Diet-induced obesity (DIO) models are widely used to investigate the metabolic and inflammatory alterations associated with obesity and to evaluate potential nutritional interventions. Among natural compounds, acorns have attracted increasing interest due to their high content of phenolic compounds and their reported antioxidant and anti-inflammatory properties [2,3]. The study aims to evaluate the possible beneficial effects in a pre-clinical model of high-fat diet (HFD). Male Sprague–Dawley rats were assigned to either a control diet or HFD (Research Diets, Inc. D12451 Rodent Diet With 45 kcal% Fat). After 8 weeks, HFD dietary regimens was supplemented for 6 weeks with an acorn extract administered in drinking water at a concentration of 7%. Body weight and food intake were recorded three times per week throughout the experimental period. At the end of the study, animals were euthanized, and the liver and white adipose tissue (WAT) depots, including perigonadal and retroperitoneal adipose tissue, were excised, weighed, and processed for histological analyses. HFD feeding resulted in a statistically significant increase in liver and adipose tissue weights compared with controls. Acorn extract supplementation attenuated organ weight gain, with the most pronounced effect observed in the liver. Histological examination revealed hepatic steatosis profile and sinusoidal dilation in HFD-fed rats. In contrast, animals receiving acorn extract supplementation exhibited partial amelioration of these pathological alterations. Given the close association between hepatic steatosis and inflammation, the hepatic inflammatory microenvironment was also investigated. Acorn extract supplementation reduced the immunoreactivity of ionized calcium-binding adaptor molecule-1 (IBA-1)-positive cells and was associated with an overall decrease in the expression of pro-inflammatory cytokines, suggesting a protective effect against HFD-induced hepatic inflammation. In WAT, acorn extract supplementation significantly attenuated adipocyte hypertrophy in both perigonadal and retroperitoneal depots compared with the HFD group. In conclusion, the reduction in liver weight, together with the improvement in tissue morphology and inflammatory markers, suggests that acorn-derived phytochemicals mitigate hepatic and adipose tissue alterations associated with diet-induced obesity. These findings support further investigation of acorn extracts as a source of bioactive compounds for potential management of obesity-related metabolic dysfunction.

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Keywords: Obesity, High-Fat Diet, Acorn Extract, adipose tissue, liver.

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Targeting Gal-3 through selective hyaluronic acid derivatives represent a promising strategy for modulating in vitro chondrocyte inflammation

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Currently, there is no cure for osteoarthritis (OA), a degenerative joint disease characterized by cartilage breakdown and macrophage-mediated inflammation, that represents a major cause of pain and disability worldwide [1]. We have recently demonstrated that a new Galectin-3 (Gal-3) binding molecule named Hylach®, obtained by combining 30% lactose with hyaluronic acid (HA), administered to in vitro inflamed human chondrocytes and spheroids, strongly reduce the expression of pro-inflammatory cytokines and Gal-3 [2]. Building on our previous demonstration of the anti-inflammatory effects of Hylach®, this study aimed to identify the molecular pathways and biological processes modulated by this Gal-3-targeting HA derivative in inflamed human osteoarthritic chondrocytes through whole-transcriptome profiling

Human articular chondrocytes isolated from joint cartilage biopsies of OA patients were exposed to the conditioned medium (CM) of U937 monocytes activated to macrophages by treatment with phorbol myristate acetate (PMA) and lipopolysaccharides (LPS), in the presence or absence of Hylach, by means of qPCR, ELISA and RNA sequencing analysis.

RNA-sequencing revealed a profound transcriptional reprogramming in OA chondrocytes exposed to macrophage-CM, with 1,264 differentially expressed genes (DEG) compared with untreated controls. Principal component analysis clearly separated inflamed, Hylach-treated, and control samples, indicating reshaping of the inflammatory transcriptional profile. Gene Ontology enrichment analysis demonstrated that inflammatory stimulation induced the activation of pathways related to cytokine signalling, inflammatory response, cell migration, leukocyte recruitment, and extracellular matrix organization. Hylach treatment markedly attenuated the enrichment of these biological processes, indicating a broad suppression of inflammation-associated transcriptional programs.

In conclusion, Hylach markedly attenuated the inflammatory transcriptional program induced by macrophage-derived stimuli in human OA chondrocytes, suppressing pathways involved in cytokine signalling, immune activation, and tissue remodelling. These findings further support Gal-3-targeting lactose-modified HA derivatives as promising candidates for modulating OA-associated inflammation. Additional studies are needed to clarify the molecular mechanisms linking Gal-3 inhibition to the transcriptional reprogramming observed in inflamed chondrocytes.

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Keywords: osteoarthritis, hyaluronic acid, inflammation, Hylach, Galectin-3, chondrocytes.

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Validation of a Detergent-Enzymatic Decellularization Protocol for Human Sural Nerve: Towards Off-the-Shelf Nerve Allografts for Peripheral Nerve Regeneration

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Peripheral nerve injuries characterized by segmental loss and critical-size gaps remain a major unmet clinical challenge. Autologous nerve grafting still represents the gold standard surgical treatment; however, donor-site morbidity, limited graft availability, mismatch in size and fascicular organization, and incomplete long-term functional recovery emphasize the urgent need for alternative regenerative strategies [1]. Decellularized nerve grafts may represent a promising approach for the treatment of critical-length peripheral nerve defects, potentially overcoming several limitations associated with autologous nerve transplantation. In this context, the present study aimed to validate the detergent-enzymatic decellularization protocol developed by the Veneto Tissue Bank Foundation (FBTV) for the development of clinical-grade human sural nerve allografts. The effectiveness of the protocol was assessed by verifying both the removal of immunogenic components (i.e., cells, DNA) and the preservation of the structural and biochemical integrity of the extracellular matrix (ECM). Particular attention was given to the maintenance of key matrix constituents and biological cues relevant for peripheral nerve regeneration. DNA quantification yielded values below the accepted threshold for decellularized tissues (50 ng/mg) [2], indicating efficient cell removal. Histological, immunohistochemical, biochemical, and scanning electron microscopy analyses confirmed the preservation of collagen, elastic fibers, glycosaminoglycans, hyaluronic acid, laminin, myelin, and the native micro- and nanoscale architecture of the decellularized sural nerve. Growth factor analysis demonstrated substantial retention of bioactive molecules following decellularization. Analysis of vascularization-related factors revealed comparable values between decellularized and native sural nerve. Moreover, a cytotoxicity extract test demonstrated that the decellularized nerve matrix does not release toxic chemical residues, maintaining the viability of mesenchymal stem cells.

In conclusion, the detergent-enzymatic decellularization protocol applied to human sural nerve successfully removed potentially immunogenic components while preserving key biochemical, structural, and biological features of the ECM. These findings support the use of decellularized human sural nerve as a promising biomaterial for peripheral nerve regeneration across critical-length defects.

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Keywords: Peripheral nerve injuries (PNIs), human sural nerve, detergent-enzymatic decellularization protocol, decellularized nerve grafts.

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Muscle-Specific Metabolic and Structural Adaptations to Mapk15 Deficiency in a Murine Model of MASLD

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Our research group previously characterized a Mapk15 knock-out (KO) mouse model showing alterations resembling metabolic dysfunction-associated steatotic liver disease (MASLD), including hepatic steatosis, insulin resistance, and increased body mass index [1]. These features were exacerbated under a Western-type diet (WD), suggesting a role for Mapk15 in metabolic adaptation to nutrient excess. Emerging evidence indicates a relationship between skeletal muscle and MASLD based on a complex bidirectional cross-talk in which skeletal muscle on one side contributes to systemic metabolic regulation protecting against MASLD progression, and on the other represents a target of metabolic dysfunction [2].

To investigate the impact of Mapk15 deficiency on skeletal muscle, we analysed two skeletal muscles, the soleus, with a predominance of oxidative fibers, and the gastrocnemius, with a predominance of glycolytic fibers, in Mapk15-wild-type (WT) and KO mice fed either a standard diet (SD) or Western-type Diet (WD). Fiber-type composition was evaluated by quantifying Myosin Heavy Chain I (MyHC I, slow twitch, oxidative fibers) and MyHC II fibers (fast, glycolityc fibers). Glycogen content, lipid accumulation, and mitochondrial function were assessed by Periodic Acid-Schiff (PAS) staining, Sudan Black B staining, and succinate dehydrogenase (SDH) histochemistry, respectively. We also quantified the cross-sectional area (CSA) of SDH+ and SDH– fibers.

Our results revealed muscle-specific responses to Mapk15 deficiency. In the soleus, KO mice showed increased CSA of oxidative fibers, while no alterations were detected in gastrocnemius. Under WD soleus muscle showed glycogen and lipid accumulation together with impaired mitochondrial function. In contrast, gastrocnemius displayed stable glycogen, lipid, and mitochondrial profiles, but exhibited an increased number of MyHC I fibers and a reduced number of MyHC II fibers. Overall, these findings indicate that the soleus muscle is more susceptible to Mapk15 loss, whereas the gastrocnemius undergoes adaptive remodelling, consistent with previously described responses to metabolic stress and endurance-like stimuli [3].

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Keywords: Metabolic Dysfunction-Associated Steatotic Liver Disease, gastrocnemius, soleus, skeletal muscle.

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Ultrastructural evidence of differential vulnerability of axons, Schwann cells, and their myelin sheaths in an ex-vivo model of peripheral nerve repair

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Ex-vivo peripheral nerve preparations are widely used as experimental models of nerve repair, particularly in functional studies involving electrophysiological recordings that require prolonged maintenance of neural tissues outside their physiological environment. However, little is known about the early ultrastructural changes occurring in peripheral nerve tissue during these procedures. In particular, the effects of ex-vivo maintenance on axons and Schwann cells remain poorly characterized. In the present study, tibial nerves were explanted from the hind limbs of neonatal rats, transected, re-apposed using different conductive adhesive matrices, and subjected to electrophysiological recordings to assess the restoration of nerve function. During these procedures, nerve segments were maintained in an oxygenated saline solution for approximately 5 hours before fixation and processing for conventional transmission electron microscopy of samples collected both proximal and distal to the repair site. Ultrastructural analysis revealed comparable alterations in myelinated fibers in all samples, consisting of early degeneration of myelin sheaths characterized by widespread loss of the normal lamellar architecture, multifocal lamellar loosening, and the formation of large interlamellar spaces. Additional alterations included inward and outward ballooning of the myelin sheath, accompanied by lamellar lysis and accumulation of amorphous material. These changes were progressively associated with degeneration of Schwann cell bodies, resembling experimentally induced Wallerian-like degenerative processes in peripheral nerve fibers. In contrast, axons generally appeared well preserved, exhibiting intact mitochondria, vesicles, and cytoskeletal elements. Notably, unmyelinated fibers remained largely unaffected, with both axons and Schwann cells retaining well-preserved ultrastructural features. These findings reveal that myelinating Schwann cells are the earliest components to undergo degenerative changes during ex-vivo maintenance, indicating a particular susceptibility to ex-vivo stress rather than incompatibility with the adhesive matrices employed. Despite these alterations, axons remained largely preserved, suggesting that degenerating Schwann cells may still retain their protective functions. Residual insulating properties may also persist, potentially supporting continued impulse conduction along nerve fibers, with preservation of unmyelinated fibers likely contributing to the maintenance of detectable electrophysiological activity in explanted nerves.

Keywords: Schwann cell, myelin sheath, peripheral nerve repair, ultrastructure.

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Continuum: From Imprint to Symbol, Scientific Exhibition at the University of Padova

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The *scientific exhibition Continuum: From Imprint to Symbol* explores the complexity and beauty of life beyond death, through photographic representations of organ imprints on gauze and cardboard. Developed within the activities of the Reference Centre for the Conservation and Use of Bodies of the Deceased at the University of Padova, the exhibition serves as a tribute to the generosity of body donors, inviting visitors to reflect on the vital cycle and on the unexpected beauty embedded in biological transformation. Beyond its artistic dimension, Continuum carries a strong educational and awareness-raising mission, emphasizing the importance of body donation for scientific research and medical training. The pictures are complemented by plastinated specimens, macrosections, and vascular casts, offering visitors a multidimensional and immersive exploration of human anatomy. Together, these exhibits highlight the remarkable complexity of biological structures and reinforce the scientific value of anatomical preservation techniques.

As part of the exhibition's educational outreach, medical students from the Universities of Padova and Treviso were engaged in an interactive learning activity using the Wooclap quiz platform, in which they were asked to identify the organs depicted in seven photographs. Results revealed an overall mean recognition rate of 84%, indicating a high level of anatomical knowledge. However, students showed greater difficulty in recognizing organs of smaller dimensions related to anatomical variation.

These findings suggest that innovative, art-science exhibitions such as Continuum may offer valuable complementary tools for anatomical education, while simultaneously fostering a broader cultural sensitivity toward body donation. Further studies with larger cohorts are warranted to confirm these preliminary results.

Keywords: Body Donation, Dissection, Ethical Value, Education.

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Intestinal alterations induced by the ingestion of micro- and nanoplastics released from orthodontic aligners

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Human exposure to micro- and nanoplastics (MNPs) is increasingly recognized as an emerging concern for environmental and human health. [1] However, the biological effects of particles released from polymer-based dental devices remain largely unexplored. [2] In a previous in vivo study, we demonstrated that exposure to MNPs derived from orthodontic aligners rapidly induces inflammatory and oxidative stress responses. [3] The present study was designed to investigate whether these molecular alterations translate into structural changes in target tissues, focusing on the effects of particle ingestion on intestinal homeostasis through an integrated molecular, histological, and histochemical approach. Hirudo verbana was employed as an in vivo experimental model due to its pronounced immune responsiveness and ease of handling. [4] MNPs generated by mechanical abrasion of two orthodontic aligner materials (OLD TC-45 and LUX CREO B4D) were administered through feeding, and intestinal tissues were analyzed after one and two weeks of exposure. Molecular analyses performed one week after ingestion revealed a slight increase in the expression of the inflammatory marker HmAIF-1 and the antioxidant enzyme superoxide dismutase (SOD), accompanied by epithelial cell flattening and a reduction in intracellular lipid droplets, suggesting the activation of an early adaptive response aimed at preserving tissue homeostasis. [5] After two weeks of exposure, a marked increase in inflammatory markers was associated with reduced SOD expression, indicating a possible impairment of antioxidant defenses. Concurrently, the intestinal epithelium exhibited progressive morphological alterations, including disruption of epithelial folds, increased extracellular matrix deposition, intracellular lipid droplet accumulation, and loss of epithelial barrier integrity, particularly in animals exposed to particles derived from LUX CREO B4D. Overall, these findings demonstrate that ingestion of MNPs released from orthodontic aligners induces a progressive intestinal response that evolves from an initial phase of metabolic and cellular adaptation to structural remodeling associated with persistent inflammation. To the best of our knowledge, this is the first evidence that plastic particles released from dental materials can impair intestinal homeostasis and epithelial barrier integrity, highlighting the importance of integrating molecular and morpho-functional evaluations into the biocompatibility assessment of dental polymers.

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Keywords: Micro- and nanoplastics, 3D-printed aligners, Tissue remodeling, Oxidative stress, Inflammation, biocompatibility.

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Molecular and Anatomical Remodeling of the Jejunum in Enteric Dysmotility

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Enteric dysmotility (ED) is a severe gastrointestinal motor disorder characterized by impaired intestinal propulsion in the absence of mechanical obstruction [1]. Although traditionally considered a neuromuscular disorder, emerging evidence suggests that additional components of the intestinal wall contribute to disease pathogenesis [2]. This study aimed to define the structural and molecular signature of idiopathic ED and to assess whether it shares remodelling features previously identified in idiopathic chronic intestinal pseudo-obstruction (CIPO) and CIPO secondary to mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) [3]. Full-thickness jejunal specimens from patients with idiopathic ED (n=10) and controls (n=10) were investigated using integrated morphometric, histochemical, immunohistochemical and molecular analyses. Vascular architecture, fibrosis, muscle layer thickness, enteric neuronal organization, expression of thymidine phosphorylase (TP), vascular endothelial growth factor (VEGF) and HIF-1α, mitochondrial DNA (mtDNA) copy number, and genomic variants were evaluated. Layer-specific mtDNA content was assessed by laser capture microdissection. ED specimens showed marked microvascular remodelling, with reduced vascular area, increased vessel density and a shift toward very small-calibre vessels. TP expression was significantly reduced, whereas VEGF and HIF-1α levels were preserved, indicating maintained tissue oxygenation despite vascular alterations. Unlike idiopathic CIPO and MNGIE, ED exhibited increased mtDNA copy number, particularly in the longitudinal muscle layer, consistent with compensatory mitochondrial biogenesis. Additional changes included increased fibrosis and collagen deposition, thinning of both muscle layers, and significant loss of myenteric and submucosal neurons with disruption of ganglionic architecture. Rare variants in collagen-related genes were identified in a subset of patients. These findings identify ED as a multicompartment disorder involving vascular, stromal, neuromuscular and metabolic elements of the intestinal wall. While sharing major remodelling features with idiopathic CIPO and MNGIE, ED appears distinguished by preserved tissue oxygenation and adaptive mitochondrial responses. This integrated anatomical framework highlights intestinal wall remodelling as a key determinant of disease phenotype in severe gut dysmotility.

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Keywords: enteric dysmotility, intestinal wall remodelling, microvasculature, fibrosis, mitochondria.

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Enhancing Periodontal Regeneration: *In Vitro* Effects of Liquid and Solid PRF on human PDLSCs

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Regeneration of periodontal tissues represents one of the main challenges of regenerative dentistry. Among the most promising biological strategies, Platelet-Rich Fibrin (PRF) has attracted growing interest due to its ability to promote healing and regeneration processes through the release of growth factors and bioactive molecules. The aim of this study was to evaluate and compare the effects of liquid PRF and solid PRF on mesenchymal stem cells derived from human periodontal tissues (hPDLSCs). For PRF preparation, peripheral blood from volunteers was collected in specific tubes and centrifugate at 1200x *g* for 8 min. hPDLSCs were harvested from healthy third molars and then used at passages 4-7. Cells derived from four different samples were treated with PRF either directly through a conditioned medium or indirectly using trans-well filter systems [1]. hPDLSCs morphology was assessed by phalloidin staining, while cell proliferation was evaluated by counting the DAPI-stained nuclei. Healing properties of liquid PRF vs. solid PRF were evaluated by scratch assay. After 5 days of treatment, hPDLSCs exposed to either liquid or solid PRF showed a significant increase ($p<0.0001$) in cell viability compared to the control group. Cytoskeletal analysis revealed an increase ($p<0.05$) in actin intensity in treated cells, compatible with an active functional state. Furthermore, both liquid and solid PRF tend to promote ($p<0.05$) cell migration, suggesting a favorable role in tissue repair processes. These preliminary results confirm the potential of PRF as biological support for periodontal regeneration. Significant differences between distinct PRF application protocols underscore the need for standardized protocols.

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Keywords: Platelet-Rich Fibrin (PRF), hPDLSCs, regenerative dentistry.

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Transgenic LIGHT/TNFSF14 knock-in mice showed improved bone mass

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Immune and bone cross talk involves different ligands including members of TNFSF, as TNFSF14/LIGHT. It is expressed by immune cells together with its receptors HVEM and LTβR. Brunetti et al. evaluated the role of LIGHT in bone remodeling showing that it is overexpressed in bone loss pathologies, however knock-out mice showed significantly reduced bone mass, rescued in Rag-/LIGHT- double knock-out mice. To better address the role of LIGHT in bone remodeling in this study we studied the bone phenotype of LIGHT knock-in mice, a humanized model with hLIGHT expressed in fl/fl mice. In this new study, we showed that compared to wild-type male 3-month mice, fl/fl mice may have significantly more trabecular bone in their distal femoral metaphysis with increased trabecular bone volume fraction (Tb.BV/TV, p=0.0317), connectivity density (p=0.05), trabecular number (p= 0.028), thickness (p= 0.029) and separation (p= 0.014). The fl/fl mice also had higher cortical area (Ct.Ar, p= 0.001), cortical bone area fraction (Ct.Ar/Tt.Ar, p< 0.0001), cortical thickness (Ct.Th p< 0.0001), and moments of inertia (pMOI p=0.02, I_{max} p=0.01, and I_{min} p=0.04). Considering as control LIGHT-KO mice, these parameters increase is more important, however the cells with the human gene are only T cells, and in the human bone diseases the major levels were shown on monocytes, and also neutrophils. In human and murine models LIGHT possible will sustain bone protection reaching a particular concentration, than became dangerous leading to bone disease, however further studies are needed to understand the mechanism.

Keywords: LIGHT/TNFSF14, Bone, mouse model, knock-in mice.

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Protective Role of Muna Compound in Ethanol-Induced Gastric Cytotoxicity

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Ethanol exposure is known to induce oxidative stress and mitochondrial dysfunction in gastric epithelial cells, leading to cellular injury, apoptosis, and impaired autophagic responses. This study investigated the protective effects of Muna Compound against ethanol-induced damage in human gastric epithelial (GES-1) cells, with particular focus on mitochondrial integrity, apoptosis, and autophagy.

Mitochondrial alterations were assessed by confocal laser scanning microscopy (CLSM) using MitoTracker Green (MTG) to evaluate mitochondrial mass and Nonyl Acridine Orange (NAO) to detect cardiolipin integrity and peroxidation. The activation of apoptotic and autophagic pathways was analyzed by Western blotting of key regulatory proteins, including Bcl-2, Bax, cytochrome c (cyt-c), Caspase-3, and Beclin-1.

Ethanol treatment markedly impaired mitochondrial function, as evidenced by a significant reduction in MTG and NAO fluorescence intensity, indicating loss of mitochondrial integrity and cardiolipin oxidation. These alterations were accompanied by decreased cyt-c signal and increased expression of the pro-apoptotic proteins Bax and Caspase-3, consistent with activation of the intrinsic apoptotic pathway and disruption of cellular homeostasis.

In contrast, co-treatment with Muna Compound significantly attenuated ethanol-induced mitochondrial damage, preserving mitochondrial structure and function, reducing cardiolipin oxidation, and maintaining cyt-c localization. Moreover, Muna Compound suppressed the ethanol-induced upregulation of Bax and Caspase-3 and promoted the restoration of apoptosis- and autophagy-related signaling pathways, resulting in improved cell survival.

Overall, these findings demonstrate that Muna Compound exerts a protective effect against ethanol-induced gastric epithelial injury by preserving mitochondrial integrity, preventing cardiolipin degradation, and modulating apoptosis and autophagy. These results highlight its potential as a promising therapeutic candidate for the prevention of alcohol-induced gastric mucosal damage.

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Keywords: GES-1 cells, Muna, ethanol, mitochondrial dysfunction, apoptosis, autophagy.

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Advancing Glioblastoma Treatment through Temozolomide-Based Combination Strategies

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Glioblastoma (GBM) is the most common malignant tumor of the central nervous system and remains one of the most aggressive human cancers, with high recurrence rates despite the current standard of care consisting of maximal safe resection followed by radiotherapy and temozolomide (TMZ)-based chemotherapy (1). Its poor prognosis is largely driven by marked intratumoral heterogeneity, aggressive biological behavior, and the ability of tumor cells to evade cell death and rapidly repopulate after therapy. Glioma stem cells (GSCs), although representing only a minor fraction of the tumor mass, are considered key drivers of recurrence and therapeutic resistance due to their capacity for self-renewal, multilineage differentiation, and tumor repopulation (2). We investigated the antitumor activity of Ipatasertib, Regorafenib, and Selinexor as potential alternatives to TMZ or candidates for combination therapy in GBM (3).

GBM biopsies were obtained through a collaboration with the Neurosurgery Unit of Tor Vergata University Hospital. GSCs were isolated from each biopsy, cultured under stem cell-selective conditions, and characterized by immunofluorescence and qPCR analysis of stemness-related genes. Drug concentrations were first optimized in established GBM cell lines and then applied to patient-derived GSCs. Cells were treated for 72 hours with the IC50 of each compound, and cell viability was subsequently assessed. The effects of each drug were compared with TMZ, and potential synergistic effects were evaluated using low-dose combinations within therapeutically relevant ranges.

As an initial step, we evaluated the cytotoxic activity of the three compounds as single agents in GBM cell lines and patient-derived GSCs. Regorafenib and Selinexor, but not Ipatasertib, effectively reduced cell viability in both models and showed greater efficacy than TMZ. Based on these results, we then assessed whether combining Regorafenib or Selinexor with TMZ could further enhance their antitumor effects. In vitro, both combinations significantly reduced cell survival compared with either agent alone. Although Ipatasertib did not significantly affect cell viability when used alone, its combination with TMZ produced a marked reduction in cell survival, consistent with a synergistic effect.

Taken together, these findings suggest that simultaneous inhibition of parallel survival pathways, including receptor tyrosine kinase signaling, nuclear export, and the PI3K/AKT axis, may enhance the therapeutic efficacy of TMZ in GBM. These results support pathway-targeted combinations as promising strategies to improve treatment response in recurrent and therapy-resistant GBM.

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Peripheral proteinopathy and Small Fiber Neuropathy as Biomarkers in Atypical Parkinsonism

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Atypical parkinsonisms are a heterogeneous group of neurodegenerative disorders that present with parkinsonian motor features, such as bradykinesia, rigidity, postural instability, and, less frequently, resting tremor, but differ from Parkinson's disease (PD) in their underlying pathology and clinical course. These include Progressive Supranuclear Palsy (PSP), a 4R-Tau pathology, and Multiple System Atrophy (MSA), an atypical alpha-synucleinopathy. Despite its distinctive neuropathological features, the in vivo diagnosis of atypical parkinsonisms remains challenging, particularly during the early stages of the disease, due to clinical overlap with Parkinson's disease (PD) and the lack of reliable biomarkers. Recent evidence suggests that pathological protein aggregates and peripheral nerve alterations may be detectable in the skin, offering a minimally invasive approach for biomarker development. This study aimed to investigate the relationship between peripheral proteinopathy and small fiber neuropathy in PSP, MSA and PD patients by assessing cutaneous tau and α-synuclein pathology, intraepidermal nerve fiber density (IENFD), plasma pTau217 levels, and clinical manifestations and neuropsychological performance. We further evaluated the potential of these biomarkers to differentiate atypical parkinsonisms from PD and healthy controls (HC). PSP patients exhibited significantly lower cervical IENFD values compared with both PD patients and healthy controls, indicating a marked involvement of peripheral small nerve fibers. Tau-positive PSP patients showed significantly reduced IENFD at both neck and leg sites compared with tau-negative patients, with large effect sizes. Further subgroup analyses demonstrated that both Tau-only and Tau-plus-α-synuclein patients exhibited lower IENFD values than pathology-negative PSP patients, whereas α-synuclein co-positivity did not confer additional reductions in nerve fiber density. In MSA patients, significant alpha-synuclein burden and lymphatic vessel disruption was present, and correlated with motor and non-motor scales, as well as disease progression. The present findings provide evidence that peripheral proteinopathy is associated with significant small fiber neuropathy, particularly in PSP, and may represent a biologically meaningful marker of disease-related neurodegeneration. Reduced IENFD, particularly in cervical skin biopsies, distinguished PSP from both PD and healthy controls and was strongly associated with cutaneous tau positivity. These results support the potential role of skin biopsy-derived pathological markers and peripheral nerve fiber assessment as accessible biomarkers for atypical parkinsonism, while highlighting the need for longitudinal studies to clarify their prognostic and diagnostic value.

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Keywords: Atypical Parkinsonism, Alpha-Synuclein, Tau protein, Peripheral Nervous System.

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Altered Serum miRNAs Expression and Oxidative Stress Markers in Male Type 2 Diabetic Patients: Potential Implications for Ectopic Biomineralization

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Background: Type 2 diabetes mellitus (T2DM) is characterized by chronic hyperglycemia and increased oxidative stress, conditions that contribute to the development of diabetic complications and ectopic biomineralization. MicroRNAs (miRNAs) have emerged as promising circulating biomarkers involved in the regulation of oxidative stress and pathological calcification pathways. In addition, melatonin, 8-isoprostane, and sclerostin have been implicated in redox homeostasis and mineralization processes, although their role in male T2DM patients remains incompletely understood. *Methods:* Serum samples were collected from 10 male patients with T2DM and 10 age-matched healthy male controls. The expression levels of miR-205-5p, miR-125b, and miR-548-3p were quantified by quantitative real-time PCR. Serum concentrations of melatonin, 8-isoprostane, and sclerostin were measured by enzyme-linked immunosorbent assay (ELISA). Statistical comparisons between groups were performed using Student's t-test after assessment of normal data distribution. *Results:* Among the investigated miRNAs, only miR-205-5p was significantly upregulated in diabetic patients compared with controls, whereas miR-125b and miR-548-3p showed no significant differences between groups. Serum melatonin levels were significantly reduced in T2DM patients ($P < 0.001$), while 8-isoprostane concentrations were significantly increased ($P = 0.028$), indicating enhanced systemic oxidative stress. No significant differences were observed in serum sclerostin levels. *Conclusions:* Male patients with T2DM exhibit a distinct circulating molecular profile characterized by increased miR-205-5p expression, reduced melatonin levels, and elevated 8-isoprostane concentrations. These findings support the involvement of oxidative stress-related mechanisms in T2DM and suggest that miR-205-5p may represent a potential circulating biomarker associated with diabetes-induced metabolic and redox alterations. The lack of significant changes in miR-125b, miR-548-3p, and sclerostin suggests a more selective contribution of specific molecular pathways in the male diabetic population.

Keywords: Type 2 diabetes mellitus, miR-205-5p, oxidative stress, melatonin, 8-isoprostane, biomineralization.

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Balancing Inflammation and Osteogenesis through Cu/Zn-Doped PEO Titanium Coatings for Improved Implant Integration

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The biological success of titanium implants depends on the complex interactions between immune response, bone regeneration and bacterial control at the implant–tissue interface, a process increasingly framed within the paradigm of osteoimmunomodulation [1]. In this study, in order to modulate osteoimmunological and osteogenic responses, plasma electrolytic oxidation (PEO) was used to generate bioactive micro–nanostructured coatings on titanium with controlled incorporation of copper and zinc ions. Titanium disks were treated by AC-PEO to obtain undoped (base) and Cu/Zn-doped coatings at two dopant levels (10-5 and 20-5). Surface wettability and bioactivity were evaluated in simulated saliva and simulated body fluid [2], and hemocompatibility was investigated through clot formation and Scanning Elecron Microscopy (SEM) analyses. Immunomodulatory effects were characterized using THP-1-derived macrophages by assessing oxidative stress, cytokine expression and polarization markers [3], whereas the osteogenic response was evaluated using MC3T3 pre-osteoblasts through viability, adhesion, collagen deposition and matrix mineralization assays. PEO produced porous, hydrophilic oxide layers that improved wettability and promoted calcium–phosphate deposition *in vitro*, while all treated surfaces supported rapid clot formation, indicating good hemocompatibility, not interfering with the physiological hemostatic process. Doped coatings reduced oxidative stress and pro-inflammatory cytokine expression compared with untreated titanium, with the highest dopant level (20-5) promoting macrophage polarization toward an anti-inflammatory M2 phenotype [4]. All surfaces sustained osteoblast adhesion and extracellular matrix formation; however, high dopant concentration slightly reduced metabolic activity and mineralization, whereas moderate doping (10-5) preserved cytocompatibility and a balanced biological profile [5]. Overall, Cu/Zn-doped PEO coatings modulated the early immune response while preserving osteogenic activity, suggesting that controlled ion incorporation can promote a balanced osteo-immunomodulatory environment and improve implant integration by supporting both tissue regeneration and controlled inflammation at the peri-implant interface.

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Keywords: Titanium implants, plasma electrolytic oxidation, Cu/Zn doping, osteoimmunomodulation, macrophage polarization, osteogenesis.

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Cone-Beam Computed Tomography Assessment of the Variability of the Endodontic Anatomy of Molars in North-West Italy

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Since complex canal configurations may increase the risk of procedural failure, a thorough knowledge of root canal morphology and its anatomical variations are essential for successful endodontic treatment, [1,2]. Cone-beam computed tomography (CBCT) provides a non-invasive, three-dimensional method to detailed visualise and measure root canal systems and accessory canals [3]. To the best of our knowledge, no CBCT-based study has yet characterised molar root canal morphology in the population of North-West Italy. Therefore, the aim of this retrospective observational study is to provide a comprehensive CBCT-based assessment of molar root canal morphology and root dimensional parameters in this population. From 445 primary CBCT scans of patients from Piedmont, Val d'Aosta and Liguria, 177 were selected on the basis of image quality and dentition completeness, yielding 1,111 evaluated teeth. Root number, canal configuration according to Vertucci's classification [4], lateral canals, apical deltas, transverse anastomoses, root curvature and root length were recorded. Associations with sex, age and dental arch were assessed through mixed-effects regression models accounting for intra-patient clustering. Molars showed significant anatomical variability. A high frequency of transverse anastomoses (42.35%) was observed, whereas lateral canals (1.30%) and apical deltas (0.98%) were rare. In two-rooted molars distal roots were predominantly Vertucci type I (90%), while mesial roots displayed high configuration variability; in three-rooted molars type I prevailed in all roots. Distal roots were significantly shorter than mesial roots, and the distal root was 2.03 mm shorter in mandibular than in maxillary two-rooted molars (p=0.0097). These findings confirm established anatomical patterns and highlight clinically relevant variations associated with tooth type and dental arch, supporting the use of CBCT [5] and individualised, anatomy-based endodontic treatment planning in order to minimise procedural risks.

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Keywords: root canal, morphology, CBCT, anatomical variations, endodontics.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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The Human Auricle Beyond Morphology: Assessing the Reliability of Anatomical Traits and Personal Descriptors

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The human auricle exhibits remarkable inter-individual morphological variability while remaining relatively stable throughout adult life, making it a potentially valuable anatomical structure for personal identification [1] particularly in challenging forensic contexts [2] where comparative data are limited to images from relatives, smartphones, and social networks [3]. Before auricular morphology can be incorporated into new frequency-based identification approaches, the reliability of its assessment must be demonstrated. This study evaluated the reliability of the morphological analysis based on a predefined set of auricular structures and of associated personal descriptors, including nevi, scars and piercings.

Three operators with different levels of expertise independently assessed 3D stereophotogrammetric images of auricles from adult Italian subjects (N=60). A standardized protocol was applied for the morphological assessment and classification of 18 distinct anatomical sub-structures (including helix, antihelix, concha, tragus, antitragus, lobule, among others). Concurrently, the auricle was divided into four macro-regions based on standard anatomical landmarks (Tragion, Superaurale, Postaurale, and Subaurale) to assess the counting and distribution of personal descriptors. Inter- and intra-observer reliability were evaluated using Cohen's κ coefficient for the morphological variables and the Intraclass Correlation Coefficient for the personal descriptors.

The reliability of the auricular morphological analysis was strongly influenced by the operator's anatomical knowledge and expertise. The expert observer achieved substantial to almost perfect agreement across the evaluated structures ($\kappa \geq 0.63$), whereas less experienced observers showed lower levels of agreement for most traits ($\kappa < 0.50$). In contrast, personal descriptors demonstrated consistently higher agreements, with satisfactory reliability levels regardless of operator expertise.

These findings highlight both the identifying potential and the methodological challenges associated with the human auricle in anatomical and forensic identification studies. While detailed morphological traits capture a high degree of inter-individual anatomical variation, their reliable assessment appears highly dependent on observer training and standardization of criteria, which may limit their suitability for frequency-based identification approaches. Conversely, personal descriptors, although less anatomically detailed, showed higher reliability and may represent more robust variables for preliminary applications in probabilistic identification frameworks. These results underscore the importance of standardized definitions and training protocols when complex anatomical features are used for forensic and anthropological purposes.

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Keywords: Auricle Morphology, Personal Descriptors, Inter-individual anatomical variability, 3D Stereophotogrammetry, Personal Identification, Forensic Anatomy.

IJAE
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Firenze University Press
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From Multimodal AI to Biological Twins: Integrating Deep Learning on Magnetic Resonance Imaging and Histology with Patient-Derived 3D Glioblastoma Models for Precision Medicine

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Glioblastoma (GBM) is one of the most aggressive primary brain tumors, characterized by poor prognosis and limited therapeutic options [1]. A major limitation is the lack of an experimental model able to reproduce tumor complexity while interpreting large-scale patient-derived data. In this context, the concept of a "biological twin" is emerging as a powerful strategy to digitally and experimentally mirror disease and investigate therapeutic approaches and responses. In our work, we implemented a deep learning (DL)-based framework to analyse multiple GBM patient-derived datasets, including magnetic resonance imaging and haematoxylin and eosin-stained histological sections from tumor biopsies. Advanced convolutional neural networks were employed to extract spatial features and identify latent patterns across radiological and histopathological domains, thereby enabling multimodal integration of macroscale tumor architecture and microscale cellular organization [2]. In parallel, we generated GBM patient-derived 3D spheroids, providing a biologically relevant and highly representative in vitro counterpart that was subjected to an extensive morpho-phenotypic characterization. The obtained results showed that this 3D model preserved key features of the native tumor, including spatial organization and cellular heterogeneity, as evidenced by the non-uniform distribution of specific molecular markers and the generation of functional cellular niches [3]. This model will be further integrated into our DL-based framework to enhance its robustness and biological relevance. Overall, by combining AI-driven image analysis with patient-derived 3D tumor models, this study proposes a translational framework for generating a GBM biological twin. This approach will allow the integration between computational and experimental oncology, with the potential to improve disease modelling, enable more accurate prediction of therapeutic responses, and advance the implementation of precision medicine strategies.

Acknowledgments: Finanziato dall'Unione europea – NextGenerationEU through the Italian Ministry of University and Research "Avviso per la concessione di finanziamenti destinati ad Iniziative di ricerca per tecnologie e percorsi innovativi in ambito sanitario e assistenziale" finanziato a valere sulle risorse previste dal Fondo complementare al Piano Nazionale di Ripresa e Resilienza - PNC0000001 - D3 4 Health Digital Driven Diagnostics, prognostics and therapeutics for sustainable Health care. Finanziato dall'Unione europea – Next Generation EU" componente M4C2, investimento 1.1, "Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)" – CUP: B53D23032960001, Implementation of a patient-derived 3D model of glioblastoma multiforme for the developing of extracellular vesicles-based drug delivery system

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Keywords: Glioblastoma, biological twin, digital twin, 3D spheroids, precision medicine.

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Morpho-molecular characterization of cell interplay in cholangiocarcinoma associated with primary sclerosing cholangitis

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Primary Sclerosing Cholangitis (PSC) is a chronic cholangiopathy characterized by inflammation and fibrosis in large intrahepatic and extrahepatic bile ducts. PSC is a known risk factor for cholangiocarcinoma (CCA)[1]; although peribiliary glands (PBGs) have been implicated in PSC-related carcinogenesis, morphological and molecular modifications that occur in the PSC to CCA progression are not well understood[2]. Our aim was to evaluate the cellular interplays of both PSC and PSC-associated CCA (PSC-CCA) by combining histomorphology with spatial proteomics. Samples from healthy controls (N=5), PSC (N=10), and PSC-CCA (N=7) were analysed. Spatial proteomic analysis was performed by GeoMx® DSP to separately study the epithelial, stromal and inflammatory cell fractions. Large intrahepatic bile and hepatic ducts from PSC patients showed increased wall thickness, collagen content, PBG area and inflammation compared to controls. In PSC-CCA, the duct wall was characterized by neoplastic glands occupying a higher area compared to PSC. In turn, a reduction in collagen fibre content and inflammation was present in PSC-CCA compared to PSC. At spatial proteomics, the immune cell fraction in PSC was characterized by an increase in myeloid, B and T cells markers compared to controls. The inflammatory profile was associated with increased expression of activation markers in the neighbouring myofibroblasts and PBGs. In PSC-CCA, a reduction in CD8 and CD56 expression within the inflammatory cells around neoplastic glands was revealed, compared to PSC, and confirmed by immunohistochemistry. In conclusion, our study provides a description of morphological changes in the progression from PSC to PSC-CCA and the modification of cell interplays among the inflammatory, stromal and epithelial cell compartments in cholangiocarcinogenesis. The extensive characterization of immuno-modulating cross-talks could clarify the role of immune evasion processes in neoplastic transformation during PSC.

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Keywords: biliary tree, digital morphology, spatial molecular analysis, cholangiocarcinoma, tumour microenvironment.

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Patient-derived 3D Glioblastoma spheroids recapitulate microanatomical complexity and enable functional investigation of tumor progression pathways

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Glioblastoma (GBM) remains the most aggressive primary brain tumor, with poor prognosis driven by extensive intratumoral heterogeneity, therapeutic resistance, and recurrence [1,2]. Patient-derived three-dimensional (3D) models offer the opportunity to recapitulate key structural and functional features of GBM while providing a clinically relevant platform for investigating disease-driving pathways [3]. We established patient-derived 3D GBM spheroids and characterized their growth dynamics, morphology, cellular organization, and ultrastructural features using scanning and transmission electron microscopy (SEM-TEM). Furthermore, morpho-phenotypical analyses of our model revealed the establishment of spatially organized functional niches, including a hypoxic core and a proliferative peripheral zone, closely recapitulating the in vivo structural organization of GBM. To explore the utility of this model for pathway-oriented studies, we investigated SCF/c-KIT signalling, a receptor tyrosine kinase pathway involved in cell growth and survival. c-KIT is a proto-oncogene encoding the stem cell factor receptor and is frequently dysregulated in several malignancies, including gastrointestinal stromal tumors and melanoma [4]. The aim of this study was to investigate the role of c-KIT signalling in GBM, with a specific focus on its interaction with the molecular chaperone HSP90. As HSP90 is known to stabilize multiple oncogenic client proteins, we hypothesized a functional association between c-KIT and HSP90 in GBM. To address this, we evaluated their interaction and co-localization using immunoprecipitation and immunofluorescence-based analyses. In conclusion, patient-derived 3D GBM spheroids recapitulate the microanatomical complexity and heterogeneity of GBM and provide a robust platform for functional investigation of tumor progression pathways. This model supports the exploration of the c-KIT-HSP90 axis as a potential therapeutic vulnerability for targeted strategies in GBM.

Acknowledgments: Finanziato dall'Unione europea – NextGenerationEU through the Italian Ministry of University and Research "Avviso per la concessione di finanziamenti destinati ad Iniziative di ricerca per tecnologie e percorsi innovativi in ambito sanitario e assistenziale" finanziato a valere sulle risorse previste dal Fondo complementare al Piano Nazionale di Ripresa e Resilienza - PNC0000001 - D3 4 Health Digital Driven Diagnostics, prognostics and therapeutics for sustainable Health care. Finanziato dall'Unione europea – Next Generation EU" componente M4C2, investimento 1.1, "Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)" – CUP: B53D23032960001, Implementation of a patient-derived 3D model of glioblastoma multiforme for the developing of extracellular vesicles-based drug delivery system

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Keywords: Glioblastoma, biological twin, 3D spheroids, c-KIT-HSP90 axis, targeted strategies.

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The intestinal barrier of young and aged mice: focus on goblet cells

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The gut barrier undergoes significant changes with age, affecting both local immunity and general homeostasis (1). However, the mechanisms regulating the interplay between the immune response and the external environment throughout life have not been fully elucidated (1). The intestinal epithelial layer, a monolayered columnar epithelial cells closely connected by tight junctions, prevents excessive contact of antigens with the immune cells and thereby also protects the gut from unwanted immune reactions (2). Specialized intestinal epithelial cells (IECs), such as goblet cells (GCs) play an essential role in gut defense, regulating the mucus layer and secrete biologically active products which make them crucial components of the innate immune system with a fundamental role in the sophisticated organization of the intestinal epithelial barrier (2). Aging is accompanied by a progressive decline of the structural and functional integrity of the epithelial barrier, resulting in increased permeability ("leaky gut") to external substances and leading to inflammation, which contributes to age-related diseases (3).

Evidence suggests associations between inflammation/senescence and the presence of chronic diseases in the elderly, thus the aim of the study has been to describe the differences in the morphological aspects of the intestinal wall of young and aged mice.

Specimens of small intestine of C57BL/6J mice were collected and intestinal sections underwent to: (i) histological procedures using hematoxylin & eosin and periodic acid-Schiff staining, (ii) immunohistochemical procedures using Muc2, Occludin (OCLN) and Claudin (CLDN), as specific marker of the epithelial barrier.

In this preliminary study, the small intestinal mucosa of young mice revealed significant differences compared to old mice: longer villi with regular shape; larger number of GCs; increase of expression of Occludin, suggesting a more structured architecture of tight junction. Our observations provide morphological insights into the interplay between IECs and the immune system, clarifying the role of GCs in GI barrier maintenance during aging. These findings show changes in the: (i) morphology of intestinal villi; (ii) density and distribution of the goblet cells; (iii) permeability profile in aged models suggesting a dysregulated communication between IECs and the immune system.

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Keywords: Gut barrier, intestinal epithelial cells (IECs), goblet cells (GCs), aging, Occludin (OCLN.

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Pain and gain in ultrastructural 3D reconstruction of the peripheral nervous system

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Serial block-face scanning electron microscopy (SBF-SEM) is an automated technique for three-dimensional (3D) specimen acquisition at ultrastructural resolution. It combines low-voltage backscattered electron imaging of subsurface layers with sequential sectioning by an in-chamber ultramicrotome, which progressively removes material along the z-axis. This approach enables the generation of high-resolution 3D datasets, providing a more comprehensive and accurate representation of biological structures.

Although SBF-SEM has been extensively applied in studies of the central nervous system, its use in the peripheral nervous system (PNS) remains relatively limited.

In the first part of this study, we addressed key challenges related to sample preparation and 3D reconstruction by developing semi-automated workflows for batch image processing and volume rendering. We also evaluated the feasibility of uranyl acetate-free -2014946362 *en bloc* staining for ultrastructural analysis of peripheral nerve and dorsal root ganglion specimens, as well as the suitability of samples originally prepared for transmission electron microscopy (TEM) for SBF-SEM imaging. Our results demonstrate that automated uranyl-free staining combined with hard resin embedding represents a viable alternative to the gold-standard manual uranyl-based protocol, although certain limitations remain. Furthermore, we showed that peripheral nerve specimens prepared using standard TEM protocols can yield high-quality SBF-SEM datasets, thereby enabling the analysis of previously embedded samples that were not originally intended for 3D imaging [1,2].

Then, since automated analysis of peripheral nerve ultrastructure is bottlenecked by heterogeneous electron microscopy datasets, where varying staining protocols and resolutions create domain shifts that confound deep learning, we developed a generalized segmentation pipeline. Using a custom image pre-processing Python script (Contrast Limited Adaptive Histogram Equalization,noise suppression and z-stack normalization) integrated into ZEISS Arivis Pro, we standardized inputs across three disparate domains: traditional osmium-based Palade, lanthanide-based uranyl-free method, and low-resolution Ellisman preparations [3].

Finally, we applied the optimized SBF-SEM protocol to a complex animal model of dysmyelinating neuropathy characterized by the formation of abnormal myelin loops with highly irregular morphology. The aim was to clarify axon–myelin interactions that are difficult to interpret from conventional two-dimensional (2D) sections. Our findings demonstrate that 3D imaging provides a far more realistic reconstruction of pathological myelin architecture. Specifically, we found that: (i) myelin outfoldings that appear focal and restricted to a subset of fibers in 2D images actually are continuous structures involving all fibers; and (ii) the observed axonal damage is caused by focal myelin infoldings that are scarcely detectable in 2D sections but result in severe axonal compression.

Overall, our findings highlight the value of SBF-SEM-based 3D reconstruction for the investigation of the PNS. However, the successful application of this technique requires the adoption of specific preparation and imaging protocols tailored to PNS tissues.

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Keywords: serial block face, scanning electron microscopy, peripheral nervous system, 3D reconstruction.

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Dental pulp-derived mesenchymal stromal cells (DP-MSCs) as a possible stromal model for studying the myeloid leukemic microenvironment

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The leukemic progression is influenced by the bone marrow (BM) microenvironment and the involvement of mesenchymal stromal cells (MSCs) in regulating leukemic cell biology needs further exploration [1]. BM-MSCs represent the gold standard for such studies, but their accessibility remains limited as a bone marrow aspirate is needed. Dental pulp-derived MSCs (DP-MSCs) instead represent a more accessible source as they are isolated from teeth extracted for orthodontic reasons, that are usually discarded. Here, we compared DP-MSCs to BM-MSCs, using the reference HS-5 stromal cell line, and the THP-1 leukemic cell line as the leukemic/myeloid counterpart.

Co-cultures were established under both direct contact and transwell conditions to discriminate signals dependent on cell-to-cell contact from those mediated by soluble factors. After 144 hours, cell populations were analyzed separately by flow cytometry using CD73 as a discriminating marker, assessing the expression of myeloid differentiation markers CD11b and CD14 and cell cycle progression via Propidium Iodide staining. Clonogenic capacity was evaluated by methylcellulose assays (MethoCult).

Our results show that both DP-MSCs and HS-5 cells induce an increase in the expression of CD11b and CD14, enhance cell cycle progression, and increase clonogenic capacity in THP-1 cells. Effects were more pronounced under direct contact conditions than in transwell settings, suggesting a stronger mechanism involving contact-dependent signals with an additional contribution of soluble factors. Quantitatively, HS-5 cells produced stronger effects than DP-MSCs, likely reflecting the greater homogeneity of the immortalized line compared to primary cells.

Taken together, these preliminary findings support DP-MSCs as an accessible and functionally relevant alternative to BM-MSCs to support hematopoietic cells, encouraging their broader adoption in in vitro models of the leukemic microenvironment. However, a broader set of experiments is needed to confirm their functional equivalence.

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Keywords: Leukemia, Mesenchymal stromal cells, Dental Pulp, Flow cytometry.

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AI-based analyses of whole slide images and multiplex imaging for the study of cell spatial relationships in inflammatory and neoplastic biliary diseases

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Inflammatory and neoplastic diseases of the liver and biliary tracts are characterized by an intricate cross-talk between inflammatory, epithelial and fibrogenetic cells. Multiple cell subpopulations have been individuated in chronic biliary diseases and biliary tract cancer[1]. The aim of the present study was to test the application of AI-based image analysis to multiplex imaging and digital histo-morphology in the study of human biliary diseases. Samples from healthy controls (N=3), Primary Sclerosing Cholangitis (PSC: N=3), and cholangiocarcinoma (CCA: N=3) were included. Serial sections were processed for H&E and immunohistochemistry (IHC). Multiple imaging was performed by SignalStar® (Cell Signaling Technologies). Stained sections were imaged by Aperio CS2 scanner and by confocal microscope. Digitalized slides were processed by HALO AI® (Indica Labs). Firstly, serial sections were registered by the HALO AI® software. The epithelial cell fraction of interest (i.e. peribiliary glands: PBG) was recognized by a previously trained AI-based segmentation model. CD8+ cells around PBGs were recognized in IHC stained sections and automatically counted. The analysis revealed a increased number of CD8+ cells in PSC compared to controls; this number was reduced in CCA compared to PSC samples. Then, CD8+ cells and segmented PBG were plotted together to study their spatial relationship. In PSC, the number of CD8+ cells closer than 100μm was increased compared to controls. In CCA samples, the number of CD8+ cells close to PBGs was reduced compared to PSC. By multiplex imaging, several subpopulations of inflammatory cells have been identified, including B, T helper, cytotoxic T, dendritic cells and macrophages. The use of HALO AI® resulted in the analysis at single cell level of the number and localization of these subpopulations with respect to the epithelial counterpart both in PSC and CCA. In conclusion, our study demonstrated the potentiality of combining multiple imaging (bright field or immunofluorescence) with AI-based image analysis system to provide novel insights on tissue microenvironment in biliary diseases.

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Keywords: biliary tree, digital morphology, artificial intelligence, multiplex imaging, tumour microenvironment.

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siRNA-Mediated Sarcoglycan Silencing: Their Role In Cytoskeletal Organization And Cell Adhesion In Human Articular Chondrocytes.

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Articular chondrocytes maintain cartilage homeostasis through dynamic interactions with the extracellular matrix (ECM), mediated by focal adhesion complexes and the actin cytoskeleton. While sarcoglycans (SGs) are well known as components of the dystrophin–glycoprotein complex in muscle tissue, their role in chondrocytes remains largely unexplored. The aim of this study was to investigate the expression of α-, β-, γ-, and δ-sarcoglycans in human articular chondrocytes and to evaluate their involvement in cytoskeletal organization and cell adhesion. Human primary articular chondrocytes were cultured in monolayer and analyzed by quantitative PCR and confocal immunofluorescence. The localization of SG isoforms was assessed in relation to F-actin and vinculin through double immunolabeling experiments. To investigate their functional role, SGCA, SGCB, SGCG, and SGCD genes were selectively silenced using siRNA-mediated knockdown, followed by evaluation of cell morphology, actin cytoskeleton organization, vinculin distribution, and focal adhesion formation. All four SG isoforms were detected at both mRNA and protein levels. Immunofluorescence analysis revealed cytoplasmic and membrane localization, with enrichment at cell–substrate interaction sites. SGs showed colocalization with F-actin stress fibers and vinculin, suggesting their integration within focal adhesion complexes. Silencing of each SG isoform induced marked morphological alterations, characterized by elongated and thinner cells, disruption of actin stress fibers, diffuse vinculin distribution, and a significant reduction in focal adhesion number compared with control cells. These findings identify sarcoglycans as novel regulators of actin cytoskeleton dynamics and focal adhesion stability in human articular chondrocytes. Loss of SG expression compromises cell architecture and cell–matrix interactions, highlighting a potential role for these proteins in cartilage homeostasis and suggesting their involvement in mechanisms underlying cartilage degeneration.

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Keywords: Sarcoglycans, Articular chondrocytes, siRNA-mediated gene silencing, Actin cytoskeleton, Focal adhesions.

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Targeting nuclear lamina alterations in glioblastoma: prelamin A accumulation as a novel strategy to impair glioma stem cell malignancy

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Glioblastoma (GBM) is the most common high-grade brain cancer. Despite timely and invasive interventions, the prognosis is very poor due to therapy resistance and tumor recurrence, highlighting the need for innovative therapeutic approaches [1].

In glioblastoma cells, the nuclear lamina structure is profoundly altered, and recent evidence indicates that pharmacologically induced accumulation of prelamin A, the precursor of lamin A, reduces the aggressiveness and stemness of glioma stem cells (GSCs), a group of cancer cells thought to drive therapy resistance [2, 3, 4].

Notably, GBM cells accumulating prelamin A show decreased expression of N-cadherin, a key factor involved in epithelial-to-mesenchymal transition (EMT). In addition, recent findings have identified a novel form of connexin, GJA1-20k (Cx20), implicated in cancer progression and potentially acting as a transcriptional regulator through binding to the N-cadherin promoter [5].

Based on these observations, this study investigates the role of prelamin A in GSCs as a foundation for novel therapeutic approaches, focusing on its potential impact on the intranuclear translocation of Cx20 and the consequent regulation of N-cadherin transcription.

In particular, prelamin A accumulation was induced using a farnesyltransferase inhibitor in glioblastoma cell lines, patient-derived GSCs and human astrocytres as control, cultured both as monolayers and as 3D spheroids. Our preliminary results suggest that drug-induced accumulation of prelamin A promotes redistribution of Cx20 toward a perinuclear localization in both glioblastoma cells and patient-derived GSCs, resembling the pattern observed in control astrocytes.

These findings suggest a link between nuclear lamina defects, prelamin A accumulation, and the Cx20/N-cadherin transcriptional pathway involved in EMT and tumor progression, potentially opening new therapeutic avenues targeting GSCs.

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Keywords: Glioblastoma, Prelamin A, Farnesyltransferase Inhibitor, Glioma Stem Cells, Connexin 20.

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Integrating Focused Shockwave Therapy into Rehabilitation for Groin Pain Syndrome: A Prospective Study in Soccer Players

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Background/Objectives: Groin pain syndrome (GPS) is a frequent and heterogeneous condition in athletes, often associated with persistent pain and functional limitation. Both focused extracorporeal shockwave therapy (f-ESWT) and exercise-based rehabilitation have been proposed as conservative treatment options, but evidence for their combined use in GPS remains limited (1). This prospective single-arm pilot study aimed to describe temporal changes in pain and function following a multimodal conservative program combining f-ESWT and structured rehabilitation in athletes with GPS, using validated groin-specific outcome measures. Methods: Thirty-one consecutive adult soccer players (mean age 28.4 ± 5.8 years; 77.4% male) with clinically and MRI-confirmed GPS underwent three weekly f-ESWT sessions (Duolith; 2400 pulses/session; 4 Hz; energy flux density 0.20 mJ/mm2) integrated within a supervised 16-week rehabilitation program (progressive adductor strengthening, core stabilization, and stretching). Outcomes were assessed at baseline (T0), 1 month (T1), and 4 months (T2): HAGOS (primary), VAS pain, and Roles and Maudsley (RM). Within-subject changes were analyzed using repeated-measures ANOVA with Bonferroni correction. Results: Statistically significant temporal changes were observed across all outcomes (all $p < 0.001$). HAGOSs changed from 47.23 ± 7.79 at T0 to 77.94 ± 16.18 at T1 and 90.00 ± 14.26 at T2 (partial $\eta 2 = 0.89$). VAS decreased from 6.81 ± 1.25 to 3.68 ± 1.11 and 1.90 ± 1.45 (partial $\eta 2 = 0.91$). RM decreased from 2.39 ± 0.50 to 1.52 ± 0.57 and 1.26 ± 0.63 (partial $\eta 2 = 0.72$). No adverse events were reported (2). Conclusions: In this single-arm pilot study, the multimodal program combining f-ESWT and structured rehabilitation was associated with temporal changes in groin-specific function and pain that exceeded established MCID thresholds. However, in the absence of a control group, these findings are purely descriptive and hypothesis-generating. The observed changes cannot be attributed to f-ESWT specifically, as the 16-week rehabilitation program likely contributed substantially to the outcomes. These preliminary observations require confirmation through adequately powered randomized controlled trials comparing the combined intervention to rehabilitation alone.

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Keywords: focused shockwave therapy, groin pain, athletes, rehabilitation, pilot study.

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Mapping ipsilateral motor control: a morphofunctional approach to the corticospinal "trifurcation" model

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The corticospinal tract is predominantly crossed, with most fibers decussating at the bulbomedullar-pyramidal junction and a minor ipsilateral component crossing at spinal segments [1]. However, anatomical and neurophysiological evidence suggests that uncrossed descending influences persist in adults and may support ipsilateral control of proximal upper limb movements and bimanual coordination [2].

Ipsilateral motor evoked potentials (iMEPs) induced by transcranial magnetic stimulation (TMS) allow for non-invasive study of the contribution of ipsilateral motor pathways. However, the anatomical significance is not univocal because the presence of an iMEP does not necessarily indicate the activation of a single anatomical pathway but may reflect the contribution of uncrossed corticospinal fibers, corticoreticulospinal circuits, or the interaction between multiple descending systems [3–5]. We therefore use the corticospinal "trifurcation" as a working anatomical hypothesis to test whether ipsilateral motor output can be stratified into reproducible morphofunctional patterns.

This study intends to construct an in vivo morphofunctional map of ipsilateral motor control by integrating TMS and functional magnetic resonance imaging (fMRI). Healthy participants will undergo bilateral TMS mapping of the primary motor and premotor cortices, with electromyographic recordings from distal hand muscles and proximal upper-limb flexor and extensor muscles. Contralateral and ipsilateral motor evoked potentials (MEPs) will be analyzed for threshold, latency, amplitude, morphology, reproducibility, and somatotopic distribution. Task-based fMRI during unilateral and bimanual movements will characterize motor network lateralization, ipsilateral cortical recruitment, and premotor/supplementary motor involvement.

We expect to detect distinct iMEP phenotypes rather than a single ipsilateral response pattern. Short-latency and reproducible responses may indicate a direct, uncrossed corticospinal component, while longer-latency responses with proximal and flexural predominance may suggest reticulospinal involvement. This multimodal approach may improve the morphofunctional interpretation of ipsilateral motor control, clarify interindividual variability in the organization of descending motor pathways, and support future studies on motor plasticity after central nervous system lesions.

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Keywords: Corticospinal tract, Trifurcation, Transcranial magnetic stimulation, Ipsilateral motor evoked potentials, fMRI.

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Polycaprolactone Improves Mucosal Wound Healing in Guided Bone Regeneration without Evidence of Inflammatory Cell Infiltration

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Polycaprolactone (PCL) is a biodegradable and biocompatible synthetic polymer that has received FDA approval for specific, long-term medical applications. It is commonly used in medical devices such as drug delivery systems, sutures, scaffolds for tissue engineering (bone/cartilage), dermal fillers[1] and recently in production of meshes for oral Guided Bone Regeneration (GBR)[2]. It has tailorable degradation kinetics and mechanical properties and ease of shaping and manufacturing enabling its use also in 3D printing with Fused Filament Fabrication (FFF) additive technique[3]. The biocompatibility of PCL also arises from its mechanical stiffness, which closely resembles that of oral mucosal tissue, as well as from its biodegradation profile, which is characterized by the production of negligible amounts of toxic catabolites. These properties yield a material that, when employed as a mesh for guided bone regeneration (GBR), exhibits a histological profile devoid of detectable inflammatory response. To address this issue, we present here a clinical case of GBR in which a maxillary bone tissue portion aimed to support dental implant placement had been lost as a consequence of infection, as shown from a radiographic analysis resulting from a Cone Beam Computed Tomography (CBCT). Following our algorithm[4], we designed and 3D printed a PCL mesh aimed to rebuilt the lost bone. A mixed autologous/xenogenic bone graft was placed to fill the defect. Then, the PCL mesh was placed to protect the graft, was fixed with some bone screws and was covered with the oral mucosa. After 3 and 6 months of healing two mucoperiosteal biopsies were harvested and processed for Optical (OM), Scanning (SEM) and Transmission Microscopy (TEM). The 3 months H&E-stained tissue showed a connective tissue beneath the epithelial layer with rich vascularization but without significant perivascular inflammatory infiltrate. Conversely, a rich inflammatory infiltrate, predominantly composed of lymphocytes, was visible around the bone xenogenic spicules. Connective tissue close to the PCL mesh revealed an intense staining with elongated cells resembling fibroblasts. 6-months tissue showed a normal oral tissue with the PCL no longer detectable, showing instead a dense meshwork of irregularly arranged collagen fibers, similar to scar tissue. SEM and TEM analysis confirmed what observed at the OM analysis with some stromal cells resembling quiescent fibroblasts, intermingled and closely apposed to the collagen fibers. In conclusion, our study showed that at 3 months after the GBR procedure, the connective tissue surrounding the mesh was free of inflammatory cells, with the fibroblasts showing a phenotypic conversion to fibrocytes in the 6-month biopsy. PCL demonstrated superior biocompatibility even compared to xenogeneic bone and may certainly be used in the future in other surgical applications beyond the dental field.

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Keywords: Polycaprolactone, Dental implants, Tissue Engineering, Bone Regeneration, Fibroblasts, Polymers.

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How age and biology shape the emotional landscape of motherhood

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Childbirth is a stressful event that triggers profound hormonal fluctuations that may be directly linked to psychological changes in the mothers [1, 2, 3]. Therefore, this study aimed to measure maternal hormonal variations during and after pregnancy to identify potential associations with dysfunctional psychological states. We measured cortisol and oxytocin levels during pregnancy and at 1, 6, and 12 months postpartum in mothers divided into two age-related groups: group 1 (20–36 years) and group 2 (37–50 years). Cortisol levels were also monitored in their infants. Saliva samples were collected from at least 16 mother-infant pairs at each time point and analyzed using ELISA. During pregnancy as well as at 6 and 12 months postpartum, maternal depression was assessed through the Edinburgh Postnatal Depression Scale (EPDS), while anxiety was evaluated using the State-Trait Anxiety Inventory (STAI). Hormonal profile analysis across all mothers revealed significant temporal hormonal fluctuations. Maternal cortisol levels were higher during pregnancy but decreased significantly at 1 month postpartum to remain consistently lower but still secreted up to 12 months ($p < 0.0001$). Conversely, maternal oxytocin levels peaked significantly at 1 month and subsequently decreased up to 12 months ($p < 0.05$). These results suggest that pregnancy, rather than the postpartum period, requires higher cortisol levels to adapt to physiological changes, whereas oxytocin is primarily required during pregnancy and the first postpartum month. When hormone levels and psychological states were compared between the two age groups, the analysis highlighted significant differences: specifically, group 2 mothers had infants with significantly higher cortisol levels at 12 months compared to group 1 (mean = 1675.42 vs. 138.40 pg/mL, $t = -2.438$, $p = 0.049$), whereas group 1 showed significantly higher depression scores at 6 months postpartum (6.69 vs. 3.75, $t = 2.350$, $p = 0.028$). Regarding the relationship between endocrine profiles and psychological states across all mothers, significant negative correlations emerged; during pregnancy, lower oxytocin and cortisol levels were associated with higher depression scores ($\tau = -0.306$, $p = 0.027$, and $\tau = -0.309$, $p = 0.026$, respectively). Lower prenatal cortisol levels were also associated with anxiety ($\tau = -0.494$, $p < 0.0001$), and this correlation persisted up to 6 months ($\tau = -0.412$, $p = 0.023$). These findings underscore the importance of incorporating complementary psychophysical assessment tools to support timely interventions for mothers with altered neurohormone profiles .

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Keywords: pregnancy, hormones, post-partum, psychologic state, motherhood.

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CircHIPK3 as a Regulator of Cytoskeletal Dynamics and Stress Vulnerability in TNBC

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype and is characterized by remarkable cellular plasticity, metabolic flexibility, and the ability to adapt to multiple forms of stress, which collectively sustain tumor progression and therapeutic resistance [1,2]. Circular RNAs (circRNAs) have recently emerged as important regulators of cellular homeostasis and stress adaptation through both transcriptional and post-transcriptional mechanisms [3]. Among them, circHIPK3 has been implicated in several oncogenic processes, although its role in coordinating adaptive stress responses remains largely unknown [4]. To define the role of circHIPK3 in proteotoxic stress adaptation in TNBC, we combined functional, imaging, and transcriptomic approaches following circHIPK3 silencing. CircHIPK3 depletion impaired the ability of cells to cope with proteotoxic stress, leading to increased cell death, ROS accumulation, UPR activation, and disruption of ER homeostasis. In parallel, circHIPK3 silencing induced a profound cytoskeletal reorganization, characterized by cortical actin remodeling, increased nuclear height, and YAP nuclear exclusion, indicating altered mechanosensitive signaling. These structural changes were accompanied by perinuclear mitochondrial clustering, consistent with global cellular remodeling. Transcriptomic profiling confirmed enrichment of actin-regulatory pathways following circHIPK3 depletion, with ROCK2 and LIMK1 emerging as candidate mediators. Accordingly, pharmacological ROCK inhibition upon circHIPK3 downregulation partially restored cytoskeletal organization, nuclear morphology, and YAP localization, suggesting players involved in ROCK signaling as circHIPK3 targets. Moreover, circHIPK3 depletion was associated with upregulation of the ER oxidoreductase ERO1α, contributing to dysregulation of ER redox homeostasis and ROS accumulation. Collectively, our findings identify circHIPK3 as a regulator of proteotoxic stress adaptation in TNBC and highlight a connection between cytoskeletal organization and stress responses. Whether cytoskeletal remodeling represents a parallel function of circHIPK3 or a determinant of impaired stress adaptation remains an open question that warrants further investigation.

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Keywords: TNBC, circRNAs, stress response, cytoskeletal dynamics.

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Chrysin promotes resolution of LPS-induced neuroinflammation by modulating glial reactivity and inflammatory response

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Neuroinflammation, mainly driven by activated microglia and astrocytes, plays a key role in the pathogenesis of several neurodegenerative disorders [1]. Chrysin, a natural flavonoid found in fruits, vegetables and mushrooms [2] has been reported to exert anti-inflammatory and neuroprotective effects [3]. In this study we investigated the impact of chrysin on lipopolysaccharide (LPS)-induced *in vivo* mouse model of prolonged inflammatory response, focusing on two different brain areas, namely caudate-putamen (CP) and hippocampus (HIP). The effects of chrysin at 72h on cytokine expression, particularly of the anti-inflammatory cytokine IL-10 and pro-inflammatory IL-6 cytokine, Toll like receptor 4 (TLR4) and phosphoinositide 3-kinase (PIK3) were evaluated by qPCR. In addition, confocal microscopy was performed using the microglial marker IBA1 and the astrocytic marker GFAP. As a result, LPS induced a marked neuroinflammatory response in both brain regions, characterized by increased IL-6 expression, modulation of innate immune signalling pathways including TLR4 and PIK3, and marked activation of microglia and astrocytes as shown by the IBA1 and GFAP staining. Chrysin alone induced a significant upregulation of IL-10, suggesting that it promotes a strong anti-inflammatory immunoregulatory environment. Notably, chrysin pretreatment in LPS-chrysin mice significantly attenuated LPS-induced neuroinflammation and reshaped late inflammatory state. Overall, these findings demonstrate that chrysin counteracts LPS-induced neuroinflammation reducing microglial and astrocytic activation and creating an immunomodulatory environment, shaping the resolutive phase of inflammation. These findings support the potential role of chrysin as an important neuroprotective compound in neuroinflammatory dysfunctions.

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Keywords: Chrysin, LPS, neuroinflammation, microglia, astrocyte, mouse model.

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Role of Phosphoinositide-specific Phospholipases C PLC in 143B human osteosarcoma: evaluation of cell adhesion and mechano-transduction

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Osteosarcoma (OS) is a rare but aggressive primarily bone cancer which affects primary children and young adults[1]. The diagnosis occurs often when the tumor is in an advanced stage, and frequently at that moment a metastatic spread of the disease has already occurred, drastically lowering the survival expectancy of the patients[1]. The OS pathophysiology is still not completely understood and recently, among the several signaling pathways involved, the phosphoinositide pathway and phospholipase C (PLC) related enzymes are starting to gain interest among the scientific community for their involvement in OS progression[2,3]. The aim of this study is investigating how PLCs modulation affects OS cell growth rate, motility and migratory capabilities through pharmacological inhibition and isoform-specific silencing.

Treatment with the PLC inhibitor U73122 significantly impaired cell growth and partially altered cytoskeletal organization in 143B OS cells. To investigate isoform-specific roles, *PLCB1*, *PLCB2* and *PLCG2* were selectively silenced using siRNAs. *PLCB2* and *PLCG2* exhibited functional cross-regulation, as silencing of either isoform altered the expression of the other and produced similar effects on focal adhesions, cytoskeletal organization, cell migration, and YAP signaling. Changes in cytoskeletal architecture and lamin expression further suggested impaired mechanotransduction. Functional assays confirmed increased invasive capacity following *PLCB2* or *PLCG2* silencing. Public transcriptomic datasets supported these findings, revealing coordinated downregulation of *PLCB2* and *PLCG2* in osteosarcoma and an association between *PLCG2* expression and patient survival. In contrast, *PLCB1* downregulation promoted adaptive cellular remodeling without inducing a fully coordinated migratory phenotype.

Taken together, these findings highlight a role for PLCβ2 and PLCγ2 in regulating OS cell behavior and suggest that PLC-dependent pathways may contribute to OS aggressiveness.

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Keywords: Phospholipase C, Osteosarcoma, Cell migration, Cell adhesion, Mechano-transduction.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Morphological and molecular analysis of PKCε expression in dorsal root ganglia in a rat model of fibromyalgia

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Fibromyalgia is a chronic pain syndrome characterized by widespread musculoskeletal pain, fatigue, and altered nociceptive processing [1]. Although both central and peripheral mechanisms have been implicated, the molecular pathways underlying sensory neuron sensitization remain incompletely defined. Protein kinase C epsilon (PKCε), a serine/threonine kinase involved in several cellular processes [2, 3], is highly expressed in sensory neurons and has been associated with hyperalgesia, ion channel modulation, and neuroinflammatory signaling [4, 5]. This study aimed to investigate PKCε expression and activation in dorsal root ganglia (DRG) in a reserpine-induced rat model of fibromyalgia. Experiments were conducted on Sprague-Dawley rats of both sexes, which were randomized to receive vehicle (acetic acid 0.5%) or reserpine (1mg/kg). To monitor the development of the fibromyalgia-like phenotype, mechanical allodynia and thermal hyperalgesia were assessed by von Frey and hot plate tests, respectively. Behavioural evaluations were performed before the first treatment on day 1, after three consecutive daily injections of reserpine or vehicle on day 4, and weekly thereafter up to day 28. Animals were euthanized on days 4 and 28, and cervical, thoracic, and lumbar DRG were collected for molecular and morphological analyses. PKCε gene expression was evaluated by RT-PCR, while total and phosphorylated PKCε protein levels were assessed by Western blot. Immunohistochemical analyses were performed to localize PKCε and phosphorylated PKCε in DRG tissue and to evaluate their colocalization with neuronal markers. Since behavioural testing, particularly the hot plate assay, showed heterogeneous results, molecular analysis was performed based on the von Frey-derived mechanical sensitivity profile of reserpine-treated animals. In reserpine-treated and responsive animals, RT-PCR showed an increase in PKCε expression in lumbar DRG compared with controls, whereas cervical and thoracic DRG displayed comparable gene expression levels. Interestingly, western blot analysis revealed increased levels of PKCe and phosphorylated PKCε in cervical and thoracic DRG of reserpine-treated von Frey-responsive rats, suggesting enhanced PKCε activation in animals displaying mechanical allodynia. At later time points, PKCε and phosphorylated PKCε levels returned to values comparable to controls, indicating that these changes may represent an early and transient response. Immunohistochemistry confirmed the quality of DRG samples, as shown by preserved sensory neuron morphology and NeuN positivity, and supported increased PKCε and phosphorylated PKCε expression in DRG from reserpine-treated and responsive rats. Overall, these preliminary findings suggest that PKCε activation may be involved in early peripheral sensory alterations associated with mechanical allodynia in a reserpine-induced fibromyalgia model. Although further studies are required to validate these results and clarify the functional role of PKCε, the present morphological and molecular evidence supports PKCε as a potential contributor to pain-processing pathways and as a candidate target for future mechanism-based studies in fibromyalgia.

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Keywords: PKCε, dorsal root ganglia, fibromyalgia, mechanical allodynia, pain, sensory neurons.

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Training the Morphological Mindset: Teaching Morphological Reasoning in the Era of Digital Imaging and AI

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In contemporary anatomy and histology education, students are increasingly exposed to large image repositories, digital platforms, and advanced imaging technologies, while explicit training in morphological reasoning often remains underdeveloped. Learners may become proficient in structure recognition without fully acquiring the interpretative skills required to describe visual evidence, relate form to function, and critically interrogate biological images. Here, we present an educational framework developed within the MorphoLAB training environment to make morphological reasoning explicit in laboratory-based education across anatomy, histology, and biomedical imaging activities. The approach is centered on the "Ten Rules of the Morphologist", a set of guiding principles designed to promote careful observation, precise description, interpretation, integration across scales, and critical questioning. The framework combines structured image observation, reflective notebook practices, and guided discussion to externalize expert reasoning and foster interpretative autonomy. Experience from laboratory-based teaching activities suggests improved descriptive precision, increased engagement with image interpretation, and greater tolerance for ambiguity when students are confronted with complex visual datasets. By shifting the focus from purely technical or recognition-based training to the cultivation of a morphological mindset, this approach proposes a transferable strategy for morphology education. In an era increasingly shaped by digital imaging and artificial intelligence, morphological literacy remains essential to transform images into biological questions, mechanistic hypotheses, and meaningful scientific explanations.

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Keywords: Morphological reasoning, Visual literacy, Artificial intelligence.

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Generation of F0 *Dtwd1* Crispants for *In Vivo* Functional Studies in Zebrafish

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Whole-exome sequencing has enabled the identification of an increasing number of rare genetic variants, many of which require functional validation to establish their pathogenic relevance. We identified a homozygous variant of uncertain significance (VUS) in *DTWD1*, c.198G>C (p.Met66Ile), in a 33-year-old male patient presenting with cerebellar ataxia, cerebellar vermis atrophy, severe kyphoscoliosis, muscle hypertrophy and hypotonia, bilateral clubfoot, conjunctival and auricular telangiectasia, congenital cataracts. Segregation analysis confirmed an autosomal recessive inheritance pattern. *DTWD1* encodes a nuclear tRNA modification enzyme involved in the formation of acp3U at position 20 of tRNAs, a modification essential for tRNA stability and translational fidelity [1]. Alterations in tRNA modification pathways have been associated with a growing group of disorders known as tRNA-modopathies, many of which present with neurodevelopmental features [2]. Given the limited understanding of *DTWD1* function and its potential role in neurodevelopmental disorders, this study aims to establish a CRISPR/Cas9-based zebrafish model to investigate the effects of *dtwd1* loss of function during vertebrate development. *Danio rerio* embryos were used as an in vivo model system [3]. A multi-sgRNA CRISPR/Cas9 strategy was developed to induce efficient mosaic disruption of *dtwd1* in F0 crispants, thereby increasing mutagenesis efficiency and enabling rapid assessment of phenotypic consequences [4]. Three sgRNAs targeting *dtwd1* were designed and optimized for CRISPR/Cas9-mediated genome editing in zebrafish embryos. Dose-response experiments identified optimal injection conditions that ensured efficient gene targeting while maintaining embryo viability. Functional validation and phenotypic characterization of F0 crispants provided novel insights into the role of *dtwd1* during vertebrate development and supported further investigation of its contribution to human disease. Further studies are underway to assess the developmental consequences of *dtwd1* disruption.

Given the limited functional and clinical evidence currently available for *DTWD1*, *in vivo* characterization in zebrafish represents a valuable experimental approach to investigate gene function and support the clinical interpretation of the homozygous VUS identified in the patient.

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The protective role of Leonurine in ameliorating astrogliosis: putative implications for BBB integrity.

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Background: Astrocytes are essential regulators of blood–brain barrier (BBB) integrity and neurovascular unit (NVU) homeostasis [1]. Under pathological conditions or following exposure to environmental pollutants action, astrocyte activation contributes to BBB dysfunction through the release of inflammatory mediators, disruption of neurovascular signaling, and induction of cellular stress responses [2]. Leonurine, a natural alkaloid derived from Leonurus species, has demonstrated antioxidant and neuroprotective properties [3], but its effects on human astrocyte dysfunction remain largely unexplored. **Methods**: Human SVGp12 astrocytes and HBEC5i brain endothelial cells were exposed to increasing concentrations of Leonurine (1-300 μg/mL) under starved condition (0% FBS). Cells were treated with Leonurine, and cell viability assay, immunofluorescent staining and western blotting analysis were performed. **Results**: After 24h of starvation condition, Leonurine did not alter the morphology of human astrocytes or brain endothelial cells. On the other hand, Leonurine treatment significantly reduced the GRP78 protein expression, an endoplasmic reticulum stress marker in human astrocytes. Concomitantly, a 30% reduction in the astrogliosis markers GFAP and S100β was observed by immunofluorescence staining. No changes in GRP78 expression or tight junction alterations were observed in HBEC5i endothelial cells. **Conclusions**: Our findings demonstrate that Leonurine attenuates astrocyte reactivity as shown by reduced GFAP and S100β expression suggesting a reduction in astrocyte-mediated inflammatory signalling, concurrent with a significant reduction in ER stress in human SVGp12 astrocytes, whereas no effects were observed in brain endothelial cells. Given the central role of astrocytes in maintaining BBB integrity, these findings suggest that Leonurine may exert protective effects on the neurovascular unit, contributing to BBB stability.

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Keywords: Leonurine, Neuro-Vascular Unit, Blood-Brain Barrier, Astrocytes, GFAP.

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Gut mucosal barrier alterations, enteric neuroplasticity and colonic inflammation in a rat model of environmental parkinsonism induced by intragastric administration of paraquat and lectins

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Accumulating evidence support the contention that Parkinson's disease (PD) can be considered a gut–brain axis disorder. Indeed, enteric α-synuclein (α-syn) accumulation, disruption of the intestinal epithelial barrier (IEB), and enteric inflammation in PD patients besides determining intestinal dysfunctions, can contribute to brain pathology via gut-brain ascending signalling. In the present study, we investigated structural and molecular changes affecting the IEB, activation of colonic inflammatory pathways, and enteric nervous system (ENS) remodeling in an environmental model of PD characterized by the progressive propagation of α-syn pathology from the gastrointestinal tract to the central nervous system. Rats received daily intragastric administration of subthreshold doses of paraquat (PQ, 1 mg/kg) and dietary lectins (L, 0.05%) for 7 consecutive days. In this model, pathological α-syn aggregates emerge firstly within the ENS and subsequently in the substantia nigra pars compacta. Indeed, gastric dysfunction precedes the onset of motor deficits characteristic of parkinsonism [1]. To assess the long-term intestinal consequences of environmental parkinsonism, animals were euthanized 12 and 16 weeks (wks) following PQ+L exposure. Proximal and distal colon were collected and analyzed for: (i) IEB integrity, through immunofluorescence staining of MUC-2 and claudin-1; (ii) activation of NLRP3 inflammasome-related pathways, evaluated by Western blot analysis of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1 and interleukin (IL)-1β, together with double immunofluorescence for Iba-1/ASC and GFAP/ASC to identify inflammasome activation in macrophages and enteric glial cells, respectively; and (iii) enteric neuronal coding, assessed by immunofluorescence for choline acetyltransferase (ChAT) and substance P (SP). Morphological and molecular analyses revealed a marked reduction of MUC-2 expression in both proximal and distal colon at 12 and 16 weeks after PQ+L administration. Claudin-1 expression was significantly decreased in the distal colon from PD rats at both time points as compared with controls. Furthermore, activatino of NLRP3 inflammasome signaling was observed in the distal colon of PQ+L-treated rats, as indicated by increased levels of ASC, caspase-1, and IL-1β, along with enhanced ASC immunoreactivity in Iba-1$^+$ macrophages and GFAP$^+$ enteric glial cells. PQ+L exposure also induced significant ENS remodeling, characterized by increased ChAT and SP immunostaining. Overall, chronic exposure to low doses of PQ and lectins resulted in persistent impairment of the IEB, activation of enteric NLRP3 inflammasome signaling, and ENS plasticity. These effects were particularly evident in the distal colon and may underlie alterations in colonic motility observed in this experimental model of environmental Parkinson's disease.

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Keywords: environmental Parkinson's disease, paraquat, gut-brain axis, intestinal epithelial barrier, gut inflammation.

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Innovative food intervention with probiotics and policosanols counteracts obesity through the modulation of enteric glial cells

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Obesity is closely associated with intestinal epithelial barrier (IEB) dysfunction, chronic low-grade inflammation and gut dysmotility [1, 2]. In this context, the modulation of IEB functions through the manipulation of gut microbiota with probiotics or bioactive natural compounds, such as policosanols, has emerged as a promising strategy to counteract obesity and related-gut dysfunctions [3]. However, the combined effects of probiotics and policosanols in the context of obesity remain poorly characterized. The present study aimed to evaluate the beneficial effects of an innovative mixture, containing probiotics and policosanols, in a murine model of high-fat diet (HFD)-induced obesity, with particular attention to IEB permeability, enteric inflammation, and gut motor abnormalities. Male C57BL/6J mice were fed either a standard diet (SD) or HFD for 8 weeks. The mixture formulation was administered by oral gavage either from the beginning of the dietary protocol (preventive treatment) or after 4 weeks of HFD exposure (curative treatment). At the end of the experimental period, body weight gain, body mass index (BMI), and metabolic alterations were assessed. In parallel, IEB integrity and permeability was evaluated through circulating lipopolysaccharide-binding protein (LBP) levels, tight junction protein expression and distribution. Colonic inflammation and enteric glial activation were investigated by analysing interleukin (IL)-1β levels and glial fibrillary acidic protein (GFAP)$^+$ cells, respectively. Moreover, fecal butyrate levels and expression of the sodium-coupled monocarboxylate transporter 1 (SMCT1) were examined. Colonic motor responses were evaluated by functional studies on tachykininergic contractions and substance P (SP) expression. HFD exposure induced significant body weight gain and metabolic impairment, associated with marked disruption of IEB integrity, as documented by reduced tight junction expression and increased circulating LBP levels. In addition, obese mice displayed decreased fecal butyrate content and reduced SMCT1 expression, together with enhanced enteric gliosis, increased IL-1β production, and elevated GFAP$^+$/IL-1β$^+$ cells in the colon. These alterations were accompanied by increased electrically evoked tachykininergic contractions and colonic GFAP$^+$/SP$^+$ cells. Treatment with the mixture significantly improved all these parameters in both preventive and curative protocol. In particular, the mixture restored butyrate availability, reduced enteric glial activation and inflammatory signaling, and normalized colonic motor dysfunctions. In conclusion, the present findings demonstrate that the combined administration of probiotics and policosanols effectively counteracts obesity-related intestinal barrier dysfunction, enteric inflammation, and gut dysmotility. These beneficial effects are likely mediated by modulation of the butyrate-dependent enteric glial pathway involving IL-1β and SP signaling.

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Keywords: food supplementation, enteric glial cells, intestinal barrier, intestinal inflammation, probiotics, obesity.

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Immunomodulatory effects of nanoplastics on human natural killer and T Cells

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Introduction: Micro- and nanoplastics are increasingly recognized as emerging environmental contaminants with potential implications for human health. Although their widespread distribution has been documented across environmental matrices, evidence of their presence in human biological samples and their effects on immune function remains limited. This study aimed to investigate the impact of nanoplastic exposure on human peripheral blood mononuclear cells (PBMCs), with a particular focus on natural killer (NK) and T cells. Methods: PBMCs from 20 healthy donors were isolated from peripheral blood by Ficoll-Paque density gradient centrifugation and cultured for 72 hours. To support NK-cell survival and maintenance, IL-15 (0.5 ng/mL) was added to the culture medium. Cells were exposed to three types of 50 nm nanoplastics (polystyrene, polyethylene, and polypropylene) at a concentration of 2 μg/mL, consistent with previously reported nanoplastic blood levels. Experiments were performed under both unstimulated and PMA/ionomycin-stimulated conditions to assess the effects of nanoplastic exposure on immune activation and function. Cell viability, activation, inhibitory marker expression, and cytokine production were evaluated. Immune phenotyping and functional analyses were performed using two 15-color flow cytometry panels including lineage-specific, activation, checkpoint, and intracellular cytokine markers. Results: Nanoplastic exposure was associated with significant alterations in activation and inhibitory marker expression across NK, NKT-like, and T-cell subsets. Compared with untreated controls, nanoplastic-exposed cells displayed increased expression of activation markers, including CD25, CD69, NKG2D, and NKp46 ($p \leq 0.05$). These changes were observed under both stimulated and unstimulated conditions, suggesting that nanoplastics may enhance immune activation and promote a NK pro-inflammatory profiles. Furthermore, the observed modulation of activation and inhibitory pathways indicates that nanoplastics can directly interact with circulating immune effector cells and influence their functional state. Conclusions: Our findings demonstrate that exposure to nanoplastics induce measurable phenotypic and functional changes in human NK and T cells. These results support the hypothesis that nanoplastics may act as immunomodulatory agents capable of altering immune homeostasis. Further studies are warranted to elucidate the molecular mechanisms underlying nanoplastic–immune cell interactions and to determine their potential clinical relevance in exposed individual.

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Spinal and whole-body posture assessed on sagittal plane during Sit-to-Stand movement in healthy young adults

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Sit-to-Stand (STS) is a complex task requiring trunk-limbs coordination [1]. Most studies investigate the kinematics of lower lims-pelvis and trunk considering the spine as a single segment and excluding head movements and whole-body biomechanics [2]. This study uses a specific 3D-Motion Analysis protocol [3] in order to evaluate whole-body and multi-segmental spinal kinematics on sagittal plane in healthy subjects during STS phases.

Twenty healthy young subjects (23.5±3.7 years) performed STS from a standardized starting position (100% knee height and about 90°hip-knee-ankle). Kinematics were captured via 3D-Stereophotogrammetry using the DB-Total marker set. Two key events were identified: Lift-Off (LO), the start of buttocks clearance, and Lumbar Reverse (LR), the inflection point where the lumbar curve transitions from lower to higher lordosis. Descriptive values of Spatial-temporal and kinematic parameters were evaluated in main events (start, Lift-Off, Lumbar Reverse, End).

LR (18.7% of the STS cycle) consistently preceded LO (27.5%), identifying a preparatory phase of detaching from the sitting position. The lumbar spine reached minimum lordosis exactly at the LR event (Lumbar Angle: 179.8°). Crucially, S2-L5 sagittal distance peaked at this instant, indicating a specific lumbopelvic flattening occurring before LO and subsequent lordosis. The trunk achieved its maximum forward inclination after LO (about 40% of STS cycle), where S2-C7 and Heel-S2-nasion angles show lowest peak. The C7-Nasion angle shows a "head-lead" strategy suggesting that neck extension is functional to maintain a horizontal visual field during all STS phases.

This is the first study to define the whole-body angle and the "Lumbar Reverse" event during STS. These normative data provide a benchmark for identifying pathological compensatory patterns on sagittal plane in pathological populations.

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Keywords: Sit-To-Stand, posture, spine, lumbar reverse, sagittal kinematics, 3D motion analysis.

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Anatomical-functional relationship between biacromial distance and foot stance width with countermovement jump performance

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Biacromial distance is an anatomical parameter that is often used as an anthropometric reference for foot stance width before specific motor tasks, such as jumping. In particular, the countermovement jump (CMJ) test is widely used to evaluate lower-limb neuromuscular performance [1] and foot placement might affect motor outcomes [2,3]. Therefore, the present study aimed to investigate how different foot stance widths affect CMJ performance assessed by an inertial measurement unit. As a secondary aim, the relationship between biacromial distance and self-selected foot stance was also explored. Twenty healthy participants performed CMJs under four randomized foot-position conditions: 0.7×, 1.0×, 1.3× biacromial width, and a self-selected stance. Before jump testing, an experienced operator identified the anatomical landmarks by locating the most lateral point of each acromion and measured the biacromial distance, which was used as the reference value. This distance was then multiplied by 0.7 and 1.3 to define the reduced and increased standardized stance widths, respectively. The self-selected stance width was measured as the inter-calcaneal distance chosen spontaneously by each participant. Three CMJs were performed for each condition, with the arms constrained alongside the body and a standardized recovery period of 30 s between trials and 60 sec between conditions. Jump variables were recorded using a wearable inertial sensor (BAIOBIT, BTS), and spatiotemporal and kinetic variables were extracted. For each condition, the mean of the three CMJ trials was analyzed. In addition, the association between biacromial distance and self-selected foot stance distance was assessed using Pearson's correlation coefficient. No significant differences were found for mean jump height ($p = 0.109$), maximal jump height ($p = 0.082$), maximal force ($p = 0.394$), rate of force development ($p = 0.068$), reactive index ($p = 0.550$), take-off force ($p = 0.254$), stiffness ($p = 0.641$), total power ($p = 0.098$), impact index ($p = 0.665$), flight time ($p = 0.106$), contact time ($p = 0.597$), eccentric phase time ($p = 0.899$), or concentric phase time ($p = 0.919$). Peak velocity was the only parameter showing a significant overall condition effect ($p = 0.044$), with the highest mean value in the biacromial distance stance. A weak negative, non-significant correlation was found between biacromial distance and self-selected foot stance (Pearson's $r = -0.291$, $p = 0.214$). Foot stance width did not significantly affect most of CMJ parameters. However, the biacromial stance showed slightly better values for jump height, peak velocity, flight time, and power, suggesting that this standardized position may help improve performance. Moreover, the absence of a significant correlation between biacromial distance and self-selected foot distance suggests that participants' spontaneous stance selection may not be directly determined by shoulder width, supporting the need to standardize foot placement in CMJ testing protocols.

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Keywords: countermovement jump, foot stance width, biacromial width, inertial measurement unit, jump performance.

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Evaluation of Facial Artery morphometry using CT Angiography vs dissection in fresh-frozen cadaveric specimens: a methodological paper.

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Background and aim: Vascular complications remain among the most severe adverse events associated with injectable aesthetic procedures.

A comprehensive understanding of the facial vascular anatomy is essential for clinicians to standardize aesthetic procedures and minimize complication rates. However, marked inter- and intra-individual anatomical variability, particularly involving the facial artery and its branches, represents a major challenge. This study aimed to assess the reliability of computed tomography angiography (CTA) for the morphometric evaluation of the facial artery through direct comparison with cadaveric dissection.

Methods: Four fresh-frozen cadaveric specimens were obtained from the ICLO Teaching and Research Center (Verona, Italy). Vascular filling was achieved using contrast medium injected through both central (facial artery) and peripheral (common carotid arteries) access routes. All specimens underwent CTA examination. In one specimen, the left lower third of the face was subsequently dissected to expose the facial artery and its branches, including the superior labial, inferior labial, and labiomental arteries. Vessel caliber and distances from selected anatomical landmarks were recorded and compared with radiological measurements. CTA datasets were further analyzed to assess arterial morphology and inter/intra-individual anatomical variability. Technical limitations encountered during imaging and specimen preparation were documented to optimize the study protocol.

Results: CTA enabled clear visualization and morphometric assessment of the principal arterial structures of the lower face. Bilateral analysis within the same specimen demonstrated a comparable arterial course, although differences in vessel caliber were observed, with facial artery diameters ranging from 2.5 mm to 4.5 mm. Comparison between radiological and dissective measurements showed an overall concordance between the two methodologies. The largest discrepancy concerned the diameter of the facial artery at its emergence (4.5 mm on CTA versus 2.5 mm on dissection), whereas measurements of the labiomental artery at the level of the mental symphysis were highly consistent (0.8 mm and 0.7 mm, respectively). Among the evaluated vessels, the facial artery exhibited the highest image quality and the strongest anatomical-radiological correspondence. Variations in contrast enhancement and morphometric measurements were detected among specimens and may reflect post-mortem tissue alterations as well as differences in specimen preparation and contrast injection protocols.

Conclusion: The present methodological study demonstrated a substantial agreement between CTA and cadaveric dissection. These findings support the use of CTA in cadaveric specimens for the assessment of anatomical variations and morphometric characteristics of the facial arterial network and suggest its potential role as an alternative to cadaveric dissection in the evaluation of facial vascular variants.

Further studies on larger sample sizes are required to validate these preliminary results and to optimize imaging and specimen preparation protocols.

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Keywords: Facial vascular anatomy, Clinical anatomy, Anatomical variability, CTA – Computed tomography angiography, Cadaveric dissection, Aesthetic medicine.

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Morphology-based profiling reveals differential endothelial responses to the endocrine disruptor Bisphenol S in healthy and type 2 diabetic donors

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Endothelial dysfunction represents a key pathological link between type 2 diabetes (T2D) and cardiovascular complications [1]. At the same time, increasing attention has focused on endocrine-disrupting chemicals (EDCs), ubiquitous environmental contaminants capable of perturbing hormonal, metabolic and cellular stress pathways. Because vascular homeostasis is highly sensitive to metabolic and endocrine signals, EDC exposure may further compromise endothelial function, particularly in individuals with pre-existing metabolic disorders. Among these compounds, Bisphenol S (BPS), widely used as a substitute for Bisphenol A, has recently raised concern for its potential effects on endothelial function, oxidative stress and mitochondrial homeostasis [2]. However, whether pre-existing metabolic disease modifies endothelial morphological responses to BPS remains poorly defined. In this study, we applied High-Content Imaging and Cell Painting-based phenotypic profiling [3] o investigate the effects of 96 h exposure to low-dose BPS (0.1 μM) in primary human arterial endothelial cells derived from healthy and T2D donors. Cells were multiplex-stained to visualize DNA, RNA-rich regions, rough endoplasmic reticulum, Golgi/membrane structures, and mitochondria. Automated image segmentation and feature extraction were followed by sparse multidimensional analysis and inferential statistics. Donor metabolic status emerged as the major determinant of endothelial morphology, clearly separating healthy and T2D-derived cells. In healthy endothelial cells, BPS induced a marked phenotypic shift involving nuclear architecture, mitochondrial spatial organization, RNA-associated descriptors and intracellular membrane features. Conversely, BPS exposure induced only limited additional phenotypic changes in T2D-derived endothelial cells, whose morphology remained largely dominated by the pre-existing diabetic state. However, additional changes were still detectable in rough endoplasmic reticulum–associated features, indicating that selected subcellular compartments may retain responsiveness to BPS exposure despite the strong baseline disease-associated phenotype. These findings suggest that pre-existing metabolic dysfunction is a major determinant of endothelial morphology and substantially influences the cellular response to environmental pollutants. While healthy endothelial cells exhibited extensive morphological remodeling following BPS exposure, diabetic cells appeared less able to undergo further phenotypic adaptation, likely because their morphology was already profoundly shaped by the diabetic condition. Overall, our results support the concept that environmental contaminants such as BPS can influence endothelial homeostasis and may further affect vascular health in the context of metabolic disease. In addition, this study highlights the utility of Cell Painting as a sensitive phenotypic screening approach for detecting subtle pollutant-induced alterations and identifying subcellular compartments that are particularly susceptible to environmental stressors.

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Keywords: BPS, Endothelial cells, Type 2 diabetes, High-content imaging, Morphology-based profiling, Cell Painting.

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Bone Marrow Phenotypic and Signaling Readouts Reveal IRE1α Inhibition as a Strategy to Enhance Venetoclax Activity in De Novo and Secondary Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is characterized by profound alterations in the cellular composition and phenotypic architecture of hematopoietic tissues, with bone marrow-derived leukemic blasts displaying marked heterogeneity in survival pathways and therapy response [1-2].

Venetoclax (VEN) has emerged as a promising option for AML patients who are ineligible for or have relapsed after standard-of-care therapies. Although ~75% initially respond, most eventually relapse due to acquired resistance, underscoring the urgent need to identify mechanisms of resistance and strategies to overcome them [3-4].

The central goal of this study is to identify chemical strategies for improving VEN efficacy in de novo AML (pAML) and secondary AML (sAML) [5].

We developed a patient-derived AML (PD-AML) ex vivo platform to identify compounds that enhance VEN efficacy. Briefly, we collect fresh bone marrow samples/peripheral blood from AML patients at both diagnosis and relapse. Samples are then treated with 1.25 mM VEN alone or in combination focused 45-compound library enriched for pathways implicated in VEN resistance, such as metabolic reprogramming, inflammation, and stress adaptation. Pre- and post- drug treatment analyses include AML cell viability/abundance, immunophenotype, and genetic subtype. Compound interaction with VEN is calculated using SynergyFinder.

To date, we have analyzed 29 PD-AML samples and observed VEN response patterns that are consistent with clinical outcomes: 38% of PD-AML are strongly responsive to VEN alone (>65% killing), 34% show intermediate (INT) sensitivity (~65-30% killing), and 28% are weakly (WEAK) responsive to VEN (<30% killing). Importantly, intermediate and refractory samples are enriched for KRAS, NRAS, FLT3-ITD, or TP53 mutations, aligning with patient-derived data. From our library, two of the top-scoring compounds are KIRA6 and 4μ8C, both of which inhibit the enzyme IRE1α, a key component of the unfolded protein response (6-7). VEN combined with KIRA6 or 4μ8C killed PD-AML cells significantly more than VEN alone, with the greatest increase in cytotoxicity observed in INT and WEAK VEN responders. In patients, VEN is used in combination with 5-azacytidine (AZA). Although both AZA and KIRA6 enhance VEN cytotoxicity, VEN+KIRA6 is significantly more effective than VEN + AZA in eliminating WEAK PD-AML cells.

We also tested VEN+IRE1α inhibitors in sAML (n=4), which also showed heterogeneous VEN sensitivity. One MPN-derived sAML (sAML_1: TP53WT; IDH2R140Q/JAK2V617F/SRSF2P95H) showed near-complete loss of viable cells with low-dose VEN, whereas therapy-related sAML with complex karyotype and TP53 deletion (sAML_2: del(7q); TP53 loss ~60% nuclei) remained >70% viable after VEN alone. However, sAML_2 was highly responsive to the combination of VEN and KIRA6. Two additional MDS/MPN-derived sAMLs showed intermediate VEN sensitivity that improved with IRE1α inhibitors. At the protein level, we observed that VEN reduced IRE1α protein in VEN-sensitive sAML_1, while IRE1α persisted after VEN exposure in VEN-resistant sAML_2. Using, two AML cell lines that represent VEN-sensitivity (MOLM-13) and VEN-resistance (HNT-34), we found that VEN alone

depleted IRE1a in MOLM-13 cells, not HNT-34. Notably, VEN+KIRA6 reduced IRE1α and MCL-1, a known VEN-resistance mechanism, enhanced apoptosis induction in HNT-34.

sAML remains an area of major unmet need, with frequent resistance to VEN-based therapy. Our data indicate that IRE1α inhibition enhances VEN activity and can overcome VEN resistance, including high-risk TP53-altered sAML. Persistence of IRE1α after VEN exposure may serve as a functional biomarker of resistance in sAML. Cohort expansion is ongoing, focusing on TP53-altered sAML.

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Keywords: AML, therapy resistance, IRE1α.

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Immunological effects of aerosols from conventional cigarettes, e-cigarettes and heat-not-burn devices in patients with rheumatoid arthritis

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Conventional cigarette smoking (CS) is a well-established risk factor for rheumatoid arthritis (RA) and for the production of anti-citrullinated protein antibodies (ACPA)[1]. However, the immunological effects of alternative smoking devices, in particular e-cigarettes (EC) and heat-not-burn devices (IQOS, HNB), remain poorly characterized, especially in patients with RA[2]. To evaluate the ex vivo effects of CS, EC and HNB aerosols on the proliferation of peripheral blood mononuclear cells (PBMCs), CD4+ T cell subpopulations (Th1, Th17, Treg, Tang), M1 macrophages (CD86+/TLR2+) and on p75NTR receptor expression, in RA patients and healthy controls. Single-centre, cross-sectional observational study. Twenty-three subjects were enrolled: 10 patients with RA (ACR/EULAR 2010 criteria) and 13 healthy controls matched for sex and age. PBMCs were isolated by density gradient and T cells purified by immunomagnetic depletion. Cells were labelled with CFSE and cultured for 72 hours in the presence of 6.25% aerosol extracts derived from CS (Marlboro), EC (Noir-Smoke, e-liquid 4 mg/ml nicotine) and HNB (IQOS with Heets Yellow Label), prepared to achieve equivalent nicotine concentrations. Cell proliferation and immunophenotype were assessed by flow cytometry. RA patients showed reduced PBMC proliferation compared to healthy controls across all experimental conditions, reaching statistical significance upon exposure to CS ($p = 0.03$). Cell death was significantly increased in RA samples after exposure to HNB ($p = 0.02$). Analysis of CD4+ subpopulations revealed that EC exposure induced a significant reduction in the proliferation of Th17 ($p = 0.04$), Treg ($p = 0.009$) and Th1 ($p = 0.04$) cells in RA patients compared to controls. Following CS exposure, a similar trend was observed for Th17, Treg and Th1, with an increase in Tang cells; after HNB exposure, Th17 and Th1 proliferation was largely comparable between groups, while Treg and Tang tended to increase in RA patients. p75NTR expression was elevated in RA patients under all experimental conditions, with statistical significance for EC ($p = 0.03$). M1 macrophage proliferation (CD86+/TLR2+) appeared increased in RA patients with EC and HNB, without reaching statistical significance.

In conclusion, these findings suggest that aerosols from EC, CS and HNB differentially modulate the adaptive immune response in RA patients. In particular, EC exposure is associated with a broad reduction in the proliferation of major CD4+ subpopulations and a significant upregulation of p75, a receptor with pro-apoptotic and neuro-immunological modulatory functions. The upregulation of p75 may represent a shared biological node linking inhaled aerosol exposure, proliferative dysfunction and inflammatory amplification in RA. Further studies on larger cohorts with multi-time-point analyses are needed to confirm these preliminary findings.

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Keywords: rheumatoid arthritis, e-cigarette, heat-not-burn, CD4+ T lymphocytes, p75NTR, immunology.

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Unmasking IPF heterogeneity through MSC fingerprinting: toward precision medicine

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Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease characterized by irreversible scarring of lung tissue and excessive extracellular matrix (ECM) accumulation [1]. Current therapies, such as Nintedanib, are prescribed without reliable tools to predict patient response, highlighting the need for a deeper understanding of the molecular mechanisms driving disease progression. Although fibroblasts are recognized as key effectors of fibrosis, increasing evidence suggests that lung-resident mesenchymal stem cells (MSCs) play a central role in sustaining the profibrotic microenvironment [2].

This study used a multidisciplinary approach to investigate the molecular and functional properties of MSCs derived from IPF patients. Lung-derived IPF-MSCs displayed constitutive hyperphosphorylation of VEGFR1 and FGFR1, sustained by an autocrine positive feedback loop driven by increased VEGF and FGF2 secretion. Nintedanib effectively disrupted this signaling network by inhibiting receptors phosphorylation.

This activated phenotype was also reflected in 3D spheroid models, where IPF-MSCs formed highly compact structures with accelerated ECM deposition and reduced structural plasticity. Nintedanib significantly reduced ECM accumulation and partially restored a more organized cellular architecture.

Despite these beneficial *in vitro* effects, clinical responses to Nintedanib vary considerably among patients [3]. These observations suggested that alternative profibrotic pathways contribute to disease heterogeneity. Immunohistochemical analyses revealed distinct signaling profiles, with some patients showing strong VEGF positivity and others exhibiting predominant activation of the classical TGF-β pathway. To further investigate this heterogeneity, Fourier Transform Infrared (FTIR) microspectroscopy identified two distinct patient endotypes based on specific spectral signatures. These findings were supported by mitochondrial functional analyses, which revealed corresponding differences in calcium handling and mitochondrial membrane potential.

Overall, the integration of molecular, biophysical, and functional data enabled the identification of distinct IPF endotypes, providing a basis for patient stratification and supporting the development of precision medicine approaches in IPF.

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Keywords: Idiopathic Pulmonary Fibrosis (IPF), Mesenchymal Stem Cells (MSCs), Extracellular Matrix (ECM), Nintedanib, Fourier Transform Infrared (FTIR).

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Tracking facial features in Marfan syndrome during growth: preliminary findings from a longitudinal morphometric study

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Marfan syndrome (MFS) is a rare connective tissue disorder caused by pathogenic variants in the *FBN1* gene and characterized by highly variable clinical manifestations. Early diagnosis is crucial given the life-threatening risk of aortic dilatation and dissection. However, diagnosis remains challenging in children without a family history, largely due to the age-dependent expression of many clinical features [1]. In a previous facial morphometric study [2], we identified subtle features that may escape routine clinical inspection and may support earlier diagnosis. To investigate the developmental evolution of the facial phenotype in MFS, an ongoing longitudinal study is evaluating pediatric patients at different periods of growth. Here, we report preliminary findings from the first three patients followed from childhood to adolescence. Three male subjects with genetically confirmed MFS underwent facial scanning during middle childhood (6–8 years), late childhood (9–12 years), and adolescence (13–17 years). 3D stereophotogrammetric images were used to quantify inter-landmark linear distances and angular measurements, which were standardized as z-scores using a reference sample of 443 healthy age- and sex-matched subjects. Age-related facial shape changes were assessed via geometric morphometrics, employing MeshMonk-based 3D image registration and Generalized Procrustes alignment to remove growth-related size effects. Per-vertex Euclidean distances were then quantified between each follow-up timepoint and baseline. From middle childhood onward, all three subjects exhibited greater facial divergence (z-scores > 2) and lower facial height index (z-scores < −1.5) compared to controls, both associated with an increased midfacial height and a shorter mandibular ramus. A mildly reduced facial width-to-height ratio, mild retrognathia, and downslanting palpebral fissures (z-scores < −1) were also observed. While facial shape remained relatively stable from middle to late childhood, adolescence was characterized by changes consistent with normal facial growth patterns, including increased prominence of the supraorbital ridges, nose, perioral region, and chin. Conversely, the malar region exhibited retrusion. These preliminary findings provide evidence that facial morphometric features associated with MFS may follow distinct manifestation patterns throughout growth. While malar hypoplasia appears to become more readily detectable during adolescence, other features, particularly facial hyperdivergence, can already be identified during middle childhood, underscoring the value of their assessment in facilitating the early diagnosis of the condition.

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Keywords: face, Marfan syndrome (MFS), growth, facial anthropometry, geometric morphometrics, stereophotogrammetry.

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Analysis of the Thermomechanical Compaction on the Root Canal of three different Bioceramic Sealers

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There are no data in the literature regarding the comparison of the marginal adaptation in the root canal of hydraulic sealers when used with a single-cone technique or through thermomechanical compaction, this study aimed to evaluate the behavior of three different endodontic sealers used with the two above-mentioned obturation techniques by evaluating the marginal gap existing between the obturation materials and the dentinal walls through scanning electron microscopy(1,2). Given this objective, a total of 84 single-rooted, straight canal teeth were selected and divided into three subgroups according to the selected endodontic sealer (AH) Plus Bioceramic Sealer (AHP), C-Root SP (CR), and One-Fil PT (OFPT). Each tooth was decoronated and instrumented with the HyFlex EDM/CM systematics up to 30.04. After irrigation procedures, the teeth of each subgroup were divided into two groups and obturated according to two different obturation techniques: the single-cone technique (SC) and the thermomechanical compaction technique (TC). After the required sealer setting time, each tooth was sectioned in three parts at 3, 6, and 9 mm from the apex, and each section was observed with a variable pressure scanning electron microscope (3,4). The marginal gap of each sample was measured using G* Power Software v3.1, and the statistical analysis was performed using the Kruskal–Wallis test, followed by a post hoc Dunn's test (5). Results showed that there were not any statistically significant differences in terms of the marginal gap between the two different above-mentioned obturation techniques for each sealer, except for the middle third of root canals, where a statistically significant difference was found for AHP, and OFPT sealers. In conclusion, the thermomechanical compaction of hydraulic sealers and gutta-percha guarantees better sealing than the single-cone technique when the root canal shape is not rounded.

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Keywords: Endodontics, VP SEM, bioceramic sealer, root canal, thermomechanical compaction.

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Anatomical trends in aesthetic breast implant surgery: implant placement planes and surgical approaches from the Italian National Breast Implant Registry

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Breast augmentation remains one of the most frequently performed procedures in aesthetic plastic surgery [1, 2]. Over recent decades, multiple surgical approaches for implant placement have been developed, each associated with specific advantages and limitations. Breast augmentation requires accurate anatomical planning in order to optimize aesthetic outcomes and minimize complications [3]. Implant positioning and surgical access are closely related to breast morphology and chest wall anatomy [4]. The aim of this study was to analyze anatomical trends in implant placement planes and surgical approaches in aesthetic breast implant procedures recorded in the Italian National Breast Implant Registry (RNPM).

A retrospective descriptive analysis was performed using RNPM data updated to December 31, 2025. A total of 30,474 aesthetic breast implant surgical procedures were analyzed, involving 29,662 patients. Primary implant procedures accounted for 75.7% of cases, while revision surgeries represented 24.2%; mixed procedures accounted for only 0.1% of interventions. Data were evaluated according to surgical indication, implant positioning plane, surgical access, and associated lipofilling procedures.

Most procedures were performed for breast hypoplasia/hypotrophy (77.3%), whereas ptotic breasts accounted for 22.7% of cases. The dual plane technique represented the most frequently adopted anatomical plane overall (54.5%), followed by subglandular (24.9%), submuscular (10.3%), and subfascial implantation (10.2%). A similar distribution was observed when procedures were stratified by diagnosis: dual plane implantation was preferred both in hypoplastic/hypotrophic breasts (56.8%) and in ptotic breasts (46.6%). In contrast, subglandular positioning was proportionally more frequent in ptotic breasts (33.2%) than in hypoplastic breasts (22.4%). Notably, submuscular implantation was less frequently performed in ptotic breasts (7.6%) compared with hypoplastic/hypotrophic breasts (11.1%), suggesting a differential anatomical strategy according to baseline breast morphology.

Autologous fat grafting was performed in only 2.6% of procedures overall, but it was more frequently associated with subfascial implantation (5.2%) compared with subglandular (3.1%), dual plane (1.7%), and submuscular approaches (1.7%), suggesting a compensatory strategy to improve soft tissue coverage in more superficial implant positioning.

The inframammary fold represented the most used surgical access (49.2%), independently from implant positioning plane, followed by the periareolar approach (28.4%) and mastopexy-pattern incision (22.1%).

Among revision procedures, the most common causes were non-device-related complications (32.8%), capsular contracture (32.0%), and implant rupture (24.1%).

The dual plane approach currently represents the predominant anatomical strategy in aesthetic breast augmentation surgery in Italy. Implant positioning and surgical access appear to be influenced by breast anatomy and soft tissue coverage requirements. Registry-based analyses may contribute to refining anatomical-surgical planning and improving long-term outcomes in breast implant surgery.

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Keywords: breast implants, implant placement plane, aesthetic breast surgery, Italian National Breast Implant Registry.

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Pericyte Tunneling Nanotubes Drive Vascular Branching via SCF/c-KIT Signaling

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In the developing human brain, forebrain pericytes of neural crest origin are emerging as key regulators of angiogenic progression at the leading edge of growing vessels. Vascular sprouting involves composite structures in which endothelial tip cells are associated with pericytes extending tunnelling nanotubes (TNTs), contributing to the formation of intervascular bridges during branching. To elucidate the mechanisms underlying pericyte TNT (P-TNT)-guided vascular growth, we investigated the spatial distribution of the c-KIT (CD117) receptor and its ligand stem cell factor (SCF), hypothesizing a non-canonical signaling pathway mediating endothelial–pericyte interactions. Human fetal brain and glioblastoma (GB) samples were analyzed using high-resolution confocal immunofluorescence microscopy and three-dimensional reconstruction. TNTs were identified in 20-µm sections using NG2 proteoglycan as a marker of immature or reactivated pericytes and collagen type IV (COL IV) as a TNT-associated extracellular matrix component, followed by morphometric quantification of NG2+ TNT density. c-KIT and SCF localization was assessed by double staining with NG2 and the endothelial marker CD31. P-TNTs were observed in association with pericytes emerging from parental vessels or extending at the distal edge of composite sprouts. TNT density was significantly higher in fetal brain compared to GB peripheral regions and markedly reduced in tumor core areas. c-KIT+/SCF+ endothelial cells were frequently located in close proximity to c-KIT–/SCF+ pericytes, indicating complementary receptor–ligand distribution. In fetal brain microvessels, c-KIT showed both membrane and nuclear localization in endothelial cells, whereas nuclear localization was barely detectable in tumor vessels. These findings identify P-TNTs as key structural elements in vascular morphogenesis, contributing to both vessel bridging and directional outgrowth. The complementary c-KIT/SCF pattern supports coordinated endothelial autocrine/paracrine and pericyte-derived paracrine/juxtacrine signaling in pericyte-driven branching, with potential relevance also in tumor vascularization.

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Keywords: Angiogenesis, Endothelial–pericyte interaction, Tunneling Nanotubes (TNTs), SCF/c-KIT signaling.

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Unraveling Cardiotoxicity: The Interplay between PLCb2 and Doxorubicin

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Anthracyclines are among the most widely used chemotherapeutic agents for the treatment of solid and haematological cancers; however, their clinical use is limited by dose-dependent cardiotoxicity, which significantly affects patients' quality of life. The most clinically relevant anthracycline is doxorubicin (DOX), which induces the production of reactive oxygen species (ROS), thereby disrupting mitochondrial activity and activating the apoptotic machinery. Nevertheless, the molecular mechanisms underlying DOX-induced cardiac side effects remain elusive, highlighting the need for further investigation.

Recent findings indicate a dynamic regulation of phospholipase Cb (PLCb) isoforms in cardiotoxicity, although their specific roles are still poorly defined. Among these, PLCb2 has been reported to be upregulated in DOX-induced models.

On this basis, the present study aimed to investigate the involvement of the PLCb2 isoform in the molecular mechanisms underlying DOX-induced cardiotoxicity. Our results provide evidence that increased PLCb2 levels are associated with the development of morphological alterations, oxidative stress, and apoptosis. Specifically, PLCb2 upregulation leads to an increase in cell size and correlates with the downregulation of DGKz and the Akt/mTOR signaling pathway. Furthermore, PLCb2 silencing appears to counteract the adverse effects of DOX, suggesting that targeting PLCb2 could exert a cardioprotective effect and represent a promising therapeutic strategy for preventing DOX-induced cardiotoxicity.

Keywords: Doxorubicin, PLCb2, cardiotoxicity, DGKz, hypertrophy.

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Hyperconnected Tumor-Brain Networks Are Associated with Poor Survival in Glioblastoma

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Glioblastoma (GBM) IDH wild-type is the most aggressive primary brain tumor in adults and is increasingly recognized as a disease that extends beyond the visible lesion through interactions with distributed brain networks [1,2]. Recent evidence suggests that GBM exploits white matter pathways to establish structural relationships with anatomically distant cortical and subcortical regions, potentially influencing tumor progression and patient outcome [2-4]. Here, we investigated the topological and anatomical organization of GBM-associated white matter networks and evaluated their prognostic significance in a large dataset of ~1000 patients coming from four independent cohorts. Using whole-brain tractography and GBM segmentations, we reconstructed and characterized tumor-brain structural connectivity networks, defined as the set of white matter tracts directly involved with the GBM lesion. Tumor-brain connectivity features were extracted and analyzed in relation to overall survival (OS). Prognostic value was evaluated using multivariate Cox proportional hazards models and machine-learning OS prediction together with age, sex, extent of resection, and MGMT promoter methylation status. Multiple connectomic measures stratified patient survival across a wide range of thresholds. Patients with higher connectivity metrics had significantly worse survival rates ($p<0.05$, two-sided log-rank tests), indicating a robust link between tumor–brain network organization and clinical outcome. Moreover, distinct connectivity-based groups were identified, showing progressively different survival outcomes. Anatomical analysis revealed distinct connectivity phenotypes characterized by different patterns of long-range interactions among cortical and subcortical regions. Notably, phenotypes with poorer OS exhibited a hyperconnected tumor-brain network architecture, characterized by extensive fronto-parietal, fronto-limbic, and fronto-subcortical connectivity, as well as increased large-scale network integration. In contrast, patients with a more favorable prognosis exhibited a comparatively hypoconnected network profile, with reduced inter-system connectivity and greater network segregation. This pattern suggests that extensive engagement of distributed white matter pathways may contribute to adverse clinical outcomes. Finally, network-informed machine-learning models outperformed clinical-only models for predicting 12-, 18-, and 24-month survival, achieving balanced accuracies up to 72%. Altogether, these findings support the concept of GBM as a distributed white matter disease whose anatomical network organization carries prognostic information beyond established clinical and molecular markers. Connectomic characterization of tumor-associated brain networks may provide novel anatomically informed biomarkers for patient characterization and contribute to the development of personalized prognostic models in neuro-oncology.

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Keywords: Connectomics, computational neuroanatomy, neuro-oncology, white matter, tractography.

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Integrating Neurosurgery and Neuroradiology into Undergraduate Neuroanatomy Teaching: A Reproducible Four-Step Model and Ten-Year Longitudinal Evaluation

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Neuroanatomy is a major source of cognitive overload and neurophobia for medical students. At the same time, neurosurgery remains marginal in preclinical curricula, with an average of roughly 1 ECTS credit in Italian medical schools. To bridge structure, function, and bedside care, we developed an integrated neuroanatomy-neuroradiology-neurosurgery module at Sapienza University of Rome, offered as a student-selected activity alongside the mandatory second-year neuroanatomy course. The module is built on a standardized, reproducible four-step cycle: (1) neuroanatomical framing of the region and its functional pathways; (2) neuroradiological integration through MRI, CT, and DTI tractography; (3) neurosurgical case discussion correlating anatomy, imaging, and surgical decision-making; and (4) guided, question-driven interactive discussion. Multidisciplinary co-teaching by an anatomist, a neuroradiologist, and a neurosurgeon is combined with case-based, problem-based, and team-based learning, as well as exposure to neuronavigation, intraoperative MRI, and simulation [1,2]. A retrospective longitudinal analysis (academic years 2016-2017 to 2025-2027) compared a standard cohort of students who attended only the traditional neuroanatomy course (n = 1812) with a supplementary-course cohort of students (n = 347) who voluntarily attended also the integrated module in addition to the standard course, using neuroanatomy oral examination grades and annual anonymous five-point Likert satisfaction items. Data were analyzed with repeated-measures ANOVA with sphericity corrections and Bonferroni-adjusted comparisons. Standard-cohort grades declined significantly and progressively ($F = 9.21$, $p < 0.001$), reaching their lowest mean in 2025 (27.47/30), whereas supplementary-cohort grades remained stable (29.13-29.70; $F = 1.23$, $p = 0.28$) and student satisfaction stayed uniformly high across all ten years (geometric means 4.10-4.54/5; $p >= 0.81$).

Early, integrated, tri-disciplinary co-teaching, anchored in a reproducible four-step cycle, sustains examination performance, is consistently valued by students over a decade, and is readily transferable to other medical schools that use existing clinical services [3].

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Keywords: neuroanatomy education, neurosurgery, neuroradiology, curriculum integration, undergraduate medical education, case-based learning, problem-based learning, neurophobia.

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Immunofluorescence and RT-qPCR analysis of sarcoglycans subcomplex in epithelial tissues in different digestive tracts.

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Sarcoglycans are transmembrane glycoproteins that play a key role in maintaining sarcolemma stabilization during muscle contraction. Several studies have demonstrated that this complex is not muscle specific and that it is also expressed in epithelial tissues as gingival, breast [1] and prostatic epithelia [2] and recently in the adipose tissue, demonstrating that these proteins are involved in cell-cell and cell-matrix interactions. In the present study, we investigated sarcoglycans expression in the epithelia of several digestive tracts and in particular, ileum, ascendant, transverse and discendent colon and ileopelvic colon. We performed immunofluorescence reactions using antibody against a-, b-, g-, d-, e- and -sarcoglycans. Moreover, in the same samples, quantitative real-time PCR was carried out to analyze gene expression of sarcoglycans. Total RNA was extracted using Trizol following standard protocol. Quantitative analysis of extracted RNA was carried out with the use of Nanodrop 1000. Reverse transcription was performed using 1 μg of total RNA with SuperScript IV Reverse Transcriptase kit (Invitrogen) and random primers according to standard protocol. cDNA obtained was used to evaluate gene expression of sarcoglycans, using β-actin as housekeeping gene for relative quantification. Our results show a different expression of sarcoglycans in the different tested tracts and in different zones of cells, demonstrating that the sarcoglycans play a different role both in cell-cell and in cell-matrix interaction. Moreover, it is important to analyse the same proteins in epithelial during pathological conditions in order to evidence the real role of sarcoglycans in interaction processes.

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Modifications of Cremaster Muscle Cytoskeletal and Sarcoglycan Complex Proteins in Retractile and Undescended Testes

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During the inguinoscrotal phase, the cremaster muscle, which develops from the gubernaculum, exerts a dynamic function in testicular descent through rhythmic contractions that propel and guide the testis into the scrotum via the inguinal canal. The specific molecular mechanisms responsible for the functional failure of this muscle in such conditions remain, nevertheless, largely unexplained. Testicular descent is a crucial process during male fetal development, and its alteration leads to cryptorchidism (undescended testis).

Cytoskeletal and sarcolemmal-stabilizing proteins, including the sarcoglycan complex, talin, and vinculin, are essential for skeletal muscle integrity and function. This study analyzes the expression and distribution of these proteins in the cremaster muscle across different clinical testicular positions to elucidate the molecular pathogenesis of associated dysfunctions.

Three groups of paediatric patients undergoing surgery were enrolled: a control group (n=10, with hydrocele or uncomplicated inguinal hernia), a group with retractile testis (n=10), and a group with undescended testis (n=10). Cremaster muscle biopsies were collected during surgery and analyzed by immunofluorescence using specific antibodies for alpha (α), beta (β), gamma (γ), delta (δ), and epsilon (ε) sarcoglycan subunits, as well as for talin and vinculin.

Immunofluorescence analysis revealed significant differences ($p < 0.05$) in the expression of the studied proteins among the groups. Compared to the control group, the cremasters of patients with undescended testis showed a marked decrease in the distribution pattern of alpha, beta, gamma, delta, and epsilon sarcoglycan subunits, as well as talin and vinculin. In contrast, in the cremasters of patients with retractile testis, an increase in the distribution pattern of all examined proteins was observed compared to the control group. Protein distribution in the undescended cremaster also appeared more disorganized, suggesting structural alterations at the cytoskeletal and membrane complex level.

The loss of muscle structural integrity and sarcolemma stability seen in undescended testes likely stems from the downregulation of these proteins, causing fiber disorganization and lower contractile force. Consequently, the cremaster muscle suffers from an intrinsic defect that impairs its ability to drive testicular migration into the scrotum. Conversely, the elevated protein levels in the retractile group may signify either a compensatory pathway or overactive contractility. Ultimately, these insights clarify the molecular mechanisms of cremasteric impairment and offer new avenues for future diagnostic and clinical interventions.

Keywords: sarcoglycans, cremaster muscle, cryptorchidism.

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Transforming Neuroanatomy Education with AEducAR3.0: A Multi-Level Learning Experience

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Neuroanatomy represents one of the most challenging areas of anatomical education because of the complexity of three-dimensional spatial relationships and the need to translate theoretical knowledge into clinically relevant topographical understanding [1]. Emerging technologies such as Augmented Reality (AR) and 3D printing have shown considerable potential for enhancing anatomy learning and spatial comprehension. Building on our previous experience with the AEducAR platform [2-3], we developed AEducAR3.0, an innovative educational platform integrating AR and 3D printing within a multi-level learning framework. The platform was designed to guide learners through three sequential stages: notional learning of cranial nerves and their relationship with the brainstem, contextual learning of nerve pathways within the skull, and topographical learning focused on clinically relevant anatomical landmarks of the face. The system combines interactive AR visualization with patient-scale 3D-printed phantoms and integrated assessment activities targeting both memory recall and knowledge application. AEducAR3.0 was evaluated in a feasibility study involving 70 second-year medical students at the University of Bologna. Learning outcomes were assessed through quizzes administered at each learning level, while students' perceptions were investigated through anonymous questionnaires and semi-structured interviews. Students achieved significantly higher scores in the contextual learning level (70% ± 4%) compared with the notional (60% ± 7%; $p = 0.04$) and topographical levels (58% ± 5%; $p = 0.02$). Learning outcomes were not influenced by gender, nationality, previous AR experience, or study habits. Questionnaire and interview findings revealed high levels of satisfaction, highlighting the usefulness of AR for understanding complex neuroanatomical relationships, increasing engagement, and supporting the development of clinically oriented anatomical knowledge. These findings suggest that AEducAR3.0 is a promising hybrid educational tool that combines AR and 3D printing to enhance neuroanatomy learning and foster the acquisition of spatial and topographical anatomical skills relevant to future clinical and surgical practice.

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Keywords: neuroanatomy, augmented reality, 3D printing, medical education, topographical learning.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Senolytics: a Novel Adjuvant Therapeutic Strategy to Reduce Recurrence in Gliomas

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Gliomas represent one of the most common and heterogeneous groups of primary central nervous system tumors, characterized by high aggressiveness [1]. Despite the current standard of care, based on surgical resection, radiotherapy, and chemotherapy, the prognosis remains poor, with a median life expectancy of approximately 15 months [2,3]. This scenario highlights the limitations of current protocols and the urgent need to identify novel strategies to enhance the efficacy of available therapies. Cytotoxic treatments aimed at eradicating the tumor mass, such as radiotherapy and alkylating agents like Temozolomide (TMZ), often trigger a stable cell cycle arrest in surviving cells, known as therapy-induced senescence (TIS). Although proliferatively inactive, these cells maintain high metabolic activity and acquire an apoptosis-resistant phenotype, thus promoting disease persistence and contributing to tumor recurrence mechanisms [4,5]. Senolytic drugs act by selectively disabling the survival networks of senescent cells, restoring their vulnerability to apoptosis [4,6]. The aim of this study was to evaluate the effect of senolytic compounds on glioma cell models previously treated with TMZ. Our results confirm that TMZ treatment induces a phenotype compatible with cellular senescence. Furthermore, sequential treatment with TMZ and senolytics led to a significant increase in apoptosis compared to TMZ alone. These findings support the potential use of senolytics as a promising adjuvant strategy to standard treatments, with the prospect of improving the therapeutic response and significantly reducing the incidence of recurrences in gliomas.

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Keywords: cancer therapy; glioblastoma; glioma; senescence; senolytics.

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NLRP3 Inflammasome Activation and Endothelial-to-Mesenchymal Transition as Potential Drivers of Renal Fibrovascular Remodeling in Systemic Sclerosis

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Systemic sclerosis (SSc) is a complex autoimmune disease characterized by immune dysregulation, microvascular injury, oxidative stress, and progressive fibrosis of multiple organs. Renal involvement, particularly scleroderma renal crisis, represents one of the most severe complications of SSc and is mainly driven by intrarenal vasculopathy, endothelial injury, and fibrovascular remodeling [1]. However, the early histopathological and molecular mechanisms underlying renal damage in SSc remain incompletely characterized [2]. Based on previous evidence from HOCl-induced murine SSc showing systemic fibrosis and renal vascular abnormalities [3], and on our recent cardiac findings demonstrating NLRP3 inflammasome activation, oxidative stress, endothelial-to-mesenchymal transition (EndMT), and perivascular matrix remodeling in the same experimental setting [4], this study investigated whether a comparable pathogenic axis is involved in the kidney. Kidney samples from HOCl-induced SSc mice and vehicle-treated controls were analyzed by hematoxylin and eosin staining, Masson's trichrome staining, and immunohistochemistry. The analysis focused on vascular, glomerular, and tubulointerstitial compartments. The marker panel included α-SMA and vimentin for myofibroblast activation and mesenchymal transition; CD31 and NOS/eNOS for endothelial integrity; TGF-β and Collagen type I for profibrotic and vasculopathic remodeling; MMP-3 and MMP-9 for extracellular matrix turnover; CD68, F4/80, and CD11b for inflammatory infiltration; NLRP3, Caspase-1, and IL-1β for inflammasome activation; and NRF2 and HMOX1 for oxidative stress response. Histological evaluation revealed increased collagen deposition, mainly in perivascular and interstitial areas, together with early glomerular and tubular alterations. Immunohistochemical profiling showed increased α-SMA and vimentin expression, associated with reduced CD31 and NOS/eNOS staining, suggesting myofibroblast activation, mesenchymal transition, and endothelial dysfunction. Increased expression of TGF-β, Collagen I, MMP-3, and MMP-9 would support active profibrotic, vasculopathic, and extracellular matrix remodeling. Moreover, enhanced expression of CD68/F4/80, CD11b, NLRP3, Caspase-1, and IL-1β would suggest activation of the inflammatory response and the inflammasome pathway, while upregulation of NRF2 and HMOX1 may indicate a compensatory oxidative stress response. Overall, this integrated approach may identify NLRP3-mediated EndMT as a mechanistic link between inflammation, endothelial injury, and renal fibrovascular remodeling in SSc.

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Keywords: systemic sclerosis, kidney, HOCl model, NLRP3, EndMT, renal fibrosis, oxidative stress.

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The Shockwave Blueprint: Decoding Tendon Repair and Regeneration from Clinical Outcomes to Cellular and Ultrastructural Mechanisms

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Extracorporeal shockwave therapy (ESWT), originally developed from extracorporeal shock wave lithotripsy, is a non-invasive form of treatment that operates by delivering acoustic waves to biological tissues [1]. Owing to its negligible side effects, typically limited to transient pain or minor hematoma, this therapy has gained increasing popularity for treating different musculoskeletal and soft-tissue disorders. Increasing evidence indicates that its therapeutic action extends far beyond a simple mechanical effect. Indeed, numerous studies have shown that shockwave (SW) treatment can stimulate a broad spectrum of biological responses in musculoskeletal tissues via mechanotransduction [2]. These include modulation of cellular activity, regulation of protein synthesis, stimulation of cell proliferation and differentiation, and enhancement of tissue repair and regenerative pathways [3]. From a clinical perspective, these biological effects translate into meaningful improvements in pain and functional outcomes across several tendinopathies. In line with current literature, our in vivo studies demonstrated sustained medium- to long-term benefits of SW in different tendon disorders, with significant reductions in pain and consistent improvements in functional scores [4]. Despite these encouraging clinical outcomes, the precise biological mechanisms underlying these effects remain incompletely understood. To address this gap, it is essential to complement clinical observations with mechanistic investigations. Within this frame, in vitro models offer a controlled environment to explore how SW stimulation influences tendon cell behavior and contributes to tissue healing and remodeling. Therefore, alongside clinical assessment, an in-depth analysis of the effects of SW on primary tenocytes, focusing on key biological processes such as cell proliferation, differentiation, and ultrastructural organization, as well as on the modulation of vesicular and intracellular trafficking, will be performed. These analyses will be conducted through an integrated approach of optical and electron microscopy, western blot, and tunable resistive pulse sensing, providing a comprehensive morpho-functional characterization of the cellular responses elicited by SW in tendon tissue.

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Keywords: Shockwave therapy, tendon regeneration, mechanotransduction, tenocyte biology, in vitro model.

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Low-Dose Exposure to Environmental Endocrine Disruptors Alters Adipogenic Programming and Impairs Insulin Responsiveness in Human Adipocytes

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Obesity is a major global health challenge closely linked to insulin resistance and type 2 diabetes [1]. Growing evidence suggests that environmental contaminants, such as bisphenol analogues and perfluoroalkyl substances, can interfere with endocrine and metabolic regulation, altering adipose tissue development and function and thereby contributing to obesity and metabolic disease [2,3]. To investigate the impact of chronic low-dose exposure on adipocyte development and insulin responsiveness, human adipose-derived stem cells were exposed to environmentally relevant concentrations of bisphenol S (BPS) and perfluorooctane sulfonate (PFOS), alone or in combination, throughout adipogenic differentiation. While lipid droplet accumulation was unaffected, indicating preserved terminal differentiation, exposure to BPS and PFOS altered the temporal expression and magnitude of key adipogenic regulators, including CEBPA, PPARγ, and the mature adipocyte marker FABP4. PFOS and combined BPS+PFOS exposure also increased IL1β expression in mature adipocytes, suggesting a shift toward a modest pro-inflammatory phenotype. Functionally, all ED-treated groups exhibited impaired insulin-stimulated glucose uptake, associated with defective GLUT4 translocation to the plasma membrane despite unchanged total GLUT4 protein levels. Notably, combined exposure induced the most pronounced effects, significantly reducing PI3K pathway activation and decreasing total AKT and ERK1/2 protein levels. Overall, these findings demonstrate that chronic exposure to environmentally relevant low doses of BPS and PFOS disrupts adipocyte transcriptional and signalling networks, promoting features consistent with impaired insulin responsiveness. The stronger effects observed following combined exposure underscore the importance of considering chemical mixtures in environmental risk assessment and support the growing recognition of endocrine disruptors as contributors to metabolic disease susceptibility.

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Keywords: Endocrine disruptors, BPS, PFOS, adipogenesis, insulin signalling, GLUT4.

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Ultrastructural cytochemistry reveals stress-associated membrane remodeling in HER2-positive breast cancer cells exposed to trastuzumab-based therapies

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Spheresomes are emerging membrane-bound compartments originally described in low grade gliomas, whose occurrence in additional cancer models suggests a broader role in membrane remodeling. However, their ultrastructural organization, histochemical properties, and relationship with therapy-induced endomembrane stress remain poorly defined. Here, we combined conventional and reduced osmium tetroxide staining with transmission electron microscopy and we targeted perturbation of endomembrane trafficking to characterize spheresomes in HER2-positive breast cancer cells exposed to trastuzumab-based therapies. Reduced osmium histochemistry revealed that spheresomes are characterized by an electron-lucent lumen surrounded by a sharply electron-dense, highly osmiophilic limiting membrane, frequently organized within larger multivesicular spheres (MVS). Compared with conventional osmium staining, reduced osmium markedly enhanced the contrast of spheresome membranes, often exceeding that of the plasma membrane, suggesting a distinct lipid-reactive organization. Both trastuzumab and trastuzumab–deruxtecan (T-DXd) increased MVS abundance, whereas a marked increase in spheresomes was selectively observed following T-DXd treatment, supporting a contribution of payload-induced stress to their formation. Pharmacological perturbation of endomembrane homeostasis further modulated MVS and spheresome organization, while the absence of BSA–gold uptake excluded a canonical endocytic origin. Spheresomes were also morphologically and histochemically distinct from lipid droplets, as confirmed by reduced osmium staining and lipid-loading experiments. Collectively, these findings highlight the potential of ultrastructural cytochemistry to uncover previously underappreciated membrane responses emerging under therapeutic stress conditions. Notably, morphologically comparable structures were identified in additional cancer cell models, including colon, pancreatic, and B-cell leukemia cells, suggesting that spheresomes may represent a previously unrecognized membrane compartment associated with membrane remodeling across diverse cellular contexts.

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Advancing Translational Anatomy Through Human Body Donor Surgical Simulation: Digital Technologies for Volumetric and Photogrammetric Analysis of Minimally Invasive Cranial Corridors

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The increasing adoption of minimally invasive cranial neurosurgery has renewed interest in the quantitative characterization of anatomical surgical corridors while promoting the integration of digital technologies into anatomical research. In this context, human body donor dissection is evolving from a predominantly descriptive discipline into a translational platform capable of supporting the development and validation of innovative surgical approaches.

This study aims to integrate human body donor surgical simulation, computed tomography (CT)-based volumetric analysis, photogrammetry, and endoscopic visualization for the investigation of minimally invasive cranial corridors. Six human body donors underwent supraorbital and mini-pterional keyhole approaches, resulting in a total of 12 anatomical procedures. Following surgical simulation, CT datasets will be acquired to perform volumetric analyses of surgical trajectories and exposed working areas according to different intracranial targets. In parallel, photogrammetric acquisition of both the operative field and endoscopic views was performed to generate three-dimensional reconstructions of the surgical corridors and their anatomical relationships.

The integration of these technologies provided objective anatomical data for the neurosurgical practice, enabling quantitative evaluation of surgical accessibility, exposure, visualization angles, and corridor geometry. Photogrammetric models further served as measurable digital anatomical replicas, enabling the extraction of morphometric parameters and the spatial relationships between surgical targets and surrounding neurovascular structures. This digital workflow improved reproducibility, facilitated comparison among minimally invasive approaches, and expanded the potential applications of cadaveric studies toward simulation, education, and technological innovation.

Preliminary observations are consistent with the growing evidence supporting keyhole neurosurgery, where reduced surgical exposure can be associated with adequate anatomical visualization and operative maneuverability. By combining surgical simulation with advanced digital technologies, this project proposes a novel framework for quantitative and translational anatomy. Such an approach may contribute to optimizing minimally invasive cranial surgery and provide anatomical foundations for future technological developments, including virtual surgical simulation and robotic-assisted microsurgery.

Keywords: Translational Anatomy, Photogrammetry, Digital Technologies, Surgical Simulation, Minimally Invasive Neurosurgery.

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Role of the RNA-binding protein SLM2 in inflammatory features in Multiple Sclerosis

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Multiple sclerosis (MS) is a neurological disease characterized by an immune-mediated inflammatory response leading to demyelination and neuronal damage in the central nervous system (CNS). Recent evidence points to T helper 17 (Th17) cells, characterised by IL-17 production, as key players in MS. Our preliminary studies have uncovered an important role for the multifunctional RNA-binding protein (RBP) SLM2 in the modulation of Th17 cells features. We demonstrated that SLM2 expression is induced during polarization of naïve T cells into Th17 cells, but not into other Th subsets in both humans and mice (Volpe et al., 2019). To test whether SLM2 expression is relevant for Th17 cell function, we analyzed circulating memory cells from wild type (wt) and *Slm2* knockout (*Slm2*-ko) mice under stimulation with anti-CD3/CD28 for up to 7 days, inducing cytokine production in the last 5 hours before the analysis by treatment with PMA and ionomycin. We found that Th17 gradually increased in the wt samples, while this increase was significantly reduced in *Slm2*-ko cells. To evaluate whether the impaired functional properties of Slm2-ko Th17 cells observed *ex vivo* could affect MS in vivo, we employed the well-established MOG35-55-induced experimental EAE model. Strikingly, ablation of *Slm2* expression strongly impacted the course of EAE, as indicated by the very mild disease course shown by *Slm2*-ko mice, with a 4-fold reduction of the mean disease score and a significant reduction of the neuropathological score in ko mice compared to wt animals. Immunophenotypic characterization of the CNS infiltrate found in wt and *Slm2*-ko EAE mice pointed out a reduced number of immune cells (CD45+) in the CNS of *Slm2*-ko EAE mice compared to wt EAE mice. Th17 cells were increased in the wt CNS during EAE, whereas this increase was significantly reduced in the *Slm2*-ko CNS. Single-cell RNA sequencing (sRNA-seq) of CD4+ T cells isolated from the spleen of EAE wt and EAE *Slm2*-ko mice allowed us to identify 12 subclusters of CD4+ T cells, including Th17 cells and Th1 cells. Although preliminary, these results strongly support a role for SLM2 in the modulation of the Th17 cell phenotype and indicate that this RBP plays an important pathogenic role during EAE. Nevertheless, further studies are needed to elucidate its mechanism of action and the full range of its impact on the inflammatory processes of EAE.

Keywords: SLM2; Multiple Sclerosis, Th17 cells.

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Surface compositional changes of titanium dental implants after electric-field decontamination (Ximplant®): significance of the oxygen decrease – a SEM-EDS study

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Peri-implantitis is the leading cause of dental implant failure, and decontaminating the surface without altering its physicochemical properties remains an unmet need [2]. Electric-field devices (Ximplant®) have shown promising antibiofilm activity [1]. Using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS), we characterised the surface elemental composition of three medical-grade Ti-6Al-5V cylinders after this treatment, focusing on the meaning of the oxygen decrease: an untreated negative control; one treated with Ximplant® in physiological saline (XF, reproducing the moist oral environment); and one treated dry (XNF). For each sample, SEM imaging and EDS map-sum spectra yielded semiquantitative elemental compositions (wt%). All samples showed the characteristic Ti-Al-V alloy profile. Compared with the control, XF showed higher Ti (59.7→ 68.5 wt%) with lower oxygen (19.0→ 13.4) and carbon (7.1→ 5.1), and the appearance of Na, Cl and P, whereas XNF was comparable to the control (Ti 56.4; O 20.6; C 7.7). The oxygen decrease seen only in the wet protocol, together with the carbon decrease and the relative titanium increase, is most consistent with removal of the carbonaceous adventitious layer – a genuine surface-cleaning effect in an electrolytic medium – rather than any change in oxidising capacity: the lower oxygen reflects loss of surface layers, not oxide reduction per se. The Na, Cl and P signals indicate concurrent saline-derived ionic residues. A non-exclusive alternative is partial electrochemical thinning of the passive TiO2 film, relevant since this nanometric oxide governs corrosion resistance and osseointegration; the unchanged dry protocol, lacking an electrolyte, supports an electrochemically driven mechanism in XF. A key limitation is methodological: EDS probes a micrometre-scale volume, while the oxide and contamination layers are nanometric [3], so it cannot localise the oxygen change (contaminant layer vs passive oxide) nor measure oxidative capacity; the atomic O/Ti ratio (0.59-1.09), far below the TiO2 value of 2.0, confirms the dominance of the bulk signal. Surface-sensitive analyses (XPS, Auger), corrosion testing and functional assays are needed to clarify its nature and biological relevance.

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Keywords: dental implants, peri-implantitis, titanium, SEM-EDS, surface characterisation, electric-field decontamination.

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Anatomical Features of the Osteomeatal Complex: A CBCT-Based Retrospective Study in Living Subjects

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The osteomeatal complex (OMC) is a key anatomical and functional unit of the sinonasal system, responsible for ventilation and mucociliary drainage of the maxillary, frontal, and anterior ethmoidal sinuses. Anatomical variations and mucosal alterations in this region may impair drainage and affect the outcome of surgical and dental procedures involving the maxillary sinus [1,2]. Cone Beam Computed Tomography (CBCT) enables detailed three-dimensional evaluation of the OMC and its variants in living subjects [3]. A retrospective observational study analyzed CBCT scans acquired at the "G. d'Annunzio" University of Chieti-Pescara. A total of 176 maxillary sinuses from adult subjects were included. The variables assessed included: presence, patency, and location of the primary maxillary ostium (PMO); presence and multiplicity of accessory maxillary ostia (AMO); ethmoidal infundibulum morphology; Schneiderian membrane status; posterior dentition, endodontic, and periodontal conditions; and demographic variables. Statistical analyses included descriptive and inferential tests and multivariate logistic regression. Statistical significance was set at $p < 0.05$. The PMO was identified in most cases, predominantly in the superior-anterior and superior-middle regions of the medial maxillary wall. PMO patency showed a significant association with Schneiderian membrane status (OR = 5.55; 95% CI: 2.15-15.15), with healthy sinuses showing higher ostial patency. Likewise, AMO presence was significantly associated with Schneiderian membrane status (OR = 4.56; 95% CI: 2.25-9.56). Age was associated with AMO presence at univariate but not multivariate analysis. No significant associations were found between PMO patency and dental, endodontic, periodontal, or morphometric variables. CBCT represents a reliable tool for the in vivo anatomical assessment of the osteomeatal complex. Schneiderian membrane status emerged as the main factor associated with both PMO patency and AMO presence, whereas demographic and odontogenic variables had no independent influence. These findings underscore the role of mucosal health in OMC function and support the use of CBCT in diagnostic and preoperative assessment.

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Keywords: Osteomeatal complex, maxillary sinus ostium, accessory maxillary ostium, Schneiderian membrane, cone-beam computed tomography.

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Schwann cell-mediated regulation of melanoma growth and vascular plasticity

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Tumor–nerve crosstalk has recently emerged as a critical driver of malignant progression. A positive association between nerve fiber density and cancer recurrence risk has been established across multiple cancer types, including prostate, gastric, colorectal, and head and neck cancers [1, 2]. Emerging evidence is beginning to clarify the role of Schwann cells (SCs), the most prevalent glial cells of the peripheral nervous system, in this process [3]. SCs found in tumor environments resemble repair SCs observed after nerve injury. A growing body of evidence suggests that these cells actively contribute to shaping the tumor microenvironment by modulating immune responses and enhancing invasiveness [4]. We previously demonstrated that SCs displaying a repair phenotype produce soluble factors that promote migration, invasion, survival, and proliferation of cholangiocarcinoma cells *in vitro* [5]. Here, we aimed to elucidate how SC-derived signals influence melanoma progression and to identify the molecular mechanisms of SC/melanoma interaction.

Immunohistochemical analyses on human melanoma sections revealed higher expression of SC markers (p75NTR/NGFR and GAP43) around melanoma cell nests compared to melanocytic nests of benign nevi. Human melanoma A375 cells and primary SCs (ScienCell Research Laboratories) were maintained under standard culture conditions for *in vitro* experiments. Migration and clonogenic assays were performed as previously reported [5]. We found that conditioned medium (CM) obtained from primary human SCs increases i) melanoma cell migration and ii) clonogenic growth of melanoma cells. In particular, we observed a statistically significant increase in the number and in the size of colonies formed by melanoma cells treated with SC-CM, compared to colonies obtained with control CM. Besides, a specific TGFβ receptor 1 (TGFBR1) antagonist (SB431542) was able to revert the clonogenic activity of SC-CM on melanoma cells, suggesting a potential involvement of TGF-β signalling. Confocal immunofluorescence microscopy also revealed SC markers (p75NTR/NGFR and GAP43) near CD31 positive vascular structures (some of which were quite abnormal), thus hypothesizing possible involvement of SCs in modulating angiogenesis and vasculogenic mimicry. In this regard, our angiogenesis assays on Matrigel indicate that SCs form capillary-like structures in the presence of melanoma cell derived CM. We can conclude that there may be two-way communication between melanoma cells and SCs, where SCs positively control melanoma growth while melanoma-derived factors can modulate SC vascular plasticity.

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Keywords: Schwann cells, melanoma, angiogenesis.

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Unveiling the Interplay between Mitochondrial Dysfunction and Mito-EVs Secretion in Pancreatic Ductal Adenocarcinoma (PDAC)

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The extracellular trafficking of mitochondrial components has been increasingly linked to tumor survival and chemoresistance across various human malignancies (1,2). Furthermore, recent evidence highlights a notable enrichment of circulating extracellular vesicles (EVs) carrying specific mitochondrial DNA (mtDNA) mutations in the serum of patients with pancreatic ductal adenocarcinoma (PDAC) (3). Building on our prior findings that demonstrated the exocytosis of fragmented mitochondria by an aggressive PDAC histotype (4), this study aims to correlate the metabolic reprogramming of PDAC cells, specifically involving mitochondrial impairment, with the EV-mediated intercellular shuttle of mitochondrial cargoes. The study was initiated using human pancreatic cancer cell lines and subsequently translated into 3D patient-derived organoids (PDOs). Interestingly, distinct mitochondrial alterations in terms of function and morphology were confirmed in human pancreatic cancer cell lines (PANC-1, BXPC-3) compared to healthy controls (HPDE), which correlated with an increased release of mitochondrial cargoes into the supernatant. In the 3D models, initial PDO stratification using Gene Set Enrichment Analysis (GSEA), combined with subsequent functional assays, revealed that advanced tumors with suppressed mitophagy adapt to mitochondrial dysfunction by actively releasing defective mitochondrial cargoes, specifically marked by TOMM20 and NDUFA4L2. Furthermore, preliminary treatment with the inhibitor MRTX 1133 suggests that the KRAS G12D mutation plays a key regulatory role in maintaining mitochondrial homeostasis through mitophagy. In conclusion, this study highlights the potential of mitochondria-derived vesicle markers for PDAC diagnosis, identifying NDUFA4L2 as a highly specific candidate due to its link to active tumor reprogramming. Future research should explore how NDUFA4L2-enriched vesicles alter surrounding stromal cells and assess whether this mechanism correlates with tumor aggressiveness to enhance patient stratification and personalized therapy.

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Keywords: PDAC, mitochondrial dysfunction, extracellular vesicles.

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Bisphenol A Effects on Cell Viability and Fibrosis in 2D and 3D Models of Myometrial, Leiomyoma and Leiomyosarcoma Cells

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Bisphenol A (BPA) is an endocrine-disrupting compound widely present in the environment and observed to be implicated in uterine pathophysiology, but its effects across benign and malignant uterine smooth-muscle disorders remain incompletely characterized. Several studies suggest that BPA and related bisphenols may promote uterine disease and extracellular-matrix remodeling through molecular mechanisms associated with fibrosis [1,2,3]. Therefore, in this study, cell viability and fibrosis-related markers [4,5] were used to compare BPA responses in myometrial, leiomyoma and leiomyosarcoma cell lines, using complementary 2D and 3D culture systems. In 2D cultures, myometrial, leiomyoma and leiomyosarcoma cell lines were exposed for 48 h to BPA concentrations ranging from 0.56 pM to 200 μM, and viability was assessed by MTT assay. Fibrosis-related transcriptional responses were evaluated by RT-qPCR for collagen 1A1 (COL1A1), fibronectin and activin A [6,7]. In 3D models, bioprinted cultures from the three cell lines were exposed with an environmentally relevant BPA concentration (56 pM) for 48 h and cell viability was analyzed by PrestoBlue assay. In addition, leiomyoma 3D bioprinted cell cultures were exposed for 7 days to 56 and 560 pM BPA concentrations, and immunohistochemical assessment of fibronectin and collagen 1A1 was performed. Our results showed that in 2D cultures, BPA reduced viability in all three cell lines only at the highest concentration tested (200 μM), whereas lower doses, including the environmentally relevant concentration of 56 pM, did not significantly affect viability. Similarly, 56 pM BPA did not significantly affect cell viability in myometrial, leiomyoma or leiomyosarcoma models in 3D, indicating that short-term low-dose exposure was not acutely cytotoxic. Despite the absence of a viability effect at low dose, BPA induced clear cell type–dependent transcriptional changes. In monolayer myometrial cells, COL1A1 and fibronectin were significantly upregulated across several BPA concentrations, whereas activin A remained substantially unchanged. Leiomyoma cells grown in 2D showed the strongest profibrotic response, with increased COL1A1 and fibronectin expression across multiple doses and significant activin A upregulation. In contrast, 2D leiomyosarcoma cells displayed a divergent pattern, mainly to high-dose exposure, characterized by reduced COL1A1 together with increased fibronectin and activin A at 200 μM. In leiomyoma 3D bioprinted cultures, immunohistochemistry for fibronectin and COL1A1 yielded qualitative information on extracellular-matrix organization after 7 days of BPA exposure at 56 and 560 pM. Compared with controls, BPA-treated 3D cultures displayed a visibly more heterogeneous distribution of fibronectin and COL1A1 immunoreactivity within the 3D constructs. These findings indicate that BPA therefore exerts cell type–dependent effects in uterine models. While acute low-dose exposure does not impair viability in either 2D or 3D systems, BPA promotes a profibrotic molecular program in myometrial and especially leiomyoma cells, whereas leiomyosarcoma shows a distinct response suggestive of malignant matrix remodeling [8] rather than conventional fibrosis. Overall, 3D bioprinted models represent a useful platform for future study on BPA-driven pathological changes in myometrial disorders.

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Keywords: Bisphenol A, myometrium, leiomyoma, leiomyosarcoma, 2D cultures, 3D bioprinted cultures, cell viability, fibrosis, extracellular matrix remodeling.

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Assessing Urinary Exosomal microRNAs as a Novel Biomarker Strategy for Monitoring Oncological and Nervous System Diseases: A Preliminary Study

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MicroRNAs (miRNAs) transported by extracellular vesicles can cross biological barriers and reflect disease stage, progression, and treatment response across multiple pathological conditions [1,2]. While most studies have focused on blood, urinary exosomes emerge as an attractive, non-invasive matrix for biomarker discovery, particularly in vulnerable patient populations, as they may capture systemic processes not restricted to the urogenital tract [3]. In this exploratory study, we investigated whether urinary exosomal miRNA profiling could serve as a biomarker source for monitoring patients with oncological or central nervous system disorders. Urinary exosomes from 24 individuals with multiple sclerosis (MS) and severe disability were analyzed for a panel of 87 miRNAs implicated in neuroinflammation, cardiovascular regulation, and MS pathophysiology, revealing biological heterogeneity within an otherwise clinically homogeneous cohort. Some miRNAs discriminated between primary and secondary progressive MS, indicating their potential utility in subtype stratification, while a panel of commonly upregulated miRNAs, most previously reported as dysregulated in MS compared with healthy controls [4], identified patients with longer disease duration and greater disability. Furthermore, three miRNAs, previously associated with tumor malignancy [5-7], were examined in 23 patients with colorectal cancer before and after surgery, showing that their urinary exosomal levels significantly correlated with the presence or absence of neoplasia, and indicating their potential use as diagnostic and/or prognostic markers.

Although preliminary, these findings provide the first evidence in humans linking urinary exosomal miRNA profiles to clinical features of colorectal cancer and MS, and support the concept that urine may serve as a non-invasive, easily accessible and repeatable source of biomarkers, capable of complementing conventional monitoring of vulnerable patients, like oncological and with severe neurological diseases, contributing to their more personalized management strategies.

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Keywords: Urinary exosomes, miRNAs, multiple sclerosis, colorectal cancer, non-invasive biomarkers.

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Activin A and CD68+ Macrophage Distribution in Human Periorbital Skin: Histological Correlations with Collagen Remodeling and Wound-Healing Response

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Activin A, a member of the transforming growth factor-β superfamily (TGF-β), has been implicated in inflammation, extracellular matrix remodeling, wound repair, and fibrotic responses [1-3]. In parallel, CD68+ macrophages represent key cellular mediators of cutaneous repair, contributing to inflammatory regulation, matrix remodeling, and tissue resolution [4, 5]. The present study aimed to investigate the histological and immunohistochemical expression pattern of Activin A in human periorbital skin and to evaluate its relationship with collagen organization, CD68+ macrophage infiltration, age, smoking status, and clinical wound-healing time. Human skin samples were obtained from 30 patients undergoing elective upper eyelid surgery, including 27 females and 3 males, with a mean age of 52 ± 11 years. Patients were stratified according to age <55 or ≥55 years and smoking status. Tissue specimens were processed for routine histology, Masson's trichrome staining, and immunohistochemistry for Activin A and CD68. Quantitative image analysis and regression models were applied to assess collagen area, immunoreactive cell distribution, demographic variables, smoking exposure, and clinical healing time. Histological analysis showed that collagen area decreased significantly with increasing age and smoking exposure, indicating age and smoke related alterations in dermal matrix architecture. Activin A immunoreactivity and CD68+ cells were mainly detected in the basal epithelial compartment and subdermal connective tissue, supporting their involvement in epithelial-stromal crosstalk and inflammatory remodeling. A positive correlation was observed between Activin A expression and CD68+ macrophage infiltration, suggesting a functional association between Activin A signaling and macrophage-mediated tissue remodeling. Both Activin A and CD68 expression tended to decrease with age, whereas higher expression levels were associated with prolonged clinical healing time. In non-smokers, collagen abundance showed a stronger correlation with Activin A expression compared with smokers, suggesting that smoking may impair physiological matrix-reparative signaling. In patients aged ≥55 years, CD68 expression decreased markedly with age while remaining associated with delayed wound healing, highlighting the potential relevance of macrophage dynamics in age-related impairment of cutaneous repair. Overall, these findings support the presence of an Activin A/CD68+ macrophage axis involved in human skin remodeling. The combined evaluation of Activin A expression, macrophage infiltration, and dermal collagen organization may provide useful tissue markers for assessing reparative remodeling, fibrotic responses, and impaired wound resolution in human skin.

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Keywords: human skin, Activin A, CD68, macrophages, collagen remodeling, wound healing, fibrosis, tissue remodeling, smoking.

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Testicular Tissue Cryopreservation for Fertility Preservation: Preclinical Validation of a Slow-Freezing Protocol in a Rabbit Model

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Advances in pediatric oncology have improved survival rates to over 80%, yet gonadotoxic treatments frequently cause permanent infertility in male survivors [1]. For prepubertal male, testicular tissue cryopreservation is currently the only available strategy to preserve spermatogonial cells prior to therapy. While autologous grafting has successfully produced offspring in non-human primate models [2], optimizing slow-freezing protocols remains essential to mitigate cryoinjury. This study evaluated a standardized slow-freezing protocol using prepubertal and postpubertal New Zealand White rabbits, followed by validation in human prepubertal samples. Histological analysis showed no significant structural alterations post-thaw, with maintained seminiferous epithelial integrity. Morphometric evaluation revealed comparable mean tubular diameters in prepubertal and postpubertal samples, with no significant variations in cross-sectional area. Immunohistochemical quantification of MAGE-A4 positive spermatogonia confirmed the presevation of germ cell populations across both developmental stages. Furthermore, DNA integrity assessed by TUNEL assay showed no statistically significant increase in fragmentation post-cryopreservation. Preliminary human validation demonstrated spermatogonial preservation in a therapy-naive patient. Overall, the results confirm that the standardized slow-freezing protocol effectively preserves tissue architecture, spermatogonial density, and DNA integrity regardless of developmental stage. Our findings support the translational efficacy of the protocol and thereby the necessity of performing testicular tissue cryopreservation before initiating oncotoxic treatments.

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Keywords: Cryopreservation, testicular tissue, fertility preservation, MAGE-A4.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
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Colorectal cancer tumor budding cells express Trop-2 as a driver of malignant invasion

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Trop-2 has been shown to drive metastatic spreading in colorectal cancer (CRC) cells upon activation by ADAM10-dependent proteolytic cleavage between residues R87 and T88 (1). Trop-2 cleavage in turn triggers E-cadherin inactivation and loss of cell-cell adhesion (2). This suggested that Trop-2 could have a direct role in the acquisition of invasive capacity of CRC. Tumor budding (TB) cells have been shown to play a role in CRC invasion, with a negative impact on disease outcome (3,4). Hence we investigated whether Trop-2 associates with CRC budding cells. We collected 80 primary cases of CRC in a test case series. Outcomes were assessed in a validation case series, that included 73 CRC patients. Our findings show that Trop-2 is expressed in essentially all CRC TB and poorly differentiated cell clusters (PDC), and this expression associates with tumor stage and disease outcome. Cell migration and matrix invasion were shown to be driven by Trop-2-dependent induction of β-catenin signaling, via expression of CDC42 and activatory phosphorylation of PKCα at Ser657 and Akt at Thr308. Trop-2, CDC42 and phosphorylated PKCα were jointly shown to have the highest prognostic impact on CRC patients. Thus we propose a model where Trop-2 expression in tumor TB and PDC cells drives CRC progression through the induction of migration and invasion determinants.

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Keywords: Colorectal Cancer, Trop-2, Tumor invasion, Signal transduction pathways.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Expression of serotonin receptors in human vagina: a translational step toward understanding female sexual dysfunction, a pilot study

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Serotonin interacts with a complex receptor network distributed throughout the central and peripheral nervous systems. It has been documented in several regions of the female genital tract, where it acts as a vasoconstrictor/vasodilator and modulates smooth muscle contraction of the genitourinary system (1-3). However, data on vaginal tissue remain scarce. Moreover, since serotonergic innervation modulates genital blood flow and lubrication, its dysregulation may therefore contribute to sexual dysfunction (1).

The present study aims to map serotonergic receptors across the vaginal mucosa, evaluate regional variability, and assess associations with menopausal status and hormonal therapy.

This observational pilot study included pre- and postmenopausal women without current or prior gynecologic malignancy. Samples included liquid-based cytology from the vaginal wall and paraffin-embedded tissue from vaginal biopsies. Western blotting and immunohistochemistry were used to assess serotonergic receptor expression and localization. Cytokeratin and β3-tubulin were used as markers of epithelial and neuronal components.

Vaginal biopsies demonstrated distinct serotonin receptor expression. 5-HT2A and 5-HT2B were predominantly localized in the vascular component, while 5-HT1A, 5-HT1B, 5-HT2B, and 5-HT5 were detected in dendritic cells and lymphocytes of the lamina propria. 5-HT2C and 5-HT6 were confined to nerve fibers. All receptor subtypes were present in nerves, and the serotonin transporter (SERT) was identified in vessels and nerves. Western blot of mucosal scrapings from 18 women (mean age 40.8 years;4 postmenopausal, 14 premenopausal, 10 on hormonal therapy) confirmed 5-HTR expression.

These results suggest that expression of serotonergic receptors within the vaginal wall may be influenced by the hormonal status. Following further validation, the study will be extended to women with female sexual dysfunction to assess 5-HT receptor patterns.

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Keywords: Serotonin, vaginal wall, sexual dysfunction.

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Biological Characterization of Fluoride- and Chlorhexidine-Loaded Electrospun Polycaprolactone Nanofibers for Oral Healthcare Applications

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Introduction: The development of biofunctionalized biomaterials for oral healthcare applications has gained increasing attention due to their potential to combine preventive and antimicrobial properties with favourable biological responses. Electrospun nanofibrous devices represent a promising platform for the controlled delivery of bioactive compounds. This study aimed to investigate the physicochemical properties, cytocompatibility with human gingival fibroblasts (HGF-1), and antimicrobial activity against *Streptococcus mutans* of polycaprolactone (PCL)-based nanofibers functionalized with fluoride (F–) and chlorhexidine (CHX).

Materials and Methods: PCL nanofibers incorporating F–, CHX, or a combination of both (F– + CHX) were produced by electrospinning. Nanofiber morphology was evaluated by scanning electron microscopy (SEM), while chemical characterization was performed using Fourier-transform infrared spectroscopy (FTIR). All analyses were conducted on the different PCL formulations. Biological evaluation included the assessment of cellular metabolic activity by the MTS assay and cytotoxicity by lactate dehydrogenase (LDH) assay at 0, 24, 48, and 72 hours. Cellular morphology was further investigated by SEM. Antimicrobial activity against *Streptococcus mutans* ATCC 35668 was evaluated by determination of viable count (Colony-Forming Unit/mL), after 24 and 48 hours of incubation.

Results: SEM analysis demonstrated a dense and interconnected fibrous architecture, with evidence of the incorporated agents without compromising the overall integrity of the nanofibers, while FTIR spectra confirmed the successful incorporation of the loaded compounds. MTS analysis revealed sustained cellular viability and progressive metabolic activity throughout the experimental period in all treatment groups. LDH assays demonstrated the absence of relevant cytotoxic effects. SEM observations confirmed the maintenance of the characteristic spindle-shaped fibroblastic phenotype, with no detectable alterations in cell morphology. PCL nanofibers loaded with F– and CHX significantly inhibited *Streptococcus mutans* ATCC 35668 growth, indicating a bacteriostatic effect.

Conclusions: The present findings demonstrate that electrospun PCL nanofibers functionalized with F- and CHX exhibit favourable physicochemical, biological and antimicrobial properties. These results support the potential use of bioactive nanofibrous coatings for oral healthcare devices and provide a foundation for further investigations on their preventive and therapeutic applications in the oral environment.

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Keywords: electrospinning, nanofibers, biomaterials, fluoride, chlorhexidine, oral healthcare.

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Human and clinical anatomy of Frida Kahlo: iconodiagnosis

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There is a strong link between art and medicine. Iconodiagnosis is the retrospective analysis of medical conditions through the artistical representation of the human body (sculptures, paintings, drawings, death masks, etc.) and was introduced by the neuropsychiatrist Anneliese Pontius in 1983 [1]. Magdalena Carmen Frida Kahlo y Calderón (1907-1954), better known as Frida Kahlo, was a Mexican painter renown for her rich artwork of which 55 are self-portraits. The latter, together with a wide available photographic collection, represents valuable visual data that helps us to better comprehend the complex artist's physical and medical conditions along time. Frida was a woman able to transform her own scars in art. Key elements of Kahlo's artwork are spinal and musculoskeletal trauma; disability; pregnancy loss and miscarriage; neurological, endocrine and dermatology issues; hidden congenital conditions, and mental illness [2-4]. At 15 years old she was accepted to the elite National Preparatory School, where she focused on natural sciences with the aim of becoming a physician, a fact that will sign her passion for anatomical details. At 18 years old a tram accident left her with multiple fractures (spine, clavicle, hip, right foot), luxation of the left elbow and a deep abdominal wound tearing her inner and outer genitals. Along life she had other health problems that forced her to be confined to bed for many months, needing to wear over 28 medical plaster and leather corsets. Her parents assembled a special bed with a top mirror, so she could see her own reflex and the room; they gave her a special easel, so she was able to paint. She declared, "I paint myself because I am often alone and I am the subject I know best." She underwent 32 surgeries in her lifetime, including multiple spinal surgeries, bone grafts, and the amputation of her right leg at the knee due to gangrene. At that time, she needed a wheelchair and crutches to be ambulatory. Consequently, she became severely depressed and anxious, and her dependence on painkillers, cigarettes and alcohol, escalated. She died in 1954 at 47 years old. The official cause of death was pulmonary embolism. Kahlo's ashes are displayed in a pre-Columbian urn at La Casa Azul, which is now a museum. Frida's pictorial self-representation renders herself portraiture like medical reports and a visual autobiography [3]. Like radiographs, artworks require a form of interpretation and Frida Kahlo challenges and develops our interpretative power. Therefore, her artwork is suitable to be used as teaching triggers within the medical curriculum. The application of iconodiagnosis in medical training is a powerful tool as well as an alternative and stimulating way to exercise students' visual and diagnostic skills [1].

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Keywords: Iconodiagnosis, medical training, visual arts, medical education, clinical anatomy, observation skills.

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In Vitro Morphological and Immunomodulatory Investigations of the Macrophage Response to Hexahydroquinoline-3-carboxylates

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Inflammation acts as a fundamental biological defense, yet its evolution into a persistent chronic state drives the global burden of major diseases, including cardiovascular disorders, diabetes, and neurodegeneration [1]. Macrophages play a leading role in inflammatory dysregulations, serving as the primary mediators of tissue homeostasis and the resolution phase following acute inflammation [2]. In this study, we investigated the immunomodulatory potential of a series of hexahydroquinoline derivatives [3], previously evaluated for their promising anti-inflammatory activity on murine 3T3 cells. Shifting the research focus to the biological response of the human immune system, we aimed to determine how these compounds influence the immunophenotype and protein expression of human macrophages, as a switch toward a pro-resolving phenotype is considered fundamental for regenerating redox equilibrium and maintaining tissue homeostasis.

To model the inflammatory response, human monocytes were differentiated into macrophages using phorbol-12-myristate-13-acetate (PMA). Cells were then treated with lipopolysaccharide (LPS) from *Escherichia coli* to trigger the inflammatory responses via Toll-like receptor 4 (TLR4) and induce classic M1 polarization. The cellular response was characterized through an integrated approach. Cell metabolic activity was assessed via MTT assays. The specific immunophenotype was analyzed using flow cytometry to monitor the surface markers CD14, CD80 (M1-like), and CD163 (M2-like). To evaluate the underlying molecular pathways of stress and inflammation, we performed Western blotting to quantify the protein expression of HSP70, COX2, and HIF1A. Furthermore, mitochondrial superoxide levels were detected to assess the restoration of redox balance. Finally, optical microscopy was employed to evaluate alterations in cell shape and morphology.

The results confirmed the high cell metabolic activity of the compounds within the macrophage model. A significant biological outcome was the restoration of mitochondrial redox balance, with superoxide levels being partially but significantly ($p<0.0001$) returned to control levels. Regarding the immunophenotype, the high expression of CD80 alongside baseline levels of CD163 indicated that, under current experimental conditions, the macrophages had not yet achieved a phenotypic switch toward a pro-resolving M2 state. However, the significant upregulation of HSP70 and preliminary findings regarding HIF1A expression suggest an activated protective mechanism that could facilitate an indirect modulation of the inflammatory response. To further explore these cellular adaptations, morpho-functional experiments are currently underway to correlate structural changes with protein expression.

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Keywords: Inflammation, macrophages, immunophenotyping, HSP70, HIF1A.

This work was produced while attending the PhD programme in Biomolecular and Pharmaceutical Sciences at the University of Chieti–Pescara, Cycle XLI, with the support of a scholarship financed by the NRRP - funded by the European Union – NextGenerationEU – Regione Abruzzo – Dipartimento Sociale – Enti Locali – Cultura PR FSE + ABRUZZO 2021-2027 "Investimenti a favore dell'occupazione e della crescita" (DM 226/2021) (Damiano Iacovozzi).

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Interplay of chronic mild stress, glial activation and neuroinflammation in an adult mouse model of Parkinson's disease

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To characterize the interplay among chronic mild stress (CMS), glia activation and neuroinflammation in an adult mouse model of Parkinson's disease (PD), 10-12 weeks old mice were randomly assigned either to control group or subjected to CMS, that was induced by exposing mice every day for 24 weeks to random stress stimuli. At the end of treatment half of control and CMS groups respectively were treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that causes the selective degeneration of the substantia nigra dopaminergic neurons, the main pathological feature of human PD [1]. Glia activation was evaluated by GFAP (for astrocytes) and IBA1 (for microglia) expression; TLR4 and CD40 expression in astrocytes and microglia were evaluated as neuroinflammation activation markers. CMS effects on mice were studied by the glucocorticoid receptor (GR) nuclear translocation and expression analysis. GR shuttles from the cytoplasm to the nucleus upon binding to cortisol, where it modulates the transcription of glucocorticoid-responsive genes. The binding to its ligand leads also to GR transcriptional regulation [2]. As expected, MPTP treatment caused a strong astrocyte activation. Moreover, GFAP expression was also increased in striata of stressed animals compared to non-stressed counterparts. Microglia of MPTP-treated animals showed an activated phenotype, together with a more intense IBA1-immunoreactivity; CMS induced an increase of IBA1 immunoreactivity, too. TLR4 expression was strongly induced by MPTP treatment; in contrast, CMS alone had no significant effects on TLR4 expression in both astrocytes and microglia, as well as in stressed MPTP-treated animals. CD40/GFAP and CD40/IBA1 double labelling immunofluorescence experiments showed that CD40 expression increased in astroglia and microglia, respectively, of MPTP-treated mice. Although CMS induced glial activation, it had no effect on CD40 expression. Animals subjected to CMS alone showed both GR nuclear translocation and increased GR expression, likely as a consequence of cortisol release, whereas MPTP had no significant effects on GR translocation or expression. In mice subjects to both CMS and MPTP treatment there was no evident GR translocation and expression increase. These results show an inverse correlation between CMS-induced GR activation and pro-inflammatory markers expression; further studies are needed to investigate the effects of CMS in MPTP-treated mouse model of PD disease.

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Keywords: neuroinflammation, Parkinson's disease, glia, TLR4, CD40.

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Clinical mapping of pelvic compartments: anatomical insights for safe and effective surgery

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The pelvic fascia and compartmental anatomy constitute a complex connective tissue framework that provides structural support to the pelvic organs while serving as a conduit for blood vessels, lymphatics, and nerves [1]. Accurate understanding and mapping of these compartments are critical for surgeons performing minimally invasive and reconstructive procedures [2]. Despite their clinical importance, terminology and descriptions of pelvic fascial layers and spaces remain inconsistent in the literature, posing challenges during surgical interventions [3]. These compartments are defined by fascial layers and ligaments, which are integral to both functional support and surgical navigability of the pelvis [4]. This review aims to provide a comprehensive clinical mapping of pelvic compartments, focusing on the fascial architecture and anatomical boundaries that guide safe dissection and preserve vital neurovascular structures.

We analyze the pelvic fascia from an anatomical perspective, detailing the organization of the endopelvic fascia, parietal and visceral layers, and defining key compartments including the retropubic (Retzius) space, paravesical, pararectal, and presacral spaces [5]. Emphasis is placed on the anatomic dissection planes and surgical spaces encountered during laparoscopic, robotic, and reconstructive pelvic surgeries. Variations in fascial continuity and compartmental borders are discussed to inform precise surgical approaches.

By integrating morphological knowledge with clinical application, this review enhances spatial orientation within the pelvis, reduces intraoperative risks, and facilitates more precise surgical outcomes. Improved anatomical insights into pelvic compartments support surgeons in achieving safer and more effective interventions for both benign and malignant pelvic conditions.

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Keywords: pelvic fascia, pelvic compartments, surgical anatomy, endopelvic fascia, retropubic space, pararectal space, minimally invasive surgery, reconstructive surgery.

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Photobiomodulation Reverses Key Hallmarks of Tendinopathy in Human Tenocytes

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Tendinopathies pose a significant challenge in musculoskeletal medicine, as existing treatments primarily focus on symptoms and rarely promote genuine tissue regeneration. The progression of tendon degeneration is closely associated with extracellular matrix (ECM) dysregulation, loss of the tenocyte phenotype, and activation of oxidative stress-related mechanisms that impair the tissue repair process [1,2]. In this context, photobiomodulation (PBM) has emerged as a promising non-invasive strategy capable of modulating cellular metabolism, mitochondrial function, and regenerative processes [3,4]. To assess PBM efficacy, we established an in vitro model of tendon injury by exposing human Achilles tendon-derived tenocytes (hAT1-tert) to triamcinolone acetonide (TCA) or dexamethasone (DEXA), two glucocorticoids (GCs) commonly used in clinical practice and that exert detrimental effects on tendon tissue [5,6]. Cells were then exposed to PBM using a GaAs diode laser (635 ± 5 nm, 4 J/cm^2, single session); additionally, the adjuvant effect of vitamin D_3 (Vit D_3) was evaluated. GCs' exposure compromised tendon biological integrity in vitro by impacting cell viability, tendon-specific marker expression, ECM synthesis, oxidative stress (ROS), NF-κB activation, and mitochondrial morphology. PBM promoted a coordinated recovery of both the tenogenic phenotype and ECM composition, with increased Scleraxis and Tenomodulin expression, normalization of Decorin and Tenascin-C levels, and improvement of the COL1A2/COL3A1 ratio. At the same time, PBM reduced ROS levels, attenuated NF-κB activation, and preserved mitochondrial morphology, supporting tendon regenerative processes. Furthermore, Vit D_3 preconditioning further enhanced the effects of PBM. These findings suggest that PBM, especially when combined with Vit D_3, enhances tendon biological recovery by coordinating cellular activities and extracellular matrix remodeling, highlighting its potential use in musculoskeletal regenerative approaches.

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Keywords: Photobiomodulation, Vitamin D_3, Glucocorticoids, Tendinopathy, Extracellular Matrix, Oxidative Stress.

Acknowledgements

This Project Has Received Funding From The Italian Ministry Of University And Research, Under The Prin 2022 Programme (20222p2naj, Cup I53d23003030006) (Finanziato Dall'unione Europea – Next Generation Eu)

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Proteomic profiling identifies a stromal TGF-β1/podoplanin axis as a driver of colorectal cancer progression

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The tumor microenvironment (TME) plays a pivotal role in the development and progression of colorectal cancer (CRC), yet the complex crosstalk among its components remains incompletely understood. Cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) have emerged as key regulators of CRC progression, but their specific contributions, particularly given their heterogeneity, are not fully elucidated [1-3]. This study identifies podoplanin (PDPN), a transmembrane glycoprotein enriched in CAFs, as highly expressed in the CRC TME, in particular surrounding the tumor, and associated with macrophage infiltration and cancer progression [4,5]. We performed mass spectrometry-based proteomic analysis on matched CRC and adjacent normal tissues from patients to identify altered signaling pathways and protein expression. The clinical relevance of PDPN expression was evaluated in CRC samples from two independent cohorts using immunohistochemistry and immunofluorescence analysis. Publicly available data from the Gene Expression Omnibus (GEO) database were analyzed to assess the association between PDPN expression and patient survival. Functional assays using direct and indirect co-culture systems investigated the influence of macrophage infiltration on stromal PDPN expression and its effect on colon adenocarcinoma cell growth. PDPN expression was significantly elevated in the stroma of the colorectal tumor tissues compared to normal tissues and correlated with M2-like macrophage infiltration. High PDPN expression was associated with reduced relapse-free survival in CRC patients. Stromal cells pre-conditioned with M2-like macrophages upregulated PDPN and more effectively supported the growth of three colon adenocarcinoma cell lines. PDPN depletion impaired the ability of stromal cells to promote tumor cell proliferation. Mechanistically, M2-like macrophage pre-conditioning induced a TGF-β1-dependent increase in YAP/TAZ nuclear localization, RhoA/ROCK/myosin-driven cytoskeletal contractility, and extracellular matrix (ECM) production in stromal cells. Inhibition of TGF-β1 signaling or ROCK activity reduced stromal support for cancer cell growth. This study reveals a novel mechanism by which the TME facilitates CRC progression and highlights PDPN as a potential prognostic biomarker and therapeutic target in CRC [6].

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Keywords: colorectal cancer (CRC), podoplanin (PDPN), tumor microenvironment (TME), extracellular matrix (ECM).

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From fat to follicle: generation of human pregranulosa-like cells from adipose-derived mesenchymal stem cells

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Age, diet, and environmental factors, as well as metabolic and endocrine disorders, significantly impact female reproduction. For this reason, many efforts have been made to study *in vitro* oocyte production by generating primordial germ cells (PGCs) [1]. However, correct meiotic maturation and growth of a PGC depend primarily on stage-specific support provided by granulosa cells (GCs) [2, 3]. This study aims to generate pregranulosa-like cells (pGLCs) from human adipose mesenchymal stem cells-derived iPSCs (hASC-iPSCs), which share the same embryonic origin as native GCs. Human ASC-iPSCs were previously established in our laboratory [1, 4]. Two protocols for pGLC production published in mice were adapted for use with human cells [5, 6]. In the first protocol [5], stepwise differentiation recapitulates embryonic development: hASC-iPSCs were treated to induce epiblast (confirmed by Brachyury), followed by intermediate mesoderm (OSR1), and coelomic epithelium (GATA4), before final pGLC induction. In the second [6], hASC-iPSCs were cultured with vitamin C and AM580 to directly generate pGLCs. Both protocols induced expression of pGLC markers FOXL2, FSHR, GATA4, LGR5, and WNT4, with significant downregulation of pluripotency markers OCT4 and NANOG, confirming differentiation. The presence of FOXL2 protein, a hallmark of granulosa lineage, was confirmed by immunofluorescence and Western blot. Notably, the two protocols generate distinct pGLC populations reflecting the two pregranulosa waves described *in vivo* [7, 8]: the first protocol yields cuboidal cells resembling PG2 precursors with high LGR5 expression, while the second protocol produces more flattened cells with high FOXL2 expression, along with CYP19A1 and OSR1, indicating PG1 phenotype. These findings provide a foundation for future studies, including single-cell transcriptomic characterization of the two pGLC populations, reconstitution of *in vitro* follicles by aggregating pGLCs with immature oocytes, and evaluation of their maturation by using microfluidic platforms that can mimic the ovarian niche [9].

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Keywords: pregranulosa-like cells, induced pluripotent stem cells, adipose mesenchymal stem cells, in vitro differentiation, folliculogenesis, FOXL2, female infertility.

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Whole XY chromosomes aneuploidy in sperms: From Morphology to Molecular Investigation

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Sex chromosome aneuploidies (SCAs) represent some of the most prevalent chromosomal variations in humans, with Klinefelter syndrome (KS) (47, XXY) being among the most common. Unlike autosomal aneuploidies, KS often originates paternally, attributed to a lack of crossover formation (CO) between XY chromosomes and subsequent nondisjunction during the first meiotic division [1]. Interestingly, fathers of KS patients show a notably higher incidence of XY aneuploidy compared to the general population [2], hinting at a genetic predisposition.

Despite its prevalence, the genetics underlying KS remain elusive. In both mice and humans, CO formation between XY chromosomes occurs within the pseudoautosomal region (PAR), initiated by double-strand breaks (DSBs) orchestrated by the protein SPO11 in tandem with TOPOVIBL. Previous studies, including our own, have highlighted the influence of both trans-acting factors (such as *Ankrd31* gene expression) and cis-acting elements (like the PAR chromatin loop/axis ratio) on recombination initiation in the PAR (reviewed in [3]).

In mice, the recruitment of DSB-promoting factors in the PAR is facilitated by a 31bp heterochromatic minisatellite (mo2) sequence, which displays significant inter-individual variability in copy number [4] . However, the impact of mo2 expansion on XY nondisjunction remains uncertain. Utilising a mouse model of XY nondisjunction developed in our lab [5], we explored the relationship between mo2 copy number and DSB formation proficiency in the PAR.

Preliminary findings reveal that a lower mo2 copy number is associated with testicular atrophy, increased spermatocyte apoptosis, and heightened rates of XY diploidy and nulliploidy in sperm. Beyond mo2, our comprehensive analysis of the PAR also reveals loci of homology mismatch that could significantly affect recombination, emphasising the structural diversity within this region. These loci are currently under further investigation, promising new insights into the mechanisms driving KS and potential therapeutic targets.

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Keywords: Germ cells, aneuploidy, nondisjunction, mouse model.

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Extracellular Vesicles-mediated propagation of Integrated Stress Response signaling promotes invasive traits in Colorectal Cancer

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Introduction. Colorectal cancer (CRC) progression is influenced by oncogenic signaling and adaptation to microenvironmental stress. A pivotal oncogenic role is attributed to KRAS mutations, occurring in 40% of metastatic CRC with the liver representing the primary site for distant metastasis. To survive and adapt to the stressful tumor microenvironment, cancer cells hijack the Integrated Stress Response (ISR), an evolutionarily conserved adaptive signaling pathway that senses and responds to cellular stress cues. Through ISR activation, cancer cells enhance their plasticity and metastatic potential.

While ISR is classically viewed as a cell-autonomous adaptive program, whether stress-adaptive states can be communicated between tumor cells and in their microenvironment remains largely unknown. Given the central role of extracellular vesicles (EVs) in intercellular communication within tumors, we hypothesize that EVs can propagate ISR signaling across the CRC tumor ecosystem contributing to the acquisition of pro-metastatic traits.

Methods. Molecular and biochemical techniques, coupled with cutting-edge imaging, were employed in 2D and 3D CRC models. Functional effects were assessed using microfluidic-based matrix invasion assays. EVs were isolated from stressed and control cells using sequential filtration and size-exclusion chromatography and characterized by TEM and nanoparticle tracking analysis (NTA). EV cargo was profiled by proteomics to define stress-associated remodeling of vesicle composition.

Results. We identified a bidirectional crosstalk between KRAS signaling and ISR, whereby ISR amplifies downstream KRAS signaling while KRAS activity is required for ISR activation. Functionally, ISR activation enhances CRC invasiveness and promotes EV release, linking stress adaptation to intercellular communication output. Importantly, EVs derived from stressed CRC cells propagate ISR signaling in both colorectal and hepatic cells, indicating propagation of stress-adaptive programs across local and distant tumor-relevant environments. This transfer induces functional reprogramming of recipient CRC cells toward enhanced matrix invasion, consistent with metastatic priming.

Proteomic profiling revealed an enrichment of translation-related components in EV cargo, including 40S and 60S ribosomal subunits and eIF2 complex components, suggesting transfer of translational machinery that may support ISR activation in recipient cells.

Conclusions. Our findings define a previously unrecognized mechanism of tumor adaptation in which EVs released upon ISR activation mediate both paracrine and long-range propagation of ISR-associated signaling that promotes invasive traits, including potential priming of the liver microenvironment. These findings identify EV-mediated ISR signaling as a mechanism contributing to CRC metastatic progression and suggest its potential as a therapeutic target.

Keywords: colorectal cancer, extracellular vesicles, Integrated Stress Response, KRAS, metastasis, invasion.

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Characterization of the PLCβ1-KDM4A interplay in acute myeloid leukemia: evidence of chromatin co-localization and genomic co-occupancy

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Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by the uncontrolled proliferation of immature myeloid cells and impaired hematopoiesis. Despite advances in the understanding of AML biology, the identification of novel molecular mechanisms involved in leukemogenesis remains crucial for the development of targeted therapeutic strategies.

In this study, we investigated the interaction between Lysine Demethylase 4A (KDM4A), an epigenetic regulator involved in AML maintenance, and Phospholipase C beta 1 (PLCβ1), a signaling molecule implicated in myeloid differentiation and disease progression, using the AML-derived THP-1 cell line. Pull-down assays employing both full-length proteins and specific KDM4A domains were performed to assess their physical interaction and identify the regions involved in complex formation.

To determine whether PLCβ1 is associated with chromatin, CUT&RUN experiments targeting PLCβ1 were carried out. In parallel, ChIP-seq analyses for KDM4A enabled the evaluation of its genomic distribution. The integration of these datasets revealed genomic regions characterized by the co-occupancy of PLCβ1 and KDM4A, supporting the existence of a functional interplay between these proteins within the chromatin context. Moreover, the expression of selected genes associated with promoter regions identified through CUT&RUN analysis was evaluated to explore the potential transcriptional relevance of this co-localization.

Overall, our findings provide novel insights into the relationship between signaling and epigenetic mechanisms in AML, demonstrating the chromatin association of PLCβ1 and its spatial convergence with KDM4A at specific genomic loci. These observations contribute to a deeper understanding of the molecular architecture underlying AML pathogenesis and may offer new perspectives for the identification of disease-related regulatory networks

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Keywords: AML, PLCβ1, KDM4A, Epigenetics, Cut&Run.

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Upper Airway and Soft Tissue Modifications Induced by incremental mandibular advancement: A Donor-Based CT Investigation

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Obstructive Sleep Apnoea (OSA) is a sleep-related chronic disorder affecting over one billion people worldwide [1], characterized by repeated episodes of upper airway obstruction during sleep, leading to intermittent hypoxemia, sleep fragmentation, and an increased risk of cardiovascular and neurocognitive comorbidities [2]. Diagnosis relies on laboratory-based polysomnography, which quantifies disease severity through the Apnoea–Hypopnea Index (AHI). Among available therapeutic options, other than surgical procedure, continuous positive airway pressure (CPAP) remains the first-line treatment; however, its long-term effectiveness is often undermined by poor patient tolerability and adherence [3]. In this context, the Mandibular Advancement Device (MAD) has emerged as an effective alternative, particularly for patients with mild-to-moderate OSA or those unable to tolerate CPAP [4,5]. MADs act by displacing the mandible and tongue base, thereby increasing pharyngeal calibre and reducing upper airway collapsibility. Nevertheless, the three-dimensional anatomical modifications induced by MAD therapy have not yet been characterised in a standardised and systematic manner, particularly in relation to the degree of mandibular advancement. To address this gap, the present study adopts an innovative body donor-based approach, which permits the use of computed tomography (CT) without the radiation exposure constraints inherent to in vivo investigations. Eight body donors underwent serial CT acquisitions at baseline and at progressive mandibular advancement steps of 2 mm up to a maximum of 12 mm, using a titratable intraoral device designed to simulate the clinical functioning of a MAD. Upper Airway Space (AS), Tongue (T) and Floor of the Oral Cavity (CF) measurements were obtained using a standardized protocol. Inter- and intra-observer reliability were assessed using the Intraclass Correlation Coefficient (ICC). Superior Airway Space (SPAS) perimeter increased significantly starting from 4 mm of protrusion ($p = 0.0156$, $r = 0.891$), while Inferior Airway Space at Gonion (G-IAS) perimeter showed significant changes from 6 mm ($p = 0.0469$, $r = 0.792$). Inferior portions of the Tongue (IT) and the Inferior Curve of the Floor of the Oral Cavity (ICF) demonstrated significant modifications already from 2 mm of advancement ($p = 0.0078$, $r = 0.891$ and $p = 0.0117$, $r = 0.817$, respectively), whereas significant differences in the Superior Curve of the Floor of the Oral Cavity (SCF) were detected only at 12 mm ($p = 0.0156$, $r = 0.842$). SPAS and G-IAS appear the most responsive airway regions, while early soft tissue changes are detected in their inferior anatomical sections. High intra- and inter-observer reliability has been observed across most measurements. Although limited by the inherent constraints of the donor model, the proposed protocol provides a reproducible and reliable framework for analysing structural effects of mandibular advancement and may support the development of more personalised and evidence-based titration strategies in MAD therapy.

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Keywords: Obstructive Sleep Apnoea, Mandibular Advancement Device, Body Donation, CT scan, Upper Airways, Anatomy.

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Cadaveric head dissection enhances anatomical knowledge of the head and neck region in first-year dental students

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Acquiring a thorough three-dimensional understanding of the head and neck region is a cornerstone of dental education, directly underpinning clinical competencies in oral surgery, implantology, and local anaesthesia. Territorial and logistical constraints increasingly limit access to cadaveric material; nevertheless, hands-on dissection remains uniquely valuable for bridging the gap between didactic learning and clinical practice. The aim of this study was to evaluate the educational impact of a structured cadaveric head dissection protocol on the anatomical knowledge of first-year dental students, compared with lecture-based teaching alone.

A cohort of 25 first-year students enrolled in the Degree Course in Dentistry at the University of Catania participated in the study. All students attended a 3-hour didactic lecture on the anatomy of the head and neck region, delivered by a Professor of Anatomy with the support of PowerPoint presentations and anatomical illustrations. Immediately after the lecture, a combined assessment comprising Multiple Choice Questions (MCQ) and image-based anatomy tests (Anatomy Labelling Test and Image-Based MCQ) was administered anonymously (Test 1). The following day, students attended a practical session in the anatomy laboratory, during which a step-by-step cadaveric head dissection protocol was guided by the same Professor. The protocol encompassed the superficial facial region, parotid and infratemporal fossa, temporomandibular joint, maxillary sinus, mandibular canal, floor of the mouth, and cranial nerves of dental relevance. Upon completion of the dissection, the identical assessment battery was re-administered (Test 2). The cadaveric heads used in this study were provided by the Section of Legal Medicine, Department G.F. Ingrassia, University of Catania, whose collaboration made the practical training possible.

Following the didactic lecture alone (Test 1), approximately 50% of students achieved a passing score. After the cadaveric dissection session (Test 2), the proportion of students reaching a satisfactory level of anatomical knowledge rose to approximately 90%, indicating a marked and clinically relevant improvement in learning outcomes.

These preliminary findings demonstrate that a structured cadaveric head dissection protocol significantly enhances the anatomical knowledge of first-year dental students, beyond what is achievable through lecture-based teaching alone. Hands-on exploration of the cadaveric head allows students to appreciate three-dimensional spatial relationships and anatomical variability, and to more readily identify clinically relevant landmarks compared with textbook study alone. Although based on a pilot cohort, these results support the systematic integration of cadaveric dissection into the dental anatomy curriculum, particularly in the study of the head and neck region.

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Extracellular Vesicles in Muscle Niche Crosstalk During Damage and Regeneration: Modulation by Photobiomodulation

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Muscle injuries are among the most common forms of trauma in physically active individuals and often require effective therapeutic strategies to ensure complete functional recovery. Beyond mechanical damage, pathological and pharmacological conditions—such as oxidative stress and corticosteroid exposure—can also induce muscle atrophy and impair regeneration. Skeletal muscle repair is a tightly coordinated process driven by crosstalk among the cell populations of the muscle niche, including satellite cells, myofibers, mesenchymal stromal cells (MSCs), and macrophages. Extracellular vesicles (EVs) are key mediators of this crosstalk, and a timely transition of macrophages from a pro-inflammatory (M1) to an anti-inflammatory/pro-regenerative (M2) phenotype is essential for the shift from inflammation to tissue remodeling. While EVs are increasingly recognized as regulators of this inflammatory-to-regenerative transition, their specific role under conditions of muscle damage remains incompletely understood.

Photobiomodulation (PBM) has recently gained attention as a non-invasive strategy to enhance muscle repair and function, with emerging evidence suggesting that it can modulate EV release and cargo composition. However, its role in shaping EV-mediated communication during both muscle injury and myogenic differentiation has not yet been clarified.This study aimed to investigate whether PBM influences EV production and functional cargo during myogenic differentiation and in response to muscle stress and to determine how EVs mediate crosstalk between myotubes and macrophages under physiological and pathological conditions. An in vitro muscle niche model was established using C2C12 myoblasts, bone marrow-derived MSCs, and RAW 264.7 macrophages. Myogenic cells were exposed to oxidative stress (H_2O_2) or glucocorticoids (dexamethasone and triamcinolone acetonide) to mimic muscle damage and atrophy, with or without PBM treatment during differentiation. To model a more complex regenerative niche, MSC-derived EVs were added to damaged myotube cultures, and their effect on macrophage polarization (M1-to-M2 transition) was assessed. EVs were isolated and characterized by size, surface markers, and molecular cargo. Our results show that both muscle damage and PBM significantly affect EV release and composition. Damage conditions promoted the secretion of EVs with a pro-inflammatory profile, whereas MSC-derived EVs attenuated this inflammatory signature in macrophages and favored an M2-like, pro-regenerative phenotype. PBM applied during myogenic differentiation modulated EV secretion even in the absence of injury, suggesting a role in priming the muscle niche toward a pro-regenerative state. These findings highlight EVs as central regulators of intercellular communication within the muscle niche under both damaging and non-damaging conditions, and identify PBM as a promising strategy to modulate EV-mediated signaling, with potential therapeutic relevance for muscle regeneration.

Acknowledgements

This project has received funding from the Italian Ministry of University and Research, under the PRIN 2022 programme (20222P2NAJ; CUP I53D23003030006) (finanziato dall'Unione europea – Next Generation EU)

Keywords: EVs, PBM, muscle damage, muscle regeneration, macrophage polarization.

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Comparison between CONV radiotherapy and FLASH radiotherapy in naïve mice: identification of markers of damage and function.

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Oncological diseases affect around 400,000 people annually in Italy [1]. Radiotherapy (RT) is used in more than 30% of total cases to reduce the size of the lesion and eliminate residual tumor cells [2]. While conventional radiotherapy (CONV-RT), characterized by gradual delivery of the therapeutic dose, often harms adjacent healthy tissues, innovative FLASH-RT delivers the therapeutic dose in a single, ultra-short session [3], achieving comparable tumor control with minimal side effects. This approach represents a promising resource for treating cutaneous melanoma, one of the most aggressive malignancies [4]. This study compared CONV-RT and FLASH-RT (35Gy) toxicity on naïve C57BL/J mouse skin and subcutaneous white adipose tissue (scWAT) as normal tissue models, using histological analyses to identify markers of damage and function. CONV-RT caused persistent skin damage, with hyperplasia, marked epidermal proliferation, and increased collagen deposition, whereas FLASH-RT largely preserved the tissue architecture. Because morphological observations showed dermal inflammatory infiltrate, immunohistochemistry for the macrophage-specific marker CD68 was performed. Morphometric analyses revealed a significant increase in both epidermal and dermal thickness in irradiated mice, with damage being more pronounced in the CONV-RT group. A significant increase in macrophage infiltration in the dermis of mice irradiated by CONV-RT was also measured. A positive correlation was identified between dermal and epidermal thickness, as well as between the number of CD68-positive macrophages and epidermis and dermis thickness. Regarding scWAT, mice treated with CONV-RT exhibited severe inflammatory infiltrate, diffuse interstitial fibrosis, and lipid vacuoles associated with delipidation zones. These features were less pronounced in FLASH-RT irradiated mice. Quantitative measurements showed a significant decrease in cell viability (indicated by a loss of the adipocyte-specific marker PLIN1), with more pronounced damage in the CONV-irradiated group. Additionally, scWAT from CONV-RT treated mice showed a quantified increase in macrophage infiltration (CD68 immunohistochemistry) and high fibrosis levels (Sirius Red staining), while FLASH irradiated mice were tissue-preserved. PLIN1 negativity positively correlated with epidermal and dermal thickening, demonstrating an overall involvement of both skin and scWAT. Collectively, these findings demonstrate that skin and scWAT homeostasis are highly sensitive to CONV-RT, whereas FLASH-RT better preserves their structure and function.

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Keywords: CONV-RT, FLASH-RT, Skin, Adipose Tissue.

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YAP-TAZ Activation in Adipose Tissue as Crucial Driver of Endocrine Resistance in Y537S-ESR1 Mutant Breast Cancer

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Somatic mutations in estrogen receptor alpha (ERa) gene (ESR1), particularly Y537S, represent a major mechanism of acquired resistance to endocrine therapies in breast cancer (BC). These mutations stabilize the ERa in a ligand-independent active conformation, leading to constitutive transcriptional activity and being associated with poor prognosis in metastatic disease [1,2]. Increasing evidence highlights the role of the tumor microenvironment (TME), in particularly adipose tissue, as an active endocrine organ in BC progression, contributing to tumor development through paracrine signaling, thereby shaping tumor behavior and therapeutic response [3,4]. Its dysfunction in obesity further enhances this crosstalk, promoting metabolic reprogramming and therapy resistance in ER+ BC [4,5]. As we showed in 2024 [6], deregulation of the Hippo pathway, with consequent activation of YAP/TAZ transcriptional co-activators, represents a key node integrating mechanical and metabolic signals from the microenvironment. YAP/TAZ activity has been recently linked to breast cancer progression, stemness, metabolic adaptation, and endocrine therapy resistance [7,8]. We performed indirect co-culture experiments *in vitro* using MCF-7 parental (P) and Y537S (YS) CRISPR-expressing cells and murine 3T3-L1 mature adipocytes, together with histological analyses in syngeneic models following orthotopic implantation of murine parental cells (generated from a transgenic mouse model, Cre-MMTV- Ki-Ras$^{(G12V)}$, representing the luminal A subtype of BC) in ovariectomized female mice maintained with a low-fat, normal-fat, or high-fat diet. Our results demonstrate that the secretome of YS mutant cells, through IGF-1 secretion, is able to activate YAP signaling and promote its nuclear translocation in mature adipocytes, sustaining their transition into myofibroblasts. Furthermore, in vivo experiments provided evidence that different dietary conditions modulate the interaction between parental breast cancer cells and adipocytes, with effects extending beyond tumor progression to include alterations of the adipose microenvironment. In particular, a high-fat diet significantly increased tumor cell proliferation, accompanied by enhanced peritumoral adipose tissue expansion and microenvironmental remodeling that favored tumor growth.

Overall, our findings highlight the existence of a bidirectional crosstalk between breast cancer cells and adipocytes, in which the Hippo/YAP pathway acts as a key mediator of tumor–microenvironment interactions. Moreover, host metabolic conditions, shaped by dietary factors, contribute to the modulation of this crosstalk, thereby promoting tumor progression and potentially fostering therapeutic resistance. These results suggest that targeting metabolic pathways and YAP/TAZ signaling may represent a promising therapeutic strategy to counteract the progression of ER-positive breast cancer and improve responsiveness to endocrine therapies.

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Keywords: YAP-TAZ, Adipose tissue, Y537S-ESR1 Mutant Breast Cancer, Endocrine resistance, mouse model.

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Differences in iron regulator proteins of biliary epithelium in acetaminophen-induced liver injury

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Ferroptosis represents an iron-dependent form of non-apoptotic cell death, discovered in 2012 and characterized by the accumulation of reactive oxygen species (ROS) and lipid peroxidation (1). It is involved in different hepatic diseases such as cancer and liver fibrosis, providing new ideas for treatments (2). Acetaminophen (APAP) is the most commonly used antipyretic and analgesic agent; however, its overdose is a model of drug hepatotoxicity resulting in acetaminophen-induced liver injury (AILI), characterized by hepatic glutathione depletion, mitochondrial protein adduct formation with oxidant stress, and release of mitochondrial intermembrane proteins, which translocate to the nucleus determining DNA fragmentation and necrosis (3). In APAP overdose, intracellular glutathione (GSH) depletion disables glutathione peroxidase 4 (GPX4) that detoxifies lipid peroxides, thereby triggering ferroptosis, which is a key driver of APAP hepatotoxicity (4). For this reason, we aimed to evaluate the possible role of ferroptosis on biliary epithelium in a rat experimental model treated with APAP (2g/kg). Firstly, we studied the morphological changes in the hepatic parenchyma after treatment and compared to control rats. Then, we assessed the presence of iron regulator proteins through immunohistochemistry and immunofluorescence. We found an alteration in the expression of protein regulators in biliary epithelium of treated animals compared to controls, such as: (i) ferritin (both heavy and light chains) that safely stores iron in the cells, (ii) transferrin receptor 1 (Tfr1/CD71), responsible for cellular iron uptake, (iii) acyl-CoA synthetase long-chain family member 4 (ACSL4) as lipid peroxidation enzyme, (iv) GPX4, as antioxidant enzyme, and (v) 4-hydroxynonenal (4-HNE), fundamental protein for lipid peroxidation. Across these experiments, we found changes in ferritin, Tfr1, ACSL4 and 4-HNE expressions, together with downregulation of GPX4 suggesting that cholangiocytes are also involved in ferroptosis during APAP-induced liver injury.

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Keywords: Biliary epithelium, cholangiocytes, ferroptosis, liver fibrosis, cell death.

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HnRNP C binding to inverted *Alu* elements protects the transcriptome from pre-mRNA circularization

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Back-splicing is a non-canonical splicing event driving circular RNA (circRNA) biogenesis. While its molecular mechanisms are partly known, global regulation in tumors remains unclear. Here, we uncover an hnRNP C-dependent mechanism that represses a broad repertoire of circRNAs in group 3 medulloblastoma (MB). HnRNP C binds *Alu* elements, preventing pre-mRNA circularization. Expression of hnRNP C modulates the balance between linear and circular splicing, ensuring efficient expression of genes that sustain the oncogenic phenotype of group 3 MB cells. In the absence of hnRNP C, introns flanking the circularizing exons generate cytoplasmic double-stranded RNAs via inverted *Alu* base pairing, triggering an interferon-induced antiviral response. These findings unveil hnRNP C as a guardian of transcriptome integrity by repressing circRNA biogenesis. Last, targeting hnRNP C in group 3 MB may trigger an inflammatory immune response, thereby boosting cancer surveillance.

Keywords: RNA-binding proteins, inverted repeated elements, circular RNA, back-splicing.

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Activity-Dependent Neuroprotective Protein (ADNP) Protects Against VEGF-Induced Corneal Barrier Disruption in Diabetic Keratopathy

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Corneal epithelial barrier dysfunction represents a critical feature of diabetic keratopathy (DK), a severe secondary manifestation of diabetes mellitus [1]. While vascular endothelial growth factor (VEGF) is a well-established mediator of retinal barrier breakdown, its precise involvement in DK development requires further investigation. This study explores the potential of activity-dependent neuroprotective protein (ADNP) in mitigating hyperglycemia-mediated damage to the cornea. In vivo assessments using a streptozotocin (STZ)-induced diabetic rat model revealed a significant reduction in ADNP immunoreactivity alongside a reciprocal elevation of VEGF expression within the corneal tissue compared to healthy controls. To model the stratified corneal architecture, rabbit corneal epithelial cells (SIRC) were subsequently maintained in an Air-Liquid Interface (ALI) culture under high-glucose (HG) conditions. Exposure to HG led to a marked impairment of the epithelial barrier, evidenced by diminished transepithelial electrical resistance (TEER) measurements and decreased expression of key tight junction (TJ) proteins, specifically ZO-1 and occludin. Notably, treatment with NAP, the minimal active peptide derived from ADNP, effectively reversed this barrier failure, recovering TEER values and upregulating TJ proteins. Additionally, NAP prevented the HG-induced depletion of the microtubule-associated proteins EB1 and Tau, underscoring its involvement in cytoskeleton stabilization. Our data indicate that NAP counteracts VEGF-driven internalization of Tau and EB1, which would otherwise trigger microtubule disassembly. Finally, NAP administration significantly accelerated the migration and wound-healing kinetics of SIRC cells under hyperglycemic stress. Taken together, these results indicate that ADNP maintains corneal epithelial barrier homeostasis and accelerates tissue repair by anchoring the cytoskeletal-junctional network.

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Deciphering the role of YAP in the leukemic bone marrow microenvironment

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Despite significant advances in targeted therapies for acute myeloid leukemia (AML), drug resistance and relapse remain major challenges, largely due to clonal selection and the protective effect of the bone marrow (BM) microenvironment. The combination of the hypomethylating agent azacitidine (AZA) with the BCL-2 inhibitor venetoclax (VEN) has become a standard regimen for AML patients who are unfit for intensive chemotherapy. Nevertheless, clinical and real-world data show that outcomes remain suboptimal, particularly for patient subgroups harboring FLT3-ITD or RAS-pathway mutations (1,2). In this context, there is an urgent need for therapeutic strategies that not only target leukemic cells directly but also dismantle the BM niche-mediated mechanisms of resistance that undermine the efficacy of current treatments. Accumulating evidence demonstrates that BM-derived mesenchymal stem/stromal cells (MSCs) play a critical role in supporting AML cell survival and promoting therapeutic resistance (3–5). Our research aims to identify the molecular pathways that enable MSCs to shield FLT3-ITD$^+$ leukemic cells from the AZA-VEN treatment. Consistent with the protective properties of the BM microenvironment, the AZA-VEN combination partially lose efficacy when FLT3-ITD$^+$ AML cells are co-cultured with MSCs. Using co-culture systems of human AML cell lines with MSCs cell lines or primary MSCs isolated from AML or healthy donor BM, we identified the co-transcriptional regulator YAP as a central element of MSC-mediated protection. By proteomic analysis and silencing RNA experiments, we demonstrate that YAP activity in MSCs maintains a low-inflammatory state by suppressing STAT1/STAT2 phosphorylation: when YAP is down-regulated, this repression is lifted, triggering a robust inflammatory response driven by increase of IFNb levels and STAT1/STAT2 activation. This inflammatory switch prevents the ability of MSCs to protect AML cells and concurrently induces a similar inflammatory program within the leukemic cells themselves. Notably, simultaneous down-regulation of STAT1 or STAT2 together with YAP partially restores the protective phenotype of MSCs. These findings reveal a previously unrecognized role for YAP as a critical regulator of microenvironment-driven therapeutic resistance.

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Keywords: Acute Myeloid Leukemia, Tumor Microenvironment, Inflammation, YAP.

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In vitro evaluation of keratinocyte barrier compatibility following exposure to a cannabidiol and β-caryophyllene-based topical patch

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The epidermis represents the first biological barrier exposed to topical medical devices; therefore, preservation of keratinocyte viability and membrane integrity is essential to ensure the safety of prolonged skin contact. In this study, an in vitro keratinocyte model was employed to investigate the compatibility of a cannabidiol (CBD) and β-caryophyllene (BCP)-based adhesive patch intended for the topical management of mild musculoskeletal discomfort. Particular attention was devoted to evaluating the effects of the active compounds on epidermal cell integrity and maintenance of barrier-related cellular features following topical exposure. Biological evaluation was performed on HaCaT human keratinocytes exposed to CBD and BCP, both individually and in combination, as well as directly cultured on the patch surface. Cell viability assays demonstrated an overall favorable biocompatibility profile under the tested conditions[1]. BCP showed good tolerability across the tested concentrations and appeared to attenuate the cytotoxic effects induced by high concentrations of CBD, suggesting a protective interaction between the two compounds[2]. Consistently, LDH assay results confirmed the preservation of membrane integrity in most experimental conditions, indicating the absence of significant cytotoxic damage. Ultrastructural analysis by transmission electron microscopy further supported the compatibility of the formulation with epidermal cells. Treated keratinocytes maintained normal morphology, intact plasma membranes, preserved intercellular contacts, and no evident apoptotic alterations. In addition, keratinocytes cultured directly on the CBD/BCP-containing patch displayed metabolic activity comparable to control conditions, with no increase in LDH release and preserved morphology observed by optical microscopy after 24 h of exposure. Lastly, BCP, CBD, and their 1:1 combination were assessed for their antioxidant properties using various methods (radical quenching, reducing power and chelating properties). CBD exhibited a superior radical scavenging capability compared to BCP. The combined formulation displayed greater activity than BCP alone in the DPPH assay. Furthermore, in the ABTS assay, the combined formulation exhibited the highest activity. In the CUPRAC assay, CBD displayed more activity than BCP, whereas the 1:1 mixture demonstrated higher activity than BCP alone. These findings demonstrate that the CBD/BCP-based patch does not compromise keratinocyte integrity under biocompatible conditions and supports the maintenance of epidermal barrier-related cellular features following topical exposure. The preservation of membrane integrity and physiological morphology suggests that the device exerts localized surface-mediated effects while maintaining compatibility with the skin barrier, supporting its potential application as a topical medical device for localized use.

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Keywords: Cannabidiol, β-caryophyllene, medical device, keratinocyte, biocompatibility.

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Ultrastructural Evidence of Gold Nanorod Endocytic Internalization in Human Cardiac Fibroblasts: Morphological Basis for Plasmonic Photothermal Therapy

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Cardiac fibrosis is driven by activated cardiac fibroblasts (CFs), which produce excessive extracellular matrix proteins causing myocardial dysfunction. Plasmonic photothermal therapy (PPTT) mediated by gold nanorods (AuNRs) represents an innovative minimally invasive strategy for selective ablation of pro-fibrotic cells [1–3]. This study investigates, from an ultrastructural perspective, the internalization dynamics of AuNRs in primary human cardiac fibroblasts (hCFs) and the subcellular compartmentalization of these nanomaterials following uptake. Primary hCFs were isolated by explant culture from surgical biopsies of left atrial appendages and treated with AuNRs (55 nm × 15 nm; longitudinal plasmon peak at 808 nm) at 50 μM, 100 μM, and 200 μM for 72 hours. After washing to remove non-internalized particles, cells were processed for transmission electron microscopy (TEM): fixed in 2.5% glutaraldehyde, post-fixed with 2% osmium tetroxide, embedded in Embed-812 resin [4]. Ultrathin sections (80–90 nm) were stained with lanthanide solution and lead citrate, imaged at 60 kV. UV-vis spectrophotometry was performed in parallel to correlate optical behavior with ultrastructural findings. TEM demonstrated effective AuNR internalization at all concentrations. Nanorods were consistently localized within endocytic-like membrane-bound vacuoles containing electron-dense clustered particles, with no free cytoplasmic dispersion, indicating a vesicle-mediated endosomal uptake route [5]. Cellular morphology was fully preserved: rough endoplasmic reticulum showed regular cisternal distension, mitochondria appeared intact, and no ultrastructural markers of apoptosis or autophagy were detected. Spectroscopic analysis confirmed a concentration-dependent redshift of the longitudinal plasmon band, consistent with AuNR clustering within vacuoles and transition to an intracellular refractive index environment. This study provides direct ultrastructural evidence of biocompatible, endocytic internalization of AuNRs in primary hCFs, with full preservation of subcellular architecture, establishing a morphological foundation for PPTT-based therapeutic strategies targeting cardiac fibrosis.

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Keywords: gold nanorods, plasmonic photothermal therapy, cardiac fibroblasts, transmission electron microscopy, endocytic internalization, cardiac fibrosis.

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Pharmacological inhibition of endolysosomal Two-Pore Channel 2 (TPC2) impairs human BRAF-mutant melanoma progression

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Two-Pore Channel 2 (TPC2) is an intracellular Ca^{2+}/Na^{+} ion channel localized on acidic organelles, including endo-lysosomes and melanosomes, and has been implicated in several cancer-related processes such as angiogenesis, metastasis, autophagy and tumour immune regulation through effects on $CD8^{+}$ T-cell cytotoxicity [1-5]. However, its role in melanoma progression remains poorly understood. This study investigated the involvement of TPC2 in human melanoma using two pairs of BRAF-mutated cell lines derived from the same patients at different stages of disease progression, representing primary (IGR39 and WM115) and metastatic (IGR37 and WM266-4) melanoma. TPC2 expression was evaluated and its functional contribution to tumour progression was assessed through analyses of cell migration, adhesion to type I collagen, and β1-integrin recycling. The effects of the novel TPC2 inhibitor SG-094, alone and in combination with the BRAF inhibitor Dabrafenib, were also examined. Metastatic melanoma cells displayed higher TPC2 expression than their corresponding primary counterparts and showed distinct migratory and adhesive behaviours. Pharmacological inhibition of TPC2 with SG-094 strongly impacted the aggressive phenotype of melanoma cells, reducing migration across all models, decreasing adhesion to type I collagen, impairing β1-integrin recycling, and lowering proliferative capacity. Notably, SG-094 also reduced the invasive potential of melanoma spheroids in three-dimensional culture models, highlighting a key role for TPC2 in melanoma progression. Combinational treatment with SG-094 and Dabrafenib did not enhance therapeutic efficacy compared to SG-094 alone. In contrast, SG-094 as a single agent showed the highest anti-tumour activity across all melanoma models. Importantly, Dabrafenib-resistant cells remained sensitive to SG-094, indicating that TPC2 inhibition can effectively bypass resistance to BRAF-targeted therapy. At the molecular level, SG-094 was associated with modulation of MITF levels, which may contribute to the functional effects observed following TPC2 inhibition.

Overall, these findings demonstrate a pro-tumorigenic role of TPC2 in human BRAF-mutant melanoma cell lines. They further suggest that pharmacological targeting of TPC2 may represent a promising strategy for the development of novel therapeutic approaches in melanoma.

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Keywords: TPC2, Melanoma, BRAF mutation, tumour progression, resistance.

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Tocopheryl quinones exert isoform-specific modulation of Nrf2 and NF-κB transcription factors in HepaRG cells and primary hepatocytes

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Tocopheryl-quinones (TQs) are oxidation products of vitamin E (α-tocopherol or α-TOH) and other tocopherols formed in vivo [1-3]. Their levels increase under hepatic lipotoxicity and oxidative stress [4-6]. Because TQ redox activity depends on chromanol-ring methylation, distinct TQ isoforms may differentially engage adaptive versus cytotoxic stress responses. Here we compared the capacity of α-, γ- and δ-TOH and their corresponding TQs in modulating the redox-sensitive transcription factors Nrf2 and NFkB in undifferentiated human pre-hepatocytes (HepaRG cells) and in primary mouse hepatocytes (PMH). In HepaRG cells, TQs but not TOHs reduced cell viability in a time- and concentration-dependent manner; this was an isoform-specific effect with desmethyl TQs, and particularly δ-TQ, producing the strongest response. When tested in short time exposure (1 h, 1 μM), these isoforms induced the highest levels of cellular oxidants confirming specific redox cycling properties. δ-TQ was the most potent inducer of Nrf2 mRNA expression, whereas α-TQ preferentially increased NF-κB mRNA. These specificities were associated with higher nuclear translocation of the corresponding transcriptional proteins and the activity of α-TQ on NF-κB mRNA expression was also confirmed in PMH and was associated with increased mRNA levels of its downstream cytokine IL-1β. TOH isoforms produced a lower response or reduced the expression of these transcription factors, particularly γ-TOH that showed significant negative modulation of NFkB/IL-1β expression in both HepaRG cells and PMH. Together, these data support the concept that TQs are not inert products of TOH metabolism but signaling molecules capable of isoform-specific modulations of stress response pathways of the hepatocyte distinct from their TOH precursors.

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Keywords: tocopheryl-quinone, vitamin E, Nrf2, NF-κB, IL-1β, redox signaling, HepaRG, primary hepatocytes.

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High-Intensity Training Induces Functional and Multi-Omic Adaptations in Older Adults with Cognitive Impairment at Risk of Frailty: Results from the CHOKO-Age Project

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Background: Cognitive impairment and frailty frequently coexist in older adults, accelerating functional decline and loss of independence. Within the CHOKO-Age project, we investigated the effects of a 12-week high-intensity training (HIT) intervention on physiological and molecular adaptations in older adults with cognitive impairment at risk of frailty. **Methods:** Participants underwent a 12-week HIT program combined with nutritional support. Functional performance, endothelial function, mitochondrial respiration, circulating biomarkers, skeletal muscle proteomics, peripheral blood transcriptomics, and plasma lipidomics were assessed before (T0) and after intervention (T1). Age-matched non-frail older adults served as reference controls for multi-omic analyses. **Results:** HIT significantly improved maximal strength (1RM), maximal voluntary contraction (MVC), endothelial function, and mitochondrial efficiency, as indicated by reduced mitochondrial leak respiration. Leptin concentrations increased following training, whereas body composition remained unchanged. Proteomic analyses revealed partial restoration of frailty-associated skeletal muscle alterations, with enrichment of pathways related to oxidative phosphorylation, mitochondrial metabolism, ribosome biogenesis, and protein synthesis. Blood transcriptomics showed concordant activation of translational and proteostasis-related processes. Correlation analyses identified coordinated molecular signatures associated with improvements in strength and metabolic responsiveness, highlighting pathways involved in proteostasis, RNA processing, mitochondrial homeostasis, cellular remodelling, and energy metabolism. Neurodegeneration-related pathways consistently emerged across omics datasets, suggesting shared biological mechanisms linking cognitive impairment, muscle dysfunction, and exercise responsiveness. In contrast, plasma lipidomic remodelling was modest. **Conclusions:** Findings from the CHOKO-Age project demonstrate that HIT induces significant functional, vascular, and mitochondrial benefits in older adults with cognitive impairment at risk of frailty. These adaptations are accompanied by coordinated systemic and skeletal muscle molecular remodeling, supporting HIT as a promising strategy to counteract frailty-related decline and promote healthy aging.

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Keywords: frailty, cognitive impairment, high-intensity training, skeletal muscle, proteomics, transcriptomics, aging, exercise responsiveness.

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Antiandrogenic Effects of Bisphenol A on Human Prostate Cells: Implications for Hormone-Dependent Disorders

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Bisphenol A (BPA) is a widely recognized Endocrine Disrupting Chemical (EDC) extensively employed in the production of polycarbonate plastics and epoxy resins, as well as in plastic bottles, food packaging and containers [1]. Over the years, the extensive use of BPA has raised increasing concern regarding its potential impact on human health, particularly on the reproductive system, due to its ability to mimic sex hormones such as 17-β-Estradiol, Testosterone, and Dihydrotestosterone, thereby altering hormone-dependent signaling pathways [2]. In the present study, we investigated the effects of low-dose BPA exposure on the normal prostate epithelial cell line PNT1A, with particular attention to its interaction with the Androgen Receptor (AR), a key regulator of prostate development, homeostasis, and physiological function [3]. Our findings demonstrated that BPA exerts antiandrogenic effects after 72 hours of treatment. Specifically, exposure to 10^{-9}M BPA reduced cellular metabolic activity, which was associated with a decreased proliferation rate. Furthermore, co-treatment with the AR inhibitor Enzalutamide (ENZA) partially attenuated the effects induced by BPA, suggesting that BPA may also interact with additional receptor pathways beyond AR signaling. Overall, these results indicate that BPA exposure, even at low concentrations and short exposure times, exerts antiandrogenic activity that may contribute to the development of hormone-dependent disorders.

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Keywords: EDCs, BPA, Prostate Disorders, AR disfunction.

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Biliary Remodeling and Hepatocyte Functional Reprogramming Define the Cholestatic Phenotype in MASLD

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Metabolic dysfunction-associated Steatotic Liver Disease (MASLD) is recognized as a heterogeneous disorder characterized by dynamic cellular and molecular alterations, mainly driven by dysregulated lipid metabolism, that shape distinct pathogenic and clinical phenotypes[1]. Progressive remodeling of the biliary compartment, accompanied by portal inflammation and ductular reaction, has emerged as a key feature of disease progression, defining a cholestatic phenotype that is increasingly associated with adverse clinical outcomes[2,3]. This study aimed to characterize cholestasis-related alterations in MASLD through an integrated histological and molecular approach. Liver tissue and clinical data from 264 MASLD patients were analyzed. Patients were stratified according to NAS score and fibrosis stage. Ductular reaction (DR) extent was assessed by immunohistochemistry for the biliary marker CK7, while targeted gene expression profiling and RNA sequencing were performed to characterize hepatocyte and cholangiocyte transcriptomic signatures. Transcriptomic analyses revealed a progressive reshaping of the hepatic cellular landscape during disease progression characterized by an expansion of the cholangiocyte compartment and a concomitant decline in hepatocyte functional programs, associated with reduced expression of genes involved in detoxification, protein secretion, metabolic processes, and bile acid homeostasis. Furthermore, biliary remodeling progressively increased across disease stages ($p < 0.0001$) and was inversely associated with the expression of genes regulating bile acid synthesis ($r = -0.43$) and transport ($r = -0.55$), suggesting that the extent of biliary remodeling closely reflects impaired hepatocellular function. These findings indicate that MASLD progression is characterized by a profound phenotypic and functional remodeling of the liver, in which cholangiocyte expansion occurs alongside progressive loss of hepatocyte function. This coordinated cellular shift may represent a pivotal mechanism underlying the development of the cholestatic phenotype in progressive MASLD.

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Keywords: MASLD, cholestatic disease, ductular reaction, biliary compartment.

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Chemotherapy effect on Human iPSC-Derived Sensory Neurons

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Human Induced Pluripotent Stem Cells iPSCs represent a revolutionary strategy for the design of new personalized experimental in vitro models [1]. So far, many iPSCs based models have been developed most of them targeting cardiac and Central Nervous System, while few models are currently available for diseases affecting Peripheral Nervous System [2, 3]. This may be particularly interesting since a very disabling side effect of several effective antineoplastic drugs is the induction of peripheral sensory neuropathy, known as Chemotherapy-Induced Peripheral Neuropathy (CIPN), which often causes the anticancer treatment interruption [4]. CIPN mainly targeting Dorsal Root Ganglia and, besides its complexity, a limiting factor for progress is the lack of reliable in vitro human models to screen putative neuroprotective molecules without interfering with the anticancer drugs. In this study, we examine the effect of four chemotherapy agents with established neurotoxic potential, Oxaliplatin, Cisplatin, Paclitaxel, and Bortezomib on iPSCs-derived sensory neurons provided by FUJIFILM Cellular Dynamics (iCell Sensory Neurons). iCells were exposed for 24 hours to different concentrations of each drug, and viability and neurotoxicity were assessed using MTT assay and neurite outgrowth analysis. Our results showed a significant decrease of neuronal survival after the exposure to all the antineoplastic agents. Moreover, all drugs impaired neurite outgrowth in a dose dependent manner. The results reported using FUJIFILM iCell® Sensory Neurons are consistent with findings previously obtained with murine DRG models [5], both in terms of reduction of neuronal survival and of neurite damage, supporting them as a valid human-based model for studying peripheral neuropathy and drug testing.

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Keywords: iPSCs, Antineoplastic drugs, CIPN, in vitro model.

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Anatomy of emotions in Darwin's work

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In his lesser known but equally important volume, *The Expression of the Emotions in Man and Animals*, published in 1872, the renowned British biologist, naturalist, and explorer Charles Robert Darwin (1809-1882) undertook a scientific study of emotions with a careful analysis of facial expressions. Through a detailed anatomical, physiological and human-comparative study of facial expressions and body language, he removed this important aspect of ethology from the less rigorous approach offered by classical physiognomy. Darwin examined the existing literature on this topic and distinguished the works that he did not consider worthy of attention from those that had inspired him. In addition to these important sources of inspiration, Darwin also availed himself of numerous and competent collaborators, including photographers and illustrators, who contributed to enriching his work. The methodological approach included the galvanization of mimic muscles and the study of facial expressions in artistic masterpieces, newborns, psychiatric patients, animals, and different ethnic groups, thanks to the distribution of a questionnaire. The numerous studies and observations and the answers obtained from the questionnaire allowed Darwin to draw the following three fundamental principles: Serviceable Associated Habits, Antithesis, and Constitution of the Nervous System. Darwin's vision was always closely tied to his revolutionary theory of evolution. Certain expressions, therefore, are hereditary, consolidated through repetition across generations, a position close to that of Lamarck, a proponent of the inheritance of acquired characteristics. On this point, Darwin was mistaken, as modern genetics has since definitively disproved this conclusion. Some fundamental expressions, such as crying and laughing, are the same across all ethnic groups. Conventional expressions (indignation, devotion, etc.) depend on cultural learning. Darwin applied the principles of natural selection to explain the evolution of emotional expression. Through observation and deductive reasoning, the data obtained allowed him to affirm that universal facial expressions exist, further consolidating the evolutionary idea that humans descend from a common ancestor. This conclusion is still widely accepted. Today it is possible to revisit the Darwinian comparison between human expressions and the expressions of non-human primates through the Facial Action Coding System (FACS), which allows to study facial expressions in a standardized way based on the anatomy of the mimic muscles [1]. Despite all the historical limitations that Darwin's work may present, we must recognize the innovative and solid method of study that stands apart from the more empiricist approaches of disciplines that were also in vogue at the time, such as physiognomy and phrenology. Modern anatomical studies confirm the role of sophisticated facial muscles, which, especially in humans, permit a wide range of expressive nuances.

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Keywords: Charles Darwin, mimic muscles, facial expressions, comparative anatomy, physiognomy.

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Capturing glioma microenvironment through patient-derived secretome metabolomics

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The glioma secretome is a key mediator of tumor–microenvironment communication and may provide clinically relevant information on tumor biology, heterogeneity, and therapeutic resistance [1,2]. However, most glioma secretome studies rely on conditioned media from in vitro models, which only partially reproduce the complexity of the human tumor microenvironment and may introduce culture-related artefacts [3]. To overcome these limitations, we established and validated a standardized patient-derived ex vivo workflow for collecting secretome directly from surgical glioma specimens. 13 glioma samples, including 10 IDH-wildtype glioblastomas (GB) and 3 IDH-mutant astrocytomas (AS), were processed immediately after resection. Tissue fragments were incubated in a physiological solution under controlled temperature conditions and gentle aeration, allowing spontaneous secretome release. Transmission electron microscopy confirmed the preservation of tissue architecture, nuclear morphology, and mitochondrial ultrastructure, supporting the biological reliability of our secretome collection method. ^{1}H-NMR spectroscopy enabled reproducible metabolomic profiling and consistently detected extracellular metabolites. Multivariate analyses revealed metabolic differences between GB and AS, and highlighted heterogeneity within GB samples. Compared with matched glioma neurosphere conditioned medium, patient-derived secretomes showed cleaner and more physiologically informative spectra, with reduced interference from exogenous medium components. This study validates a scalable patient-derived approach for glioma secretome collection and molecular characterization, providing a translational platform to investigate extracellular metabolic reprogramming and tumor microenvironment dynamics in glioma.

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Keywords: glioma, tumor microenvironment, NMR metabolomics, patient-derived models, precision medicine.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Spatial architecture of DCLK1-positive tumor cells within the immune microenvironment of hepatocellular carcinoma: histological and immunohistochemical characterization

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Hepatocellular carcinoma (HCC) represents one of the most prevalent and lethal malignancies worldwide. Doublecortin-like kinase 1 (DCLK1) has emerged as a putative cancer stem cell marker in several gastrointestinal solid tumors. Furthermore, recent evidence has implicated DCLK1$^+$ tumor cells in tumor immune escape mechanisms. DCLK1$^+$ cells tissue distribution and spatial relationship with the immune tumor microenvironment (TME) remain poorly characterized. This study provides a morphological description of DCLK1-expressing tumor cells and their spatial organization relative to immune infiltrates, leveraging both human and experimental tissue models. 10 human HCC specimens arising on a background of metabolic dysfunction-associated steatotic liver disease were histologically characterized for the spatial relationship between DCLK1$^+$ cells and inflammatory infiltrates. Moreover, human HCC-derived organoids were generated to further investigate DCLK1 cell behaviour and expression patterns. Histological analysis of human HCC specimens revealed a distinctive spatial organization, with DCLK1$^+$ cells consistently residing in close proximity to immune infiltrates. In organoids, DCLK1 expression was selectively confined to the central core, delineating a morphologically distinct subpopulation with different lineage marker expression. Orthotopic HCC model was established in C57BL/6J mice by intrahepatic injection of PM299L cells following partial hepatectomy with anti-PD-1 monoclonal antibody or control solution. In the murine model, anti-PD-1 treatment significantly remodelled the TME architecture, with concurrent expansion of CD3$^+$, CD4$^+$, and FOXP3$^+$ infiltrating cells and a significant reduction in the DCLK1$^+$ tumor area fraction. These findings provide histological clue for a spatially and functionally defined suggested between DCLK1-expressing tumor cells and the immune TME in HCC, supporting DCLK1 as a morphologically traceable marker of potential relevance for the immunological landscape of this malignancy.

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Keywords: HCC, DCLK1, immune tumor microenvironment, mouse model.

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Enhanced Osteogenic Response on Nanotextured and Three-Dimensional Porous Titanium Surfaces: A Comparative In Vitro Study

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Titanium is widely employed in orthopedic and dental implantology due to its excellent biocompatibility and mechanical properties. However, surface modifications and three-dimensional (3D) architectures have been proposed to further improve cellular responses and osseointegration [1, 2]. The present study compared the biological performance of three titanium substrates: conventional smooth titanium discs, acid-treated nanotextured titanium discs, and 3D titanium microspheres obtained from a highly porous titanium scaffold designed to mimic the trabecular architecture of bone.

Cell adhesion and osteogenic behavior were evaluated through scanning electron microscopy (SEM) and immunofluorescence analyses. Attention was devoted to focal adhesion formation using vinculin as a specific marker of cell–substrate interactions. SEM observations revealed marked differences in cell morphology and spreading among the investigated surfaces. Quantitative and qualitative analyses demonstrated enhanced cellular adhesion on nanotextured titanium compared with smooth titanium. Notably, the 3D titanium microspheres exhibited the most favorable biological response, promoting extensive cell attachment, spreading, and organization throughout the porous structure. Vinculin immunolabeling confirmed a greater development of focal adhesion complexes on nanotextured and 3D substrates, indicating stronger cell–material interactions. These findings demonstrate that while titanium intrinsically supports cellular adhesion, both nanotexturization and biomimetic 3D architecture significantly enhance its osteogenic potential.

The combination of surface nanotopography and three-dimensional porous organization represents a promising strategy for the development of next-generation implants aimed at improving osseointegration and long-term clinical outcomes.

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Keywords: titanium; nanotextured surfaces; porous titanium; 3D scaffolds; osseointegration; focal adhesions; vinculin; SEM.

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Imaging and molecular characterizaton of a new 3D bioprinted glioblastoma model for drug screening

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Glioblastoma Multiforme (GBM) is the most aggressive primary brain tumor. Treatment is highly difficult due to its infiltrative nature, high intra- and inter-heterogeneity, and resistance to standard therapies.

Despite significant efforts to develop new strategies against GBM, current treatment relies on the Stupp protocol, which consists of surgical removal followed by radiotherapy and temozolomide chemotherapy [1].

Conventional 2D models, not taking into account the Extracellular matrix (ECM) signals in Tumour MicroEnvironment (TME), fail to replicate the complex physical, mechanical, and biochemical properties of GBM, limiting their effectiveness for animal-free drug testing.

3D bioprinting allows for the creation of advanced in vitro tumor models that accurately mimic the biochemical and structural properties of the ECM, modulating the functional complexity of the GBM TME and helping to bridge the current gap [2].

Although the role of glycans is still largely unexplored, it is known that the glycosignature, including sialic acids in post translational modifications of proteins and sulphate glycosaminoglycans (GAGs) in proteoglycans, is involved in the functional modulation of cell fate within the TME. In particular, the disruption of these physiological glycosignatures is strongly linked to the onset of pathological conditions, including the promotion of tumor growth, immune escape, immunosuppression, and invasion by modulating cell signaling, cell-matrix adhesion, and extracellular matrix remodeling [3,4].

In this study, the application of hydrogel-based 3D Bioprinted GBM model (3DGlycoGBM) and the use for drug screening applications is presented. Using cutting-edge imaging and molecular techniques such as multiplex immunofluorescence (IF) analysis and high resolution nano-computed tomography (nano CT) analysis we have characterized the 3D bioprinted hydrogel with incorporating patient-derived GBM cells. The 3DGlycoGBM in vitro model, with different glycosignatures, selected using data obtained from ML-guided analytical pipeline GlycoMatrix_GBM demontrates that glycosignatures influences GBM cell growth, matrix deposition and drug response, concomitantly emphasizing the critical necessity of meticulous model validation for the drug preclinical screening.

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Keywords: Glioblastoma, 3D bioprinted in vitro model, glycosignature, drug preclinical screening.

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Synovial fluid-derived extracellular vesicles as mediators of endothelial dysfunction in musculoskeletal diseases

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Chronic inflammation plays a central role in the pathophysiology of numerous musculoskeletal disorders, including osteoarthritis (OA), the most common degenerative joint disease worldwide, and a major cause of disability[1]. Early diagnosis, prevention, and treatment of the initial stages of OA and pre-arthritic conditions, such as femoroacetabular impingement (FAI), are essential to reduce progression to end-stage hip OA and the consequent need for total hip replacement. In recent years, extracellular vesicles (EVs) have gained increasing attention not only as potential biomarkers for several inflammatory pathologies, but also as key mediators of intercellular communication and active contributors to disease mechanisms[2]. A growing body of evidence indicates that inflammatory conditions are associated with an alteration in the structure of the endothelial barrier[3]. Based on these observations, this study aimed to provide new insights into the involvement of synovial fluid-derived microvesicles in the onset and progression of inflammatory processes, with a specific focus on the endothelial compartment. EVs were isolated from the synovial fluid (SF) of patients exhibiting different degrees of inflammation. They were subsequently characterized, and their functional impact on human umbilical vein endothelial cells (HUVEC) was assessed using multiple experimental approaches. Our findings show that HUVEC exposed to synovial fluid-derived EVs display enhanced wound closure, increased angiogenic activity, and elevated expression of adhesion molecules such as ICAM-1 and VCAM-1. Moreover, the interaction between small extracellular vesicles and endothelial cells induced a structural rearrangement of the endothelial barrier, as demonstrated by altered localization of VE-cadherin and β-catenin. Collectively, these results indicate that synovial fluid-derived EVs exert a pro-inflammatory effect on the endothelial barrier, with potential implications for the pathophysiology of musculoskeletal diseases.

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Keywords: OA, FAI, EVs, Angiogenesis.

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CAM Model and *Osteosarcoma "Avatar"* for cancer research: development of Osteosarcoma Spheroid Models on Chick Chorioallantoic Membrane for the Study of Tumour Progression

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The chick Chorio-Allantoic Membrane (CAM) model is a well-established experimental platform used as alternative to the animal testing to asses behaviour of molecule/scaffold both in physiological and pathological conditions. It is a highly vascularized extraembryonic membrane connected to the developing embryo's circulatory system, offering several advantages, including easy accessibility for manipulation and monitoring, rapid experimental outlines, cost-effectiveness, and reduced ethical constraints compared with mammalian *in vivo* models. These characteristics make the CAM a valuable tool for cancer research and preclinical studies[1].

The aim of this study is to develop a three-dimensional (3D) osteosarcoma model based on tumour spheroids to be implanted on CAM in order to investigate key hallmarks of tumour progression. In particular, we aim to evaluate induction of angiogenesis and tumour aggressiveness by assessing cellular proliferation and local invasion. Osteosarcoma spheroids will be generated from different cell lines to better reproduce the structural and functional complexity of the tumour microenvironment. Following implantation onto the CAM, vascular recruitment, tumour growth and invasive behaviour will be analysed through molecular, morphological and histological approaches. Particular attention will also be devoted to the role of the nuclear envelope, whose structural and functional alterations have increasingly been associated with cancer progression. Changes in nuclear envelope components, including nuclear lamins and nuclear pore complexes, can affect chromatin organization, gene expression, genome stability, and cellular mechanotransduction, thereby promoting tumour aggressiveness, invasion, and metastatic dissemination[2].

The use of spheroid cultures allows overcoming many limitations associated with conventional two-dimensional cell cultures. Indeed, 3D spheroids, as cancer "avatar", more closely mimic the native architecture of tumour tissue, preserving cell–cell and cell–extracellular matrix interactions and generating physiologically relevant gradients of oxygen, nutrients, and metabolites[3]. The combination of osteosarcoma spheroids with the CAM model therefore represents a promising and versatile platform for studying tumour biology in a biologically relevant *in vivo-like* environment.

Beyond osteosarcoma, the proposed experimental approach could be adapted to a wide range of solid tumours, providing a reproducible and flexible model for investigating angiogenesis, tumour growth, invasion, and interactions with the surrounding microenvironment. Furthermore, the CAM-spheroid platform may serve as a valuable preclinical tool for evaluating the efficacy of novel anticancer strategies, including chemotherapeutic agents, targeted therapies, anti-angiogenic compounds, and combination treatments. By enabling rapid screening of therapeutic approaches in a physiological-like setting, this model has the potential to support translational cancer research and contribute to the development of more effective treatment strategies.

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Keywords: Chorioallantoic Membrane, Spheroid, Osteosarcoma, cancer invasion, tumor model.

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PACAP Mitigates Hyperglycemia-Induced Inflammatory and Mophological Alterations in a 3D Air–Liquid Interface Model of Diabetic Keratopath

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Diabetic keratopathy represents a frequent ocular complication of diabetes mellitus [1] and is characterized by structural and functional alterations of the corneal epithelium, including chronic inflammation, impaired barrier integrity and delayed wound healing [2]. Increasing evidence suggests that pituitary adenylate cyclase-activating polypeptide (PACAP) plays a protective role in corneal homeostasis and wound healing. The present study investigated the ability of PACAP to counteract inflammatory and morphological changes induced by hyperglycemic conditions in a three-dimensional air-liquid interface (ALI) model of the corneal epithelium. Human corneal epithelial cells were cultured under ALI conditions to obtain a epithelial barrier closely resembling the native corneal surface. Exposure to high glucose was used to reproduce key features of diabetic keratopathy. The effects of PACAP treatment were evaluated through morphological analysis, barrier thickness assessment, and measurement of inflammatory markers, including IL-1β, TN-αand phosphorylated NF-κB. PACAP treatment significantly attenuated these molecular changes, reducing pro-inflammatory cytokine levels and limiting NF-κB activation, an important mediator of inflammation. In addition, PACAP improved epithelial morphology and partially restored barrier thickness compared with untreated hyperglycemic cultures. The ALI model successfully reproduced relevant morphological and inflammatory features of diabetic keratopathy. PACAP exerted marked anti-inflammatory and barrier-protective effects, supporting its potential as a therapeutic candidate for the prevention and treatment of corneal complications associated with diabetes [3].

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Keywords: PACAP, Cornea, Diabetic keratopathy, Inflammation, Cytokines, NF-kB.

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An Inflammaging-Related SASP and NLRP3 Signature Predicts Poor Prognosis and Metastasis in Elderly Cancer Patients

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Aging is a complex, progressive, and irreversible biological process characterized by profound structural and functional declines [1]. Central to aging and its associated pathologies is "inflammaging", a systemic, low-grade chronic inflammation [2]. A primary driver of this phenomenon is the Senescence-Associated Secretory Phenotype (SASP), a pro-inflammatory secretome released by cells undergoing replicative senescence. By compromising cellular homeostasis, SASP impairs the body's ability to adapt to environmental stressors [3]. Consequently, this persistent chronic inflammation acts as a key driver in the pathogenesis of major age-related conditions, including neurodegenerative disorders, cardiovascular diseases, and cancer [4-6]. In this study, we evaluated the SASP and inflammatory secretory profiles in the serum of a cohort of 110 cancer patients aged over 65 years. We analyzed these profiles in relation to various comorbidities and assessed their overall impact on patient survival. Serum concentrations (pg/ml) of specific cytokines (CCL2, IL-1β, MMP-3, CXCL10, IL-6, TK1, and TNF-α) were quantified using a custom Luminex multiplex assay on the Bio-Plex 200 platform, while NLRP3 levels were determined via a commercial ELISA kit. Within our cohort, IL-6 and CXCL10 levels were significantly higher than those of IL-1β and TNF-α. Expression increased for the progression markers MMP-3 (involved in tissue remodeling and metastasis), CCL2 (MCP-1), and TK1, too. Additionally, a significant overexpression of NLRP3 was detected. Biomarker levels significantly correlated with specific patient comorbidities. Specifically, metastasis at diagnosis correlated with TNF-α, TK1, IL-6, IL-1β and CCL2. Additionally, neurological disorders associated with TK1 and CXCL10, while cardiomyopathies correlated with IL-1β. Survival analysis showed that high expression of IL-6, TNF-α, TK1, CXCL10, CCL2, and NLRP3 significantly predicted poorer DFS ($p < 0.05$). Additionally, elevated IL-6 and CCL2 levels were strong predictors of shortened OS ($p < 0.01$). Multivariate analysis revealed that IL-6 and CCL2 acted as independent negative prognostic factors for DFS. Regarding OS, IL-6, CCL2, TK1, and NLRP3 all emerged as independent indicators of poor prognosis. In conclusion, chronic inflammation, driven by senescent cell signaling (IL-6, CCL2), accelerates cancer progression and metastasis, particularly in older patients. High levels of these markers, along with TK1 and NLRP3, independently predict shorter survival and increased mortality risk.

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Keywords: Aging, Cancer, SASP, NLRP3, IL-6/CCL2 axis.

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The Petrotympanic Canal (Civinini's Canal): An Integrated Digital, Dissective, and Evolutionary Approach

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The petrotympanic canal, historically identified as Civinini's or Huguier's canal, represents a crucial anatomical interface linking the middle ear to the temporomandibular joint (TMJ) [1–3]. Despite its early description, its structural complexity and functional significance have frequently been underestimated in classical literature. This study provides a comprehensive re-evaluation of the structure through a multidisciplinary approach that integrates digital anatomy (3D reconstructions from CT scans), dissective anatomy (macroscopic dissection), and evolutionary anatomy.

Morphometric analysis of 195 recent human skulls confirms the canal's constant presence, revealing a funnel-shaped architecture with an average intermediate diameter of 0.5 mm. Detailed statistical analysis demonstrates that the structure is sexually monomorphic and lacks significant bilateral asymmetry. Conversely, a comparative assessment across 27 primate specimens reveals a striking evolutionary pattern: the canal is consistently present in humans and great apes but systematically absent in non-anthropoid, quadrupedal primates [4-6].

This distribution strongly suggests that Civinini's canal is a derived feature associated with craniofacial reorganization linked to bipedal or facultative bipedal posture and to the evolutionary reduction of the postglenoid process [7]. Functionally, rather than acting primarily as a mechanical shield, the canal should be interpreted as a specialized conduit enabling anatomical and functional continuity between two closely related systems. It transmits a neurovascular and ligamentous complex (including the chorda tympani nerve, anterior tympanic artery, and the discomalleolar and anterior malleolar ligaments), thereby allowing dynamic interactions between temporomandibular joint mechanics and the ossicular chain. Within this framework, mandibular (AML) and disc-ligamentous (DML) dynamics can be understood to functionally "interface" with the malleus, supporting a coordinated biomechanical relationship between TMJ activity and middle-ear function [8-9].

From a medical perspective, these findings clarify the anatomical basis for the correlation between TMJ dysfunctions and otological symptoms, such as tinnitus and otalgia, and support the role of the petrotympanic canal and fissure in mediating TMJ–middle-ear interactions [10-11]. In addition, they identify the canal as a potential pathway for the spread of inflammatory or neoplastic processes between the infratemporal fossa and the middle ear, with relevant implications for surgical planning and the prevention of iatrogenic damage in otological and maxillofacial procedures.

From an evolutionary perspective, the selective presence of Civinini's canal in humans and great apes points to a specialized osteofibrous adaptation emerging within the hominoid clade. This pattern is consistent with broader cranial base remodeling associated with bipedalism, altered TMJ–middle ear spatial relationships, and the increasing integration of masticatory, vestibular, and auditory demands in anthropoid evolution. Within this framework, Civinini's canal can be interpreted as a small but informative marker of the complex biomechani-

cal, sensory, and developmental constraints that shaped hominid craniofacial architecture.

In conclusion, by combining digital and traditional techniques, this study elevates the canal of Civinini from a simple descriptive landmark to a key indicator of functional integration between contiguous anatomical systems, reframing it as both a clinically relevant structure and a potential trait of evolutionary significance.

This integrated anatomical, clinical, and evolutionary perspective offers essential insights into the organization and function of this osteofibrous system, helping to clarify the structural context within which related pathologies may arise and progress.

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WWT Nucleopeptide as a Self-Assembling Supramolecular Scaffold: Preliminary Evaluation in a Fibroblast-Based Wound Repair Model

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Chronic wounds and impaired tissue regeneration remain significant clinical challenges, highlighting the need for innovative biomaterial approaches capable of supporting cellular repair processes [1, 2]. Nucleopeptide-based supramolecular systems represent an emerging class of bioactive materials that combine molecular recognition, self-assembly, and biological functionality [3, 4]. Here, we investigate the wound repair-related potential of WWT, a self-assembling nucleopeptide, in a fibroblast-based cellular model. WWT was synthesized through Fmoc-based solid-phase peptide synthesis incorporating nucleoside chemistry. Characterization of structural integrity, molecular mass, and chemical purity was established via liquid chromatography-mass spectrometry (LC-MS). The supramolecular organization of WWT was assessed by circular dichroism (CD) spectroscopy, supporting the formation of ordered assemblies driven primarily by stacking and aromatic interactions. Computational docking simulations were used to model molecular packing arrangements underlying this supramolecular network. The biological response to WWT was preliminarily assessed using IMR90 human lung fibroblasts. WWT showed no detectable cytotoxicity based on MTT assays. Immunofluorescence analysis further indicated preservation of cellular architecture, including normal cytoskeletal, mitochondrial, and endosomal organization. In scratch migration assays, treatment with 1 μM WWT significantly enhanced fibroblast migration over 24 hours compared with controls, indicating a distinct potential to promote wound closure. Together, these findings demonstrate that WWT represents a biocompatible, highly structured, and pro-migratory candidate for advanced wound healing and tissue regeneration applications.

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Keywords: nucleopeptide, supramolecular assembly, mass spectrometry, fibroblast migration, wound repair.

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Mitochondria-Lysosome Contact Site Remodeling in NPC1- and NPC2-Deficient Cell Models

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The spatial architecture and communication maintained at organellar membrane contact sites (MCS) contribute to intracellular homeostasis [1, 2]. While lysosomal dysfunction is a recognized hallmark of Niemann-Pick Disease Type C (NPC), how the absence of specific NPC proteins reorganizes the physical networks connecting mitochondria and lysosomes remains incompletely understood. Using engineered HEK293 and ARPE19 cell lines, we combined high-resolution imaging, transmission electron microscopy (TEM), and proximity ligation assays (PLA) to quantify the extent of contact sites between mitochondrial membranes and late endosomes/lysosomes (LE/Lys), while using mass spectrometry to profile whole-cell sphingolipid alterations in the HEK293 model. Morphological profiling and PLA quantification revealed a reduced contact site area between mitochondria and lysosomes in NPC2-deficient HEK293 cells relative to wild-type controls. Concurrently, the acidic vacuolar compartment underwent substantial remodeling, presenting as enlarged, lipid-dense organelles filled with free cholesterol and sphingolipids, as determined by fluorescence microscopy and mass spectrometry. In contrast, the broader mitochondrial network architecture remained structurally preserved. Preliminary TOM20-LAMP1 PLA analyses in ARPE19 cells revealed divergent inter-organellar dynamics between NPC1- and NPC2-deficient cells. While mitochondria-lysosome contact sites were slightly decreased in ARPE19 *NPC2*$^{-/-}$ cells compared to their wild-type counterparts, consistent with our observations in the HEK293 model, these contact zones were increased in ARPE19 *NPC1*$^{-/-}$ cells. Alongside this structural divergence, our findings suggest that organelle interface destabilization might be a contributor to NPC pathogenesis that extends beyond lipid storage, while highlighting distinct, protein-specific roles for NPC1 and NPC2 in regulating mitochondrial-lysosomal membrane contact site dynamics.

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Keywords: Niemann-Pick Disease Type C, mitochondria-lysosome contact sites, NPC1, NPC2, organelle remodeling, sphingolipidomics, lysosomal storage disorder.

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Ct Angiographic Evaluation Of Anatomical Variations Of The Celiac Trunk, Superior Mesenteric Artery And Inferior Mesenteric Artery

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Anatomical variations of the major unpaired branches of the abdominal aorta represent an important consideration in abdominal surgery, transplantation, and endovascular interventions [1,2]. Detailed knowledge of the morphology and branching patterns of the celiac trunk, superior mesenteric artery (SMA), and inferior mesenteric artery (IMA) is essential for accurate preoperative assessment and for minimizing the risk of vascular complications [3,4]. This retrospective study analyzed 60 abdominal computed tomography angiography (CTA) scans acquired using 128-slice technology at the University Institute of Radiology in Skopje. The examinations had been performed for clinical indications unrelated to the present study and were subsequently evaluated to assess the origin, morphology, and branching patterns of the celiac trunk, SMA, and IMA, with variations classified according to the Uflacker system [5]. The classic celiac trunk trifurcation pattern (Uflacker Type I) was identified in 95% of cases, whereas Uflacker Types II, V, and VI were each observed in 1.67% of cases. No anatomical variations of the IMA were detected. The results demonstrate a predominance of standard anatomical configurations of the celiac trunk, SMA, and IMA within the studied population, with only occasional vascular variations. Furthermore, the findings confirm the value of 128-slice CTA as a reliable imaging modality for the identification of mesenteric arterial anatomy and its variants, facilitating safer surgical planning and reducing the likelihood of procedure-related complications.

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Keywords: computed tomography angiography (CTA), celiac trunk, superior mesenteric artery, inferior mesenteric artery, anatomical variations.

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Bone maturation following guided bone regeneration with polynucleotides and hyaluronic acid: histomorphometric outcomes and the osteogenic role of the periosteum

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Guided bone regeneration (GBR) is a well-established surgical technique for restoring alveolar bone defects prior to implant placement. However, achieving predictable and adequate bone regeneration remains clinically challenging, driving the development of new devices — such as meshes with optimized geometries for improved space maintenance — and bioactive agents – such as polynucleotides (PNs) and hyaluronic acid (HA)- aimed at enhancing the regenerative process. The periosteum, a highly vascularized connective tissue covering bone surfaces, also harbors osteoprogenitor cells known to contribute to bone formation and remodeling. This study had two aims: to characterize the histomorphometric features of regenerated bone following a GBR protocol combining a new mesh design with PNs and HA, and to evaluate the potential benefit of periosteal coverage in cases of clinically suboptimal bone maturation. Fifteen patients with atrophic alveolar crests were treated with a graft - combination of autologous bone (50%) and deproteinized bovine bone mineral particles (50%) with hydrogel formulation containing PNs and HA- covered with a new titanium mesh and a resorbable membrane. Upon surgical re-entry at 8 to 11.5 months, ten patients (Group 1) presented regenerated bone that was deemed clinically adequate for implant placement and underwent core biopsy at that time point. The remaining five patients (Group 2) clinically exhibited undermineralized bone considered unsuitable for implant placement; in these cases, the membrane was removed and the periosteum was sutured directly over the regenerating site for an additional 4 months, with the aim of exploiting its osteogenic potential. Biopsies were subsequently taken at 13 months total healing time. Morphometric parameters — including percentages of lamellar bone, woven bone, osteoid matrix, residual biomaterial, and medullary spaces — were quantified histologically and descriptive statistics was performed with these data. To assess inter-group comparability despite the difference in total healing time, Spearman correlation analysis was performed within Group 1 between healing time and all morphometric parameters. A comparative analysis between groups was performed using the Mann-Whitney U test. At the observation, samples of group 1 showed an active but incompletely mature regeneration. Histomorphometric data on tissue fractions were: woven bone 25.17%, lamellar bone 10.09%, osteoid matrix 2.37%, biomaterial 22.01% and medullary spaces 40.35%. No significant correlation was found for any parameter, including lamellar bone ($\rho=-0.31$, $p=0.38$) and woven bone ($\rho=-0.32$, $p=0.37$), and timepoint suggesting that bone maturation had reached a plateau within the 8–11.5 months range. On this basis, the comparative analysis was performed between groups and exhibited significantly higher lamellar bone (20.55% vs 10.09%, $p=0.005$) and significantly lower woven bone (12.91% vs 25.17%, $p=0.008$) in group 2 compared to group 1, indicative of markedly more advanced bone maturation. No significant differences were observed in osteoid matrix, residual biomaterial, or medullary spaces. These findings suggest that periosteal coverage of a clinically suboptimal GBR site promotes bone remodeling and accelerates the transition from immature to mature lamellar bone, likely through the osteogenic contribution of periosteal progenitor cells. The periosteum may therefore represent an effective biological strategy to rescue inadequate GBR outcomes and improve bone quality prior to implant insertion.

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Keywords: alveolar bone, guided bone regeneration, polynucloetides, titanium mesh, periostium, bone graft, deproteinized bovine bone mineral particles.

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Dupilumab triggers cell-cycle checkpoint reactivation via the IL-4Rα/p21 axis in T-cell Acute Lymphoblastic Leukemia

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Introduction**:** T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematologic malignancy characterized by uncontrolled proliferation and frequent relapse [1]. Although IL-4Rα has been implicated in tumor progression in several malignancies, its role in T-ALL cell-cycle regulation and phenotypic stability remains poorly understood [2]. This study investigated the biological and molecular consequences of IL-4Rα blockade by Dupilumab, focusing on cell-cycle checkpoints, leukemic cell remodeling, and the clinical relevance of the IL-4Rα/p21 axis. Methods**:** Jurkat T-ALL cells were treated with Dupilumab (1 nM–100 μM) using single (1×; 0 h) and repeated (2×; 0 h and 24 h) administration protocols, and analyzed after 72 h. Cell proliferation, cell-cycle distribution, apoptosis, and CD4 surface expression were evaluated by flow cytometry, while the MAPK (p-ERK), NF-κB (p65), and p53/p21/p18 checkpoint pathways were analyzed by Western blotting. Clinical and transcriptomic validation was performed through *in silico* analysis of the TARGET T-ALL cohort (n = 814). Results**:** Dupilumab induced a dose-dependent inhibition of Jurkat cell proliferation under both treatment schedules, accompanied by marked cell-cycle remodeling. Following a single administration (1×), cell-cycle analysis revealed a marked G0/G1 accumulation at 50 μM (73.6% vs 43.0% in controls), whereas the highest concentration (100 μM) promoted S-phase enrichment (41.8%). Repeated administration (2×) further suppressed cell growth, shifted cell-cycle progression toward G2/M accumulation at 100 μM (12.2% vs 4.8% in controls), and concomitantly increased late apoptosis up to 32.2%. At the molecular level, these effects were associated with the activation of p-ERK and NF-κB signaling and robust upregulation of the p53/p21/p18 tumor suppressor network, indicating the restoration of cell-cycle checkpoint control. Phenotypic analysis demonstrated substantial remodeling of leukemic cells, with CD4 surface expression decreasing to 26.0% following the 1× protocol at 100 μM compared to controls. Notably, whereas the 1× regimen predominantly induced phenotypic modulation and cytostasis, repeated exposure shifted the cellular response toward apoptotic commitment while maintaining low CD4 expression. Transcriptomic and network analyses of the TARGET cohort identified a significant positive correlation between *IL4R* and *CDKN1A* (p21) expression (p = 0.0026), highlighting p21 as a central regulatory hub within the IL-4Rα-associated network. Furthermore, patients with high *IL4R* expression exhibited significantly poorer overall survival compared with those with low *IL4R* expression ($p = 9.27 \times 10^{-7}$). Conclusions**:** Pharmacological inhibition of IL-4Rα induces dose-dependent molecular, phenotypic, and cell-cycle remodeling in Jurkat T-ALL cells. While single exposure predominantly promotes cytostasis and phenotypic reprogramming, repeated administration drives checkpoint activation and apoptotic commitment. The IL-4Rα/p21 axis emerges as a critical regulator of leukemic cell fate and a clinically relevant prognostic pathway, supporting further investigation of Dupilumab as a therapeutic strategy for high-risk T-ALL.

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Keywords: T-ALL, Dupilumab, IL-4Rα, Cell cycle checkpoints, CD4 downregulation.

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Progesterone-Induced Hybrid Epithelial–Mesenchymal Phenotypes Enhance Regenerative and Immunomodulatory Functions of Amniotic Epithelial Cells

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Epithelial-mesenchymal plasticity (EMP) is crucial during embryonic development, tissue regeneration, and disease progression or neoplastic transformation [1]. In this study, we demonstrate that progesterone (P4) delays EMP in amniotic epithelial cells (AEC), favoring the emergence of hybrid epithelial-mesenchymal (E/M) phenotypes [2]. These hybrid cells coexpress epithelial and mesenchymal characteristics, display distinct surface markers, and maintain intermediate levels of key EMP regulators. Compared to their fully mesenchymal AEC counterparts, hybrid AECs exhibit greater collective migration capacity, upregulation of stemness-associated transcription factors, and enhanced immunomodulatory properties both in vitro and in vivo. Their regenerative potential was validated by inducing tendon differentiation in vitro on PLGA fleeces and in sheep tendon injury model, where hybrid AEC transplantation accelerated the early regeneration phase [3,4,5]. This mode of action was associated with a timely transition from inflammation to proliferation, mediated by macrophage polarization and extracellular matrix remodeling. Our results reveal that P4 maintains AECs in functionally more potent hybrid E/M state, conferring both regenerative and immunomodulatory benefits. These findings offer new insights into the physiological regulation of EMP and support the therapeutic relevance of hybrid AECs as promising candidates for next-generation cell therapies in regenerative medicine.

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Keywords: Epithelial-mesenchmal plasticity, Progesterone (P4), Hybrid E/M phenotype, Amniotic epithelial cells (AECs), Immunomodulation, Regenerative medicine.

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CCR2-dependent monocytes gut-homing contributes to obesity-related enteric neuroplastic changes

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Increasing evidence indicates that enteric inflammation contributes to bowel motor dysfunction in obesity[1]. Of interest, in response to high-fat diet (HFD) exposure, activation of intestinal immune cells drives neuroplastic changes within the enteric nervous system (ENS), contributing to enteric dysmotility[1]. This inflammatory condition is further sustained by CCR2-dependent recruitment of circulating monocytes into the intestinal mucosa, promoting the progression of chronic enteric inflammation[2]. Based on this background, we investigated whether CCL2/CCR2-dependent recruitment of circulating monocytes drives colonic motor dysfunction in HFD–induced obese mice. Wild-type C57BL/6J mice were fed HFD or standard diet (SD) for 8 weeks. Throughout HFD exposure, animals were treated with the CCR2 antagonist RS504393 (2 mg/kg/day, orally) or vehicle, while body weight and BMI were monitored. Colonic motor function was assessed by measuring *in vivo* faecal output and evaluating *in vitro* colonic contractility through substance P (SP)-induced contractile responses, along with analysis of SP-positive neurons. Immunohistochemical analyses were performed to quantify $CD64^+$/$Iba\text{-}1^+$ macrophages within the myenteric plexus, as well as CCL2 expression in enteric neurons (HuC/D^+) and glial cells ($GFAP^+$). Gut vascular barrier integrity was investigated by assessing PV-1 expression (vascular permeability marker) and circulating lipopolysaccharide-binding protein (LBP) levels. HFD mice showed an increase in body weight and BMI. In HFD mice, altered *in vivo* colonic transit was observed, along with an increase *in vitro* tachykininergic contractions and increased myenteric SP immunopositivity. HFD mice showed marked infiltration of monocyte-derived macrophages ($CD64^+$, $Iba\text{-}1^+$) within the muscularis layer, closely associated with SP-positive nerve fibers and myenteric plexus. In addition, CCL2 expression was increased in both enteric neurons and glial cells, together with impaired gut vascular barrier function. Treatment with CCR2 antagonist attenuated weight gain and significantly improved colonic transit. Moreover, in HFD mice treated with CCR2 inhibitor, CCR2 blockade normalized tachykininergic hyperactivity, reduced SP expression, decreased colonic CCL2 levels, and limited monocyte/macrophage accumulation in the enteric neuromuscular layer. In conclusion, our findings support a pivotal role for the CCL2/CCR2 axis in the recruitment of circulating monocytes to the gut in obesity, promoting neuroimmune remodelling within the ENS and subsequent colonic dysmotility. Accordingly, targeting CCR2-dependent monocytes gut homing may represent a promising therapeutic strategy for obesity-associated gastrointestinal dysfunction.

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Keywords: colonic motility, high fat diet, obesity, substance P, tachykininergic neurotransmission, *muscularis* macrophages, CCL2/CCR2 pathway, monocyte gut-homing.

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A multi-component formulation enhances skin cell function and delays senescence through multi-level biological modulation

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Skin regeneration involves complex, coordinated biological processes including extracellular matrix (ECM) remodelling, cell migration, proliferation, and redox homeostasis. Multi-component formulations combining bioactive molecules such as Nicotinamide Adenine Dinucleotide (NAD^+), hyaluronic acid (HA), an epithalamic tetrapeptide and a represent a promising strategy to modulate these pathways simultaneously. However, their integrated biological effects on human skin cells have not been fully characterised. To evaluate the biological effects of a multi-component formulation, on cell viability, ECM-related gene expression, wound healing capacity, and transcriptional responses in both 2D and 3D human skin cell models. Three human skin cell lines were used: Human Dermal Fibroblasts (HDF), human epidermal keratinocytes (HaCaT), and epithelial-like cells derived from human skin (NCTC). Cells were exposed to the multi-component formulation, its individual constituents (NAD^+, HA, tetrapeptide,), or H_2O_2 1% as a cytotoxic negative control. Cell viability was assessed after 24 hours by flow cytometry using a live/dead staining protocol. Gene expression of ECM-related genes (HAS1, HAS2, HAS3, Hyal1, Hyal2) was quantified by qPCR after 24 hours of treatment in 2D cultures. Wound healing capacity was evaluated using a scratch assay in HDF and NCTC cells, with microscopic analysis at 5h and 24h. A 3D in vitro skin model was generated using HDF and NCTC cells on transwell inserts and after one week the constructs were treated for 96 hours and gene expression was assessed for ECM, oxidative stress, and metabolic pathway genes (SOD2, PLIN2, NFκB2, PPARG). The multi-component formulation did not reduce cell viability in any of the three cell lines tested, with viability profiles comparable to or slightly above those observed with the individual constituents. H_2O_2 markedly reduced viability, confirming assay sensitivity. qPCR analysis in 2D cultures revealed a coherent modulation of hyaluronidase synthase and hyaluronidase genes (HAS1–3, Hyal1–2) across all cell lines, with a broader and more coordinated pattern compared to individual components alone. In scratch assays, wound closure was enhanced at 24 hours in cells treated with the multi-component formulation compared to unstimulated controls, with performance comparable to or exceeding that of individual constituents, indicating improved migratory and/or proliferative capacity. In 3D skin models, the formulation induced a broader transcriptional response than HA alone, encompassing not only ECM remodelling genes (HAS family, Hyal genes) but also genes involved in oxidative stress regulation (SOD2), lipid metabolism (PLIN2), and inflammatory signalling (NFκB2, PPARG).

In conclusion, these findings demonstrate that the multi-component formulation, combining NAD^+, hyaluronic acid, is well tolerated and supports skin cell function through multi-target biological modulation. The coordinated effects on ECM dynamics, wound closure, and redox/metabolic gene expression, particularly evident in 3D models, suggest a synergistic action of its constituents that extends beyond simple ECM support. These results provide a preclinical basis for further investigation of this formulation in skin regeneration and anti-ageing applications.

Keywords: skin regeneration, extracellular matrix, NAD^+, hyaluronic acid, bioactive peptides, wound healing, 3D skin model, gene expression.

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CD34$^+$ Hematopoietic Progenitors Display A Tissue-Remodeling Signature Associated With Bone Marrow Fibrosis In Myelofibrosis

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Myelofibrosis (MF) is the most aggressive Philadelphia-negative myeloproliferative neoplasm, characterized by excessive proliferation of malignant CD34$^+$ hematopoietic stem/progenitor cells, profound alterations of the bone marrow microenvironment, progressive fibrosis, and chronic inflammation [1]. Abnormal activation of stromal cells induced by proinflammatory cytokines released by the megakaryocytic clone [2], and proliferation of neoplastic fibrocytes [3] have been pinpointed so far as the main events underlying the increased deposition of reticulin and collagen fibers. The role of CD34+ cells as potential drivers of the fibrotic process has not been fully investigated yet, although it has been demonstrated that they could overexpress specific cytokines in patients with higher fibrosis grading (G) [4-6]. Here, we performed RNASeq analysis on immunomagnetically isolated CD34$^+$ cells from MF patients (prePMF, overt-PMF, secondary-MF) to identify transcriptome differences according to disease subtype, grading of BM fibrosis (G ≤1 vs. >1), and exposure to JAK-inhibitor therapy (therapy naïve, MF-TN *vs* MF-JAKi). CD34+ cells from healthy donors (HD) served as controls. Sequencing was performed using Novogene total RNASeq solution on Illumina NextSeq 500/550 platform on a Mid-Output kit V2.5 (300 cycles). Pathway enrichment analysis was performed using GeneOntology and KEGG. Genes were considered differentially expressed when showing a fold change >2 and a *P* value <0.05. To identify the most impactful pathways, a GeneRatio ratio cut-off of 0.02 was set as a reference. We identified 8353 differentially expressed genes (DEGs) among MF-TN *vs* HD, 583 among pre *vs* overt PMF, 2425 among MF with fibrosis G > 1 *vs* ≤ 1, and 1112 among MF-TN vs. JAKi. The GO pathway analysis between MF-TN patients and healthy controls highlighted alterations in processes related to cilium organization, microtubule dynamics, and cell division. In parallel, differentially expressed genes included markers associated with extracellular matrix remodeling (*BMP8B, COL4A2, COL4A5*) and coagulation (*F7, F8*), suggesting the involvement of CD34+ cells in the of the BM microenvironment alterations. Intriguingly, in overt-PMF TN *vs* pre-PMF TN patients, the GO pathway analysis revealed that immune-inflammatory responses, wound healing, leukocyte migration, and ECM/cell-adhesion pathways displayed the highest differential enrichment. Consistently, DEGs were related to coagulation, platelet activation (*F12 GP5, GP6, GP9*), ECM-receptor interaction, and the interaction of cell adhesion molecules (*CCN1*). These results were confirmed by comparing patients with MF-G > 1 vs. ≤ 1. Notably, when comparing MF-TN versus MF-JAKi, GO analysis highlighted that JAKi therapy modulates gene sets involved in immunoinflammatory pathways, cell chemotaxis, and extracellular matrix organization, in addition to cytokine production. Our analysis reveals for the first time that pathways associated with wound healing, osteogenesis, and extracellular matrix remodeling are differentially expressed in CD34$^+$ cells from MF and correlate with the grading of BM fibrosis. Notably, JAKi therapy modulates the expression of several genes involved in tissue remodeling and angiogenesis, suggesting that its biological effects extend beyond the suppression of cytokine signalling and may also affect microenvironmental remodeling processes relevant to disease progression.

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Keywords: Myelofibrosis, CD34+, gene profile, extracellular matrix, microenvironment.

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Generation and characterization of a P4hb-Y395C mouse model of Cole–Carpenter syndrome and therapeutic proof-of-concept

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The Cole Carpenter Syndrome (CCS) is a neglected autosomal dominant disease affecting bone, which becomes brittle. [1] CCS is caused by the heterozygous p.Y393C mutation in the P4HB gene encoding for the protein disulfide isomerase A1 (PDIA1) [2]. To date, there are no studies showing how the mutation affects the skeletal and the disease has no cure. Based on this, we generated a mouse model carrying the mouse homolog P4hb Y395C mutation. Skeletal phenotyping revealed pronounced osteopenia and increased bone fragility in mutant mice across multiple ages, associated with an impaired bone quality ($p<0.05$). Consistently, CCS mice exhibited reduced serum procollagen type I N-terminal propeptide (PINP) levels (-60%; $p<0.01$), indicating decreased type I collagen biosynthesis. Primary osteoblasts isolated from CCS mice showed lower expression of osteoblast-associated markers (Col1α1 -26%, p=0.003; Ocn -37%, p=0.04), whereas osteoclast differentiation was increased (+2.5fold, p=0.005). At the subcellular level, Western blot and immunofluorescence analyses conducted on cellular models carrying the P4HBY393C revealed a reduction in type I collagen expression (-50%;p=0.009) and secretion (-70%;p=0.01) along with a higher PDIA1 cytosolic stabilization and Bip1 and p62 expression (+3 fold and +1.8fold, respectively; $p\leq0.05$) compared to WT cells suggesting the presence of ER stress and an impaired autophagic flux. Similar results were obtained in primary CCS mouse osteoblasts. Alteration in the type I collagen path and cellular trafficking was further confirmed by unbiased proteomic analysis performed in CCS femurs ($p<0.05$). Finally, we explored mutation-specific therapeutic strategies. Specifically, we developed an allele-specific siRNA approach identifying sequences capable to selectively silencing the mutant P4HB allele in vitro (-50%, $p<0.05$) and ex vivo in CCS femurs (-53%, p=0.007) without affecting the WT allele. Collectively, our study establishes a robust preclinical model for P4HB-related CCS, defines defective collagen I biosynthesis as a central pathogenic mechanism, and identifies mutation-specific therapeutic strategies.

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Keywords: Rare Bone Disease, Target-therapy, Osteoblast, Bone Fragility.

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Epigenetic and Antioxidant Preconditioning Enhances the Regenerative Potential of Amniotic Epithelial Cells for Type 1 Diabetes

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Type 1 Diabetes (T1D) is an autoimmune disease characterized by pancreatic β-cell destruction, resulting in insulin deficiency and chronic hyperglycemia, which can lead to severe systemic complications. Among the stem cell sources investigated for regenerative medicine applications, perinatal stem cells represent a promising option due to their low immunogenicity and ethical accessibility. In particular, amniotic epithelial cells (AECs) have attracted increasing interest because of their ability to differentiate into insulin-producing cells. However, their clinical application is limited by the progressive loss of viability and proliferative capacity during in vitro expansion[1].

This study evaluated a rejuvenation strategy based on epigenetic modulation and antioxidant supplementation to improve AEC culture performance and enhance their differentiation potential toward insulin-producing cells. AECs were preconditioned with valproic acid and a combination of antioxidants (L-carnitine, N-acetylcysteine, and 2-phospho-L-ascorbic acid). Following treatment, oxidative stress, cellular senescence, and stemness-related markers were assessed[2].

Preconditioned AECs were subsequently differentiated into insulin-producing cells using a three-stage protocol. Differentiation efficiency and cellular functionality were evaluated through immunofluorescence analyses and glucose-stimulated insulin release assays.[3] To further mimic the pancreatic islet microenvironment, differentiated AECs were co-cultured with Umbilical Cord Mesenchymal Stromal Cells to generate pseudoislet-like spheroids[4].

Preconditioning improved key biological properties of AECs, including metabolic activity, resistance to oxidative stress, and reduction of cellular senescence, while preserving their differentiation competence. In addition, co-colture promoted the formation of compact and organized pseudoislet-like structures.

In conclusion, these findings suggest that cellular rejuvenation strategies may enhance the therapeutic potential of AECs for pancreatic regenerative medicine and cell-based approaches for T1D.

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Mechanisms of assembly of plasma membrane-associated platforms in migrating cells

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Cell motility requires a tightly coordinated and continuous rearrangement of cell-matrix adhesions and the cytoskeleton to drive front-rear polarization and protrusion formation[1]. This process is modulated by plasma membrane-associated platforms (PMAPs), which are dynamic intracellular networks composed of the scaffold proteins ERC1/ELKS, Liprin-alpha1, and LL5s[2]. These macromolecular complexes support the turnover of integrin-mediated focal adhesions at the cell front; however, the precise molecular mechanisms governing the hierarchical assembly and disassembly of specific PMAPs during cell migration remain unknown. Recently, we demonstrated that DYRK3-mediated phosphorylation of Liprin-alpha1 regulates PMAP formation at the leading edge, thereby altering cell motility[3]. Based on the dynamic nature of PMAPs at the cell front, we hypothesized that their assembly is driven by liquid-liquid phase separation (LLPS) and that ERC1 acts as the primary driver of this complex[4]. ERC1 contains coiled-coil domains and intrinsically disordered regions (IDRs), which are key structural determinants for LLPS, and it has been shown to form condensates with liquid-like properties. Furthermore, we investigated whether PMAP protein turnover and the recruitment of components required for mechanosensitive responses depend on low-affinity intermolecular interactions mediated by preassembled homodimers. By combining molecular biology, biochemical, and functional analyses, we identified specific intermolecular interactions and examined the effects of perturbing these interactions on the dynamics of ERC1 and PMAP functional cluster assembly. Our results reveal that mutating specific regions of ERC1 alters its biophysical properties, disrupting its proper localization. Consequently, this impairment compromises PMAP assembly at the leading edge and significantly impacts cell motility. This study provides a comprehensive understanding of the mechanisms driving the formation of dynamic cytoplasmic condensates, revealing a general biophysical principle applicable to the assembly of diverse supramolecular networks. Crucially, these findings offer a novel perspective for the rational design of targeted therapeutic strategies aimed at interfering with pathological cell motility, such as tumor invasion and metastasis.

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Keywords: liquid-liquid phase separation (LLPS), biomolecular condensates, cell motility and cancer cell invasion.

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Morphological and Ultrastructural Characterization of Teratoma-Derived Teeth

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In 1869 Rudolph Virchow coined the term "teratoma" to describe cystic structures containing such diverse tissues as skin, hair, bone, and teeth. These latter are relatively uncommon findings in teratomas, and research into their precise morphology and ultrastructure remains scarce and/or hardly accessible and/or outdated [1-5]. Because a proper bone and tooth formation requires a complex interplay of signals between different cell lineages, a condition that seems unlikely to be maintained in this setting, we studied the ultrastructural details of teratoma-derived teeth and their supporting bone using high-resolution electron microscopy.

Six teeth- and bone-containing specimens were selected from a vast collection of teratomas, and we sincerely thank Prof. E. Fulcheri (Genoa University) for his generous donation. All specimens were subjected to the simplest treatment possible. At visual inspection most teeth exhibited an apparently normal architecture with limited deformities, appearing more often as incisors and occasionally as molars. Under the electron microscope all specimens showed a well-developed enamel with densely packed prisms, and the dentine showed regular, parallel, normal-appearing dentinal tubules. The roots were covered by acellular cementum, with significant cementocytes present only near the root bifurcations as usual. The alveolar bone formed a normal lamina dura containing a well-developed vascular network. Since the specimens were originally not intended for ultrastructural studies and were not adequately processed, soft tissues such as the periodontal ligament and predentin were unsuitable for the present study. There was ample evidence of osteoclastic and odontoclastic activity, this latter involving not only the bone but also the dental cementum down to the dentinal tubules.

On the other hand, sparse mineralization defects were a very common feature. These included enamel pits, occasional poorly mineralized areas in both enamel and dentin, and a few atypical mineralization nuclei, all possibly due to a discontinuous availability of energy and metabolites in the difficult environment of the cyst. Even in presence of these structural abnormalities teratomas can contain surprisingly good and normal-looking teeth and bone, implying that a good level of signaling control was maintained in these atypical conditions.

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Keywords: Teratoma, Dental tissues, Ultrastructure, SEM.

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Chronic exposure of human microglia (HMC3) to polystyrene nanoplastics: a morphological and ultrastructural study

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Plastic pollution by micro- and nanoplastics (MPs and NPs) has reached global proportions, with particles now detected in several tissues, organs and within the central nervous system (CNS). Most literature models rely on acute exposure, in which cells receive high NP doses for a short time, showing that nanoplastics enter cells and induce oxidative stress and inflammation [1]. The presence of MPs/NPs in human cells has also been linked to ultrastructural organelle alterations typical of metabolic stress, both in vivo in placental tissue [2] and in vitro in trophoblast cells [3]. However, since environmental exposure is chronic, our aim was to develop a model of human microglia subjected to chronic NP exposure.

We treated the human microglial line HMC3 with commercial spherical polystyrene 50 nm NPs. Acute viability tests (25–400 μM; 24–96 h) showed that 70% of cells remained viable after 96 h at 400 μM. To simulate chronicity, cells were exposed for 14 days with continuous NP administration (400 μM) at each medium change (days 4, 7, 11, 14). At each time point, cells were analysed by fluorescence microscopy (IBA1 labelling; form factor as activation parameter) and by transmission electron microscopy (TEM) to assess NP uptake and subcellular alterations.

After 14 days, cells showed a statistically significant shape change versus controls ($P = 0.0002$), shifting from a ramified (surveillance) to a rounded (activated) morphology. TEM confirmed NPs internalisation and accumulation within endosomes/phagosomes/phagolysosomes, associated with dilated endoplasmic reticulum and swollen/pycnotic mitochondria, consistent with previous observations [1,2]. In line with few recent evidence, these findings indicate that chronic exposure to polystyrene NPs, even at sub-toxic levels, drives microglial activation and sub-lethal stress [4,5], providing a CNS-relevant model that mirrors real-life conditions better than acute paradigms.

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Keywords: nanoplastics, microglia, HMC3, chronic exposure, neuroinflammation, transmission electron microscopy, ultrastructure.

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From adaptive organelle stress to epithelial attrition: a dose-dependent ultrastructural progression of cigarette smoke-induced injury in murine alveolar organoids

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Cigarette smoking remains the leading preventable cause of chronic obstructive pulmonary disease (COPD) and pulmonary emphysema, yet the subcellular events underlying alveolar injury remain incompletely defined, and a morphological correlate of the transcriptionally defined arrested transitional alveolar state is still missing. We provide a dose-dependent ultrastructural characterization of cigarette smoke extract (CSE)-induced injury in murine alveolar organoids. Three-dimensional organoids were generated from murine alveolar type 2 (AT2) cells as previously described [1], expanded and differentiated in defined media. The control group was exposed to standard medium, the treated group was exposed for 24 h to increasing CSE concentrations (0.5%, 1% and 2%, v/v). Samples were processed for transmission electron microscopy (TEM) by glutaraldehyde and osmium fixation, tannic-acid treatment and epoxy resin embedding; ultrathin sections were contrasted with a uranyl-acetate alternative and lead citrate. Within well-preserved fields, all cells were exhaustively classified by two independent trained observers, irrespective of morphological state to avoid selection bias, along orthogonal dimensions: viability, epithelial phenotype, mitochondrial alterations and rough endoplasmic reticulum (rER) stress. We observed that cellular viability declined progressively with dose, while the transitional alveolar compartment expanded at the expense of both AT2 and AT1 cells, bearing a disproportionate share of cell death at the highest exposure. Mitochondrial lesions accumulated in stage-specific combinations, and rER cisternal dilation emerged as a near-universal, phenotype-permissive feature already detectable at sublethal doses. Together, these ultrastructural findings define a coherent three-stage progression: from early adaptive organelle stress, through a critical inflection point, to dose-dependent epithelial attrition. TEM thus provides the morphological substrate for the molecularly defined block of AT2-to-AT1 transition reported in this organoid model [1], identifying the transitional compartment as its ultrastructural correlate.

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Keywords: alveolar organoids, cigarette smoke extract, transmission electron microscopy, alveolar type 2 cells, transitional alveolar cells, mitochondrial injury, rough endoplasmic reticulum stress, emphysema, ultrastructural classification, dose-response.

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Intestinal Barrier Remodelling in Inflammatory Bowel Disease: A Multidimensional Analysis Integrating Histomorphology, Microbiota and Nutrition

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Intestinal barrier dysfunction in inflammatory bowel disease (IBD) is increasingly recognised as a complex remodelling process involving epithelial integrity, stromal architecture, microbial communities and nutritional determinants, whose interplay remains understood.[1] This preliminary study aimed to characterise these interactions through an integrated assessment of mucosal histomorphology, tight junction organisation, microbiota composition and dietary patterns.[2,3] Fifty patients with ulcerative colitis (UC) or Crohn's disease (CD), together with healthy controls (HC) were evaluated. Intestinal permeability and microbial metabolism were assessed by fecal zonulin and urinary indican. Mucosal architecture and collagen deposition were analysed by H&E and Masson's trichrome staining, while CLDN-3, CLDN-4 and ZO-1 expression was assessed by immunohistochemistry. Gut microbiota composition was investigated by 16S rRNA sequencing, and dietary intake by 7-day food diary and Mediterranean Diet Serving Score (MDSS).

IBD mucosa showed epithelial erosion, crypt distortion and inflammatory infiltration. Collagen deposition was significantly higher in CD than UC ($p=0.0044$), indicating enhanced fibrotic remodelling. CLDN-3, CLDN-4 and ZO-1 expression was significantly altered ($p<0.001$, $p<0.05$ and $p<0.0001$, respectively). CLDN-3 inversely correlated with collagen deposition ($r=-0.5933$, $p=0.0058$), whereas ZO-1 positively correlated with fibrosis in CD ($r=0.7155$, $p=0.0302$). Histological severity assessed by the Geboes score was associated with increased CLDN-3 ($p=0.0002$) and reduced ZO-1 ($p=0.0027$), suggesting a selective remodelling of intercellular junctions driven by the interplay between mucosal inflammation and fibrostenotic changes, rather than a uniform loss of barrier proteins.

Microbiota analysis revealed reduced Faith phylogenetic diversity in UC compared with HC ($p=0.0003$), and Shannon diversity correlated with GS ($r=0.7143$, $p=0.0368$), linking microbial ecology to mucosal remodelling. Dietary analysis showed low fibre intake (12.18±6.83 g/day) and poor Mediterranean diet adherence (MDSS 8.78±4.76). Elevated urinary indican ($p<0.0001$) and increased fecal zonulin further supported altered microbial metabolism and barrier dysfunction, reinforcing the link between functional, histological and microbial changes.

These preliminary findings support intestinal barrier remodelling in IBD as an integrated process involving: (i) enhanced collagen deposition; (ii) alterated permeability profile; (iii) a correlation with microbial ecology; and (iv) dysfunction in microbial metabolism.

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Keywords: Gut-brain axis, IBD, Barrier remodelling.

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Translatome analysis of glutamatergic neurons in a 3D human organoid model of medulloblastoma

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Medulloblastoma is among the most aggressive paediatric brain tumours and remains associated with significant mortality and recurrence (1). It is a highly heterogeneous disease, currently classified into distinct molecular subgroups, among which SHH and Group 3/4 medulloblastomas are believed to have a cerebellar developmental origin (2). Childhood brain tumours are often interpreted as the consequence of altered developmental programs, making the identification of lineage-specific mechanisms of tumour initiation a critical step toward improved disease modelling and therapeutic development. In this context, several lines of evidence suggest that cerebellar glutamatergic neurons, including granule cell precursors and unipolar brush cells, may contribute to medulloblastoma initiation. These neuronal populations originate from ATOH1-positive progenitors located in the rhombic lip, a neurogenic structure of the developing cerebellum (10,11). However, the early molecular mechanisms driving transformation of ATOH1-derived glutamatergic neurons remain poorly understood. Although genetically engineered mouse models have provided fundamental insights into medulloblastoma biology, the human cerebellum differs substantially from the mouse cerebellum (3). Human organoid technology therefore represents a powerful complementary approach to model human central nervous system tumours in a 3D developmental context (4,5). Recent advances in cerebellar and tumour organoid technologies now allow the generation of human models that reproduce key features of tissue architecture, cellular heterogeneity and tumour growth (7,8). Here, we established human cerebellar organoids that reproduce key features of cerebellar differentiation, including ATOH1-lineage derivatives, granule cell-like populations and Purkinje cell-like neurons. To model Group 3 medulloblastoma, cerebellar organoids were electroporated at day 35 of differentiation with MYC and OTX2, together with a Venus reporter, using a PiggyBac-based strategy (6–8). This approach induced rapidly expanding tumour-like lesions within the organoids. Venus-positive tumour cells showed high proliferative activity, as indicated by Ki67 and PCNA expression. Importantly, tumour tissue could be frozen and thawed while maintaining its viability and tumour-inducing capacity when reintroduced into approximately day-90 cerebellar organoids, suggesting that this system may allow for the study of tumour adaptation to organoid microenvironments of different developmental stages. To investigate the molecular mechanisms active in the glutamatergic lineage during tumour initiation, we are developing a cell-type-specific translatomic approach based on Translating Ribosome Affinity Purification (TRAP), which allows the isolation of ribosome-associated mRNAs from defined cell populations (9). For this purpose, we generated a viral construct, p277-ATOH1-RPL29-GFP, designed to drive the expression of a GFP-tagged ribosomal protein selectively in ATOH1-lineage glutamatergic neurons. This strategy will permit the visualization of targeted cells and the immunoprecipitation of ribosome-bound transcripts specifically from glutamatergic neurons within control and tumour-bearing cerebellar organoids. The recovered ribosome-associated mRNAs will be processed for high-throughput RNA sequencing to identify the active translational programs associated with early tumour initiation and progression.

Overall, this work establishes a human 3D cerebellar organoid model of Group 3 medulloblastoma and introduces a lineage-specific translatomic strategy to dissect molecular programs active in ATOH1-derived glutamatergic neurons during tumour development. This platform may help identify early determinants of medulloblastoma tumorigenesis and provide a basis for future patient-specific modelling and therapeutic testing.

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Impairment of Brain Tumor Stem Cell Growth and Improved Response to Temozolomide Therapy Through Eg5 Inhibition

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Glioblastoma (GBM) is the most common malignant primary brain tumour in adults [1]. Despite the current standard of care, consisting of tumour surgical removal followed by chemotherapy and ionizing radiation (IR), GBM remains incurable [2]. A rare population of multipotent self-renewing cells, the brain tumour stem cells (BTSCs), are responsible of hyperaggressive phenotypes of GBM, thanks to their abilities of proliferate, spur the growth of new tumours and evade the current therapeutic approaches [3]. A driving force to stimulate BTSC proliferation is an abnormal activity of cell-cycle machinery, thus targeting individual cell-cycle components may represent an effective anti-cancer strategy. Eg5 is a mitotic motor protein essential for the correct formation of the bipolar spindle during mitotic cell division [4]. Literature reports high expression of Eg5 in GBM tumours and high correlation with BTSCs markers. However, the precise role in GBM tumorigenesis and particularly in BTSCs remains poorly studied. Thus, the aim of the present work is to investigate the role of Eg5 on multiple patient-derived BTSCs through its pharmacological inhibition (Eg5i) by using an Eg5 inhibitor, K858. In addition, a possible combination treatment between K858 and the current standard of care, Temozolomide (TMZ), has been investigated, with 24 h of K858 pre-treatment, followed by TMZ administration. Results demonstrate that: I) Eg5i impairs BTSC growth with a strict correlation with the proliferative rate of cells; II) BTSC growth impairment could be attributed to cell cycle arrest in G2 phase, molecularly driven by an up-regulation of p-Ampk; III) Eg5i reduces BTSC stemness markers expression, reducing the aggressiveness of tumour; IV) cell cycle arrest in G2 phase, due to Eg5i pretreatment, sensitizes BTSCs to TMZ-induced DNA damage, overcoming GMB resistance. The functional relevance of these findings to GBM *in vivo* is under investigation. Our data suggest that Eg5 could be a promising target for GBM therapy. Furthermore, a combined therapy of Eg5i with the current standards of care could be a valuable alternative to overcome BTSC resistance and counteract GBMs.

This communication was produced with the support of MUR National Innovation Ecosystem – Recovery and Resilience Plan (PNRR) Italy – Vitality (CUP D73C22000840006).

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Keywords: Glioblastoma, Brain Tumor Stem Cell, Eg5, Temozolomide, tumour growth.

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Pro-angiogenic response OsteoBiol GTO®-mediated through COX-2/PGE2/VEGF axis: an *in vitro* study

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One of the most frequently performed procedures in dentistry is tooth extraction. Among the potential complications associated with this procedure there is a partial resorption of the alveolar socket, due to a loss of mechanical stimulation, associated with a collapse of hard and soft tissues, with subsequent impairment of dental implant osteointegration [1]. Thus, socket preservation techniques are widely studied, highlighting several biomaterials for this purpose with pro-osteogenic properties [2]. An essential prerequisite of biomaterials for bone formation and remodeling is the ability to support angiogenesis, which is a crucial step of tissue repair [3]. OsteoBiol GTO® is an innovative xenograft biomaterial composed of 80% heterologous cortical-cancellous granular bone combined with 20% TSV gel, a mix of porcine-derived type I and III collagen enriched with polyunsaturated fatty acids. *In vitro* models shed light on the extraordinary pro-osteogenic GTO® properties [4,5]; however, no studies are currently available investigating the direct effect of GTO® on the endothelial compartment. Thus, the aims of the present work are: I) by using EA.hy926 endothelial cell line (ECs), set up an *in vitro* model to test GTO® directly on ECs, comparing different soaking preparation methods (Original Soaking, Centrifuged Soaking, Diluted Soaking) and concentrations (1, 5, 10, 20 mg/ml); II) quantify collagen release from GTO®; III) investigate the pro-angiogenic response induced by GTO® on ECs. Results demonstrate that, despite the biocompatibility of the different soaking preparation methods and concentrations, cell viability assay and collagen release analysis highlighted 10 mg/ml Original Soaking as the best condition to treat ECs. Collagen released under this condition presumably triggers COX-2/PGE_2/VEGF pro-angiogenic pathway. Indeed, a significant increase of COX-2 protein expression with PGE_2 secretion is recorded, followed by an up-regulation of VEGF protein expression and eNOS phosphorylation at ser1177, culminating in a high tube-like structure formation. By demonstrating biological effects that extend beyond osteoconduction, these findings broaden the current understanding of GTO® and further support its use in clinical strategies for socket preservation and guided bone regeneration.

This communication was produced with the support of MUR National Innovation Ecosystem – Recovery and Resilience Plan (PNRR) Italy – Vitality (CUP D73C22000840006). Moreover, this research has been supported by Tecnoss Dental S.r.l. Company, Giaveno, Italy.

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Keywords: Osteobiol GTO®, angiogenesis, endothelial cells, bone regeneration, dental biomaterials, collagen.

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Boron Neutron Capture Therapy on Healthy 2D and 3D Skin models induces reduced proliferation and upregulated repair proteins

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Boron Neutron Capture Therapy (BNCT) is an innovative binary radiotherapy for tumors resistant to conventional treatments, combining a ^{10}B-enriched compound as boronophenylalanine (BPA) with thermal neutron irradiation [1,2].

The skin represents a critical dose-limiting healthy tissue due to unavoidable beam exposure [3]. This study evaluates the effects of BNCT and conventional photon irradiation on epidermal barrier integrity regulated by tight junctions like Claudin-1 (CLDN-1) and ZO-1 [4] using integrated 2D and 3D in vitro models. HaCaT cells (spontaneously transformed human epidermal keratinocytes) in proliferative/differentiated conditions were exposed to photon (0.5–4 Gy) or neutron (0.5–4.4 Gy) radiations. Clonogenic assays showed a dose-dependent survival decrease, while BPA alone did not induce cytotoxicity.

Photon irradiation altered epidermal barrier integrity, causing CLDN-1 mislocalization, from cell membrane to cytoplasm, and decreased ZO-1. Immunofluorescence analysis showed a dose-dependent increase in γH2AX foci and 53BP1, indicating respectively the presence of DNA damage and the repair pathways activation. BrdU incorporation assays revealed a dose-dependent reduction in proliferative activity. In a 3D Reconstructed Human Epidermis model (RHE, SkinEthic™) exposed to photons (6–108 Gy), morphological alterations were observed, with epidermal layers thinning and reduced proliferation as assessed by BrdU incorporation. Immunohistochemistry showed modifications in Keratin 10, Filaggrin, pan-Cytokeratins differentiation markers and ZO-1, suggesting radiation-induced effects on epidermal turnover and epidermal barrier re-organization.

Ultrastructural TEM analysis revealed increased cellular disorganization in the treated samples compared with the controls. Despite these alterations, no evident radiation-dependent changes were observed in intercellular junctional structures, such as desmosomes, suggesting preservation of the overall tissue histoarchitecture.

After BPA and neutron treatment, RHE showed reduced proliferation and upregulated repair proteins as PCNA (Proliferating Cell Nuclear Antigen), consistent with previous 2D in vitro model observations. These integrated approaches characterized radiation-induced biological responses in healthy skin, with the aim to deepen dose-effect relationships to maximize BNCT efficacy while minimizing toxicity versus normal tissue and preserving the healthy skin barrier.

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Keywords: Boron Neutron Capture Therapy (BNCT), Epidermis, HaCaT cells, Transmission Electron Microscopy.

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Morphological Effects of Extracellular Vescicles Released by HaCaT Keratinocytes in an Inflammatory Psoriatic Microenvironment

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The complex interplay among proinflammatory cytokines, keratinocytes, and immunocompetent cells is the etiopathogenetic basis for the development and progression of psoriatic plaque lesions [1]. In this complex scenario, the setting of simplified experimental models is useful to dissect the specific contribution of each component. With regard to keratinocytes, emerging research suggests that extracellular vesicles (EVs) can represent key players in psoriasis [2,3,4]. We investigated the biological effect(s) of EVs derived from HaCaT cells, a line of spontaneously transformed human keratinocytes. HaCaT cells were i) induced to differentiate by adding 1.8 mM $CaCl_2$ to the culture medium for 4 days and ii) stimulated with a psoriatic MIX of interleukins (IL-17A, IL-22, IL-23, and TNF-alpha) for 24 hours (T24) [5]. EVs were isolated from the medium of both non-stimulated cells (EVs CTR) and MIX-stimulated cells (EVs MIX) and then incubated with HaCaT cells for a further 24h. The EVs-induced effect was analyzed through BrdU incorporation assay, intercellular adhesion considering claudin 1 (CLDN-1) and zonula occludens 1, (ZO-1) and keratinocyte differentiation for keratin 10 (K10). EVs were analyzed by transmission electron microscopy (TEM). The cell proliferation analysis with BrdU showed that the percentage of HaCaT cells in the DNA synthesis phase was reduced by the proinflammatory cytokine MIX and EVs MIX. CLDN-1 showed an intense fluorescence in the controls at 24h, which decreased in samples treated with both cytokine MIX and its derived EVs-MIX. The distribution of ZO-1 remained stable across all conditions, with no significant alteration compared to the controls. The direct inflammatory stimulus and its associated effect mediated by EVs induced a marked K10 downregulation at 24h, thus indicating an early alteration in the keratinocyte differentiation program. These results demonstrate that extracellular vesicles derived from keratinocytes under inflammatory stress (EVs MIX) play a pivotal role in propagating psoriatic-like alterations, effectively replicating the direct effects of the proinflammatory cytokine MIX. Taken together, the striking similarity in the trend between the direct cytokine MIX and the derived EVs MIX confirms that extracellular vesicles act as key pathological vectors [6]. By transferring a harmful molecular cargo, they are capable of independently altering differentiation and weakening keratinocyte adhesion, thereby mimicking and amplifying the early events of the formation of psoriatic plaque lesions.

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Keywords: cytokines, cell differentiation, cell proliferation, cell adhesion.

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Muscle spindle remodelling in experimental fibromyalgia: evidence of inflammation, oxidative stress and neural alterations

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Fibromyalgia is a chronic pain syndrome characterized by widespread musculoskeletal pain and altered sensory processing. Although central sensitization is considered a major pathogenic mechanism, increasing evidence suggests that peripheral tissues actively contribute to the persistence of nociceptive signaling and pain maintenance [1]. Recent studies have highlighted the involvement of peripheral nerve fibers and neuroimmune mechanisms in fibromyalgia pathophysiology [2,3]. Among peripheral sensory structures, muscle spindles represent highly specialized proprioceptive receptors whose role in fibromyalgia remains largely unexplored.

In the present study, we investigated muscle spindle alterations in a reserpine-induced rat model of fibromyalgia. Histological and immunohistochemical analyses were performed to evaluate markers associated with inflammation, oxidative stress and neural integrity. Muscle spindles from reserpine-treated animals showed a marked increase in the expression of inflammasome-related proteins, including NLRP3, caspase-1 and IL-1β, indicating the establishment of a local inflammatory microenvironment. In parallel, increased NRF2 immunoreactivity suggested activation of endogenous antioxidant pathways in response to oxidative stress, a process increasingly recognized as a key contributor to fibromyalgia pathogenesis [4].

The concomitant activation of inflammatory and oxidative pathways suggests that muscle spindles undergo substantial neurobiological remodeling in experimental fibromyalgia. The consequent peripheral sensitization processes could potentially contribute to abnormal proprioceptive signaling and chronic pain maintenance. These findings support the concept that muscle spindles are not passive structures but active participants in the peripheral mechanisms underlying fibromyalgia. Overall, our results identify muscle spindles as a potential anatomical substrate linking inflammation, oxidative stress and neural remodeling in fibromyalgia, providing further evidence for the contribution of peripheral sensory structures to the pathophysiology of this complex disorder.

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Keywords: Fibromyalgia, muscle spindle, Skeletal muscle, Inflammasome, Oxidative stress, Neural remodeling.

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Development and Assessment of a Decellularized Human Bone Scaffold for Skeletal Repair and Reconstruction

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The extracellular matrix (ECM) plays a fundamental role in biological tissues by providing structural support and delivering biochemical signals that influence key cellular processes, including adhesion, proliferation, migration, and differentiation. In bone tissue, the ECM accounts for approximately 90–95% of the total volume. It surrounds and supports resident cells, provides mechanical strength and rigidity, and preserves the intricate three-dimensional organization required for normal skeletal function. Replicating the structural and biological complexity of native bone ECM remains a major challenge in tissue engineering and regenerative medicine. Although synthetic biomaterials have made considerable progress, they are still unable to fully reproduce the heterogeneity and functional characteristics of natural bone ECM. For this reason, biologically derived scaffolds have attracted increasing interest, as they retain native biochemical signals and microarchitectural features that are highly beneficial for bone repair and regeneration [1]. This study describes a robust and reproducible approach for producing decellularized extracellular matrix (d-ECM) from human trabecular bone, with potential applications in tissue engineering, regenerative medicine, and orthopedic research.

Bone specimens were collected from the humeral heads of patients undergoing orthopedic surgery (n = 9). Samples were first decalcified in a 14% EDTA solution, then sectioned and processed using a decellularization protocol based on sodium dodecyl sulfate (SDS), Triton X-100, and antibiotics under continuous agitation [2]. The success of decellularization was assessed by histological examination and quantification of residual double-stranded DNA (dsDNA), which served as an indicator of remaining cellular material. The preservation of the scaffold's structure and biochemical composition was further evaluated using scanning electron microscopy (SEM), histochemical staining, immunohistochemistry, and quantitative assays to verify retention of key matrix components and overall architecture. In *vitro* biocompatibility was investigated using human adipose-derived mesenchymal stem cells (hADMSCs) isolated from sixteen patients undergoing abdominoplasty. Cells were seeded onto the d-ECM scaffolds, and viability was assessed using Trypan Blue exclusion and CellTiter-Glo luminescence assays, which measure cell survival and metabolic activity. The decellularization protocol resulted in complete removal of cellular nuclei and yielded low residual dsDNA content (30.6 ± 2.14 ng/mg), confirming its effectiveness. Histological stains, including Masson's trichrome, Mallory, and Van Gieson, together with quantitative assays, demonstrated preservation of the ECM's three-dimensional organization and protein composition. Moreover, hADMSCs cultured on the bone d-ECM scaffolds showed viability above 90%, indicating strong cytocompatibility.

This study establishes an efficient protocol for generating structurally intact and biocompatible decellularized ECM from human trabecular bone. The findings support the potential use of this scaffold for bone repair, skeletal reconstruction, and regenerative medicine applications.

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Keywords: Decellularized Extracellular Matrix, Human Adipose-Derived Mesenchymal Stem Cells, Decellularized Human Bone Scaffold.

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Development of Skeletal Muscle Analogues via Optimization of Different Hydrogels and Scaffolds

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Skeletal muscle tissue engineering holds immense promise for volumetric muscle loss treatment and in vitro disease modelling. However, fabricating functional, highly organized muscle constructs remains a challenge [1,2]. In this context, a particularly promising field is the combination of different scaffolds (such as hydrogels), to create three-dimensional structures that mimic the complex architecture of native muscle. This study investigates the use of different hydrogels provided by CELLINK® combined with a murine myoblast cell line (C2C12) to evaluate the synergistic effects on cellular viability and myogenic maturation. C2C12 cells were encapsulated into three distinct hydrogel formulations —GelXA Fibrin + $CACl_2$, CELLINK GelMA, CELLINK GelMA + $CACl_2$— seeded on different scaffolds and analysed morphologically at 24 hour and days 1, and 7 utilizing a Live/Dead cell staining assay. Following the preliminary ink screening phase, the fibrinogen-based hydrogel from CELLINK® was selected as the gold standard for printing muscle cells. Our next step was to combine the constructs with stretchable scaffolds, enabling the use of a bioreactor to simulate muscle mechanical contraction. We used PDMS (Polydimethylsiloxane) as a highly versatile silicone elastomer widely utilized in muscle tissue engineering (SMTE), not only because its biological compatibility, but also because of its mechanical properties [3]. To optimize the cell-scaffold interface, the PDMS supports were conditioned with two different coatings: collagen and Matrigel. The latter is a solubilized extracellular matrix derived from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, used to promote cell adhesion. C2C12 cells were seeded onto these conditioned supports, the resulting constructs were maintained in culture for a period of 1 week to evaluate viability and muscle differentiation, using morphological and qRT-PCR analyses, at days 3 and 7 of culture. A Live/Dead kit and phalloidin staining, a highly selective marker for actin filaments were used. The results on the "collagen-coated" samples revealed significant technical criticalities, as high autofluorescence was observed, which masked the signal emitted by the fluorescent probes. In contrast, the Matrigel-conditioned samples allowed a clear visualization of the cells seeded on the muscle constructs as early as 3 days of culture, confirming the presence of viable and elongated cells within the construct. Molecular biology analysis conducted at 7 days compared the transcriptional profile of C2C12 cells seeded on PDMS-Matrigel constructs with respect to control cells seeded on plastic (2D). The results showed that the Myoferlin, Myosin, and Myomaker genes exhibited a statistically significant higher expression in 2D samples compared to the constructs, indicating that the myogenic differentiation process in PDMS/hydrogel samples is still delayed. Consequently, the 3D differentiation protocol requires further optimization. Future analysis will focus on modulating conditioning parameters and introducing mechanical stimulation using the bioreactor, in order to promote proper myogenesis in the 3D constructs.

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Keywords: hydrogels, bioreactor, live/dead assay, qRT-PCR, constructs.

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On the Road to Muscle Regeneration: Shockwave-Induced Exosome-Like Vesicles as Drivers for Myogenic Differentiation

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Mechanical stimulation is increasingly recognized as a potent regulator of intracellular communication in muscle cells, with extracellular vesicles (EVs), and particularly exosomes, emerging as key mediators of these processes [1]. Previous studies demonstrated that mechanical strain of myoblasts increases the production of exosomes with enhanced proliferative and myogenic functions [2]. Building on this concept, accumulating evidence suggests that mechanical cues can activate mechano-dependent secretory programs, promoting the release of vesicles enriched in pro-differentiating and pro-regenerative mediators. Among mechanical stimuli, shockwave (SW) therapy represents a clinically relevant form of mechanotransduction. Notably, a few recent studies indicate that SW induces a robust secretion of exosomes from cardiomyocytes and endothelial cells, suggesting that EVs may act as paracrine mediators of SW-driven cardiac repair [3,4]. However, the underlying cellular and molecular mechanisms are largely unresolved. To address this gap, our study investigates whether SW stimulation modulates EV biogenesis, intracellular trafficking, and exosome release in an in vitro model of skeletal muscle. Therefore, murine C2C12 myoblast cells will be cultured under standard growth conditions and exposed to SW using a clinical device (DUOLITH®, Storz Medical AG) with a water bath configuration adapted from Holfeld et al. (2014) [5]. SW parameters will be applied based on preliminary optimization (EFD 0.1 mJ/mm², 3 Hz, 500 impulses), with untreated cells serving as controls. EVs released at defined time points (3h, 6h, and 12h) will be isolated by differential ultracentrifugation and characterized by Tunable Resistive Pore Sensing (TRPS) to quantify particle concentration and size distribution. In parallel, transmission electron microscopy (TEM), light microscopy, and immunofluorescence analyses will be integrated to dissect the dynamics of intracellular vesicle trafficking and exosome biogenesis. This multimodal approach will allow us to clarify the potential role of exosomes as early mediators of SW-induced myogenic and regenerative signaling.

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Keywords: Shockwave therapy, myoblasts, exosomes, extracellular vesicles, transmission electron microscopy.

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In vitro Engineering of Vascular Grafts using Human Decellularized Blood Vessels Endothelialized with Human Adipose-Derived Mesenchymal Stromal Cells

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Ischemic heart disease (IHD) is still the leading cause of illness and death worldwide, and coronary artery disease (CAD) represents its most common form. Coronary artery bypass grafting (CABG) is among the most effective surgical treatments for patients with severe CAD. However, the outcome of CABG strongly depends on the availability of suitable vascular grafts, which continues to be a major clinical limitation [1]. This study aims to generate bioengineered vascular grafts using human decellularized blood vessels (Hd-BV) repopulated with human adipose-derived mesenchymal stromal cells (hADMSCs).

Human blood vessels were obtained from patients undergoing limb amputation surgery (n = 20) and decellularized using SDS, Triton X-100, and antibiotics [2]. The efficiency of decellularization was evaluated through histological analysis and quantification of residual double-stranded DNA content. hADMSCs were isolated from subcutaneous adipose tissue collected from patients undergoing abdominoplasty (n = 16). These cells were characterized before and after in vitro endothelial differentiation [3] by observing morphological changes and assessing the expression of mesenchymal and endothelial markers using semi-quantitative real-time PCR and immunocytochemistry. The Hd-BV scaffolds were then seeded with hADMSCs and maintained in culture for up to four weeks. Scaffold cytocompatibility and cell viability were assessed using trypan blue exclusion and CellTiter-Glo luminescence assays. Endothelialization of the Hd-BV scaffolds was examined by immunofluorescence and semi-quantitative real-time PCR. H&E staining showed that the extracellular matrix architecture of Hd-BV scaffolds was preserved and confirmed effective decellularization through the absence of nuclei. This was further supported by the very low residual dsDNA content (13.07 ± 4.662 ng/mg dry tissue). Masson's, Mallory's, Movat and Gomori's staining confirmed preservation of the vascular structure and retention of key extracellular matrix proteins, including collagen and elastin, which contribute to the biochemical and mechanical properties of the vessel wall. hADMSCs displayed the typical spindle-shaped morphology and expressed mesenchymal stromal cell markers, including CD105, CD71, CD166, CD44, and CD90. Following endothelial induction, the cells adopted a cobblestone-like morphology and expressed endothelial-associated markers, such as VEGFR2, CD31, CD102, Tie-1, and Tie-2. Cytocompatibility and viability assays showed that more than 90% of cells within the Hd-BV scaffolds remained viable. Notably, hADMSCs formed a layer along the tunica intima of the vascular lumen and expressed endothelial-specific markers.

These findings support the feasibility of endothelializing decellularized human blood vessels with hADMSCs. This approach may contribute to the development of patient-specific, autologous bioengineered vascular grafts for use in coronary artery bypass grafting.

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Keywords: Coronary artery disease, Human adipose-derived mesenchymal stromal cells, Vascular grafts.

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Role of the SAM68/XRN2 Complex in RNA Processing Regulation in Prostate Cancer

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Aberrant regulation of alternative splicing (AS) and alternative polyadenylation (APA) contributes to prostate cancer (PC) progression and hormone therapy resistance (1-4). We previously showed that the MYC-regulated RNA-binding protein SAM68 and the exonuclease XRN2 cooperate to reprogram APA in PC by promoting proximal polyadenylation site usage, resulting in 3′UTR shortening and enhanced translation of cell cycle-related mRNAs (3). Bioinformatic analyses revealed that SAM68/XRN2 target transcripts are enriched in chromatin and nuclear matrix (NM) fractions. Moreover, depletion of SAM68 or XRN2 altered the subcellular distribution of unspliced transcripts, suggesting a role for this complex in coordinating RNA processing with transcript release. To investigate its global impact, we performed PolyA+ RNA-seq on cytoplasmic, nucleoplasmic, chromatin and NM fractions from control, SAM68- and XRN2-depleted LNCaP cells.

Notably, SAM68-only targets and SAM68/XRN2 common targets displayed significantly higher RNAPII elongation rates than unregulated genes. Furthermore, loss of SAM68 and XRN2 increased RNAPII Thr4 and Ser2 phosphorylation and enhanced RNAPII processivity, indicating accelerated transcription elongation.

Collectively, these findings suggest that the SAM68/XRN2 complex regulates RNAPII elongation dynamics, coupling transcription with RNA maturation. Increased elongation rates may reduce the efficiency of co-transcriptional RNA processing, favoring nuclear retention of incompletely processed transcripts and promoting post-transcriptional splicing and 3′ end processing.

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Keywords: PC, SAM68, XRN2, alternative splicing, alternative polyadenylation.

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Integrating Point-of-Care Ultrasound (POCUS) into Medical Education: Bridging Anatomy and Bedside Clinical Competence

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Human anatomy is the cornerstone of medical education. However, the traditional lag in ultrasound training for medical students has created an educational bottleneck. While Point-of-Care Ultrasound (POCUS) is globally recognized as one of the most effective, cost- and time-saving bedside tools of the 21st century, often referred to as the "Visual Stethoscope", its formal integration into core medical curricula remains limited [1]. In countries like the United States, POCUS is already deeply institutionalized within medical schools and residency programs, serving as an essential diagnostic and procedural extension of the physical examination across specialties such as emergency medicine, cardiology, and nephrology [2]. Conversely, in the Italian academic framework, the systematic use of POCUS during undergraduate medical training represents a highly innovative and necessary paradigm shift to modernize classical medical semiotics. This proposal outlines a structured POCUS Training Program (PTP) designed for Italian medical and surgery students. The goal is to move beyond passive learning by embedding clinical ultrasound directly into standard educational hours, thereby enhancing the understanding of living anatomy, preventing inappropriate imaging use, and fostering early clinical reasoning. The proposed curriculum introduces a progressive, credit-bearing framework (1 CFU per module) spanning the 3rd, 4th, and 5th years, running parallel to clinical clerkships. To optimize resources and reduce learner performance anxiety, the program utilizes a "Peer Tutor" (PT) model. Selected students are trained in clinical environments (e.g., radiology, cardiology, pulmonology, emergency medicine) and vetted through rigorous examinations to become certified tutors. A dedicated "SonoLab" equipped with educational-discounted machines serves as the practical hub. The training follows a comprehensive, multi-system approach:

a) Core Diagnostic Protocols: Students master foundational diagnostic workflows, including the eFAST protocol for trauma (evaluating perihepatic, perisplenic, pelvic, and cardiac views) [3], the BLUE protocol for rapid respiratory failure assessment [4], and FoCUS (Focused Cardiac Ultrasound) utilizing limited views like the subcostal and parasternal axes [5].

b) Anatomical and Systems Coverage: Training encompasses airway anatomy, volume status, pulmonary, vascular (DVT), abdominal, and renal sonography.

To ensure rigorous competency prior to independent clinical decision-making, students must complete a strict verification process:

1. Attendance of at least six core didactics and completion of standard online learning modules.
2. Development of an ultrasound image portfolio (comprising 20 cardiac, 5 pulmonary, 5 volume status, 5 DVT, 3 renal, and 5 abdominal studies showcasing both normal anatomy and pathology). This portfolio allows for spaced repetition, improving long-term skill retention.
3. A final, hands-on practical examination appraised by program faculty.

Implementing a structured POCUS curriculum represents a milestone for Italian medical education. By transforming abstract anatomical knowledge into dynamic, real-time clinical visualization, this program equips future physicians with vital 21st-century skills, bridging the gap between basic morphological sciences and advanced bedside patient care.

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Keywords: Point of-care ultrasound, medical education, peer tutor.

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Skull-to-face spatial correlation: a preliminary 3D geometric morphometric study

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The craniofacial complex comprises a skeletal component overlaid by soft tissues, and understanding how and to what extent skeletal morphology is reflected in the overlying soft tissues is fundamental for normal and pathological craniofacial morphology characterization, surgical planning, and forensic facial approximation. Existing literature largely relied on sparse-landmark approaches or analysed the two components separately, limiting insight into the nature and extent of skull-to-face relationship [1,2]. To overcome such limitations, the present study aims to investigate the spatial skull-to-face correlation employing 3D spatially dense geometric morphometric (GMM) approaches.

Thirty-one anonymised head CT scans (17M, 14F) were semi-automatically segmented and pre-processed [3,4] to later establish dense correspondence via the MeshMonk toolbox [4]. Asymmetry calculation, spatial correlation be-tween individual craniometric landmarks and soft tissues, joint Principal Component Analysis (PCA), and Partial Least Squares Regression (PLSR) were performed to obtain an in-depth investigation of the hard and soft tissues and their correlation, covariation and predictability.

Average asymmetry was comparable between hard (1.12 mm) and soft tissues (0.99 mm), although the latter showed higher asymmetry in specific areas, as the orbital and nasal regions, which are not represented in the skeletal tissues. Landmark-to-face correlation revealed distinct patterns: some landmarks showed localised influences (e.g., gonion and alare), while others had widespread effects across anatomically distant regions (e.g., glabella and pogonion), as also supported by joint PCA. The latter also revealed spatially coherent symmetric variation, and a more complex relationship for the asymmetric component. Partial Least Squares Regression showed that skull geometry explains approximately 20% of facial soft tissue shape variance, with increased explainability where the two tissues are in close anatomical proximity.

These preliminary findings reveal that dense GMM approaches could provide more comprehensive understandings on the complex and regionally variable skull-to-face relationship. Furthermore, the study lays the methodological foundations for larger-scale studies, whose results can have direct implications in craniofacial surgery and orthodontic planning, personalized treatment prediction, and improved forensic facial approximation.

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Keywords: Three-dimensional imaging, Geometric Morphometrics, Craniofacial morphology, Hard-Soft Tissues Correlation, Maxillofacial surgery, Forensic Facial Approximation.

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Morphological characterization of collagen fibers in breast tissue by SHG microscopy and deep learning segmentation

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Fibrillar collagen is a major component of the extracellular matrix (ECM) and undergoes significant structural remodeling during tumor progression, including increased deposition, cross-linking, and spatial disorganization [1, 2]. Quantitative characterization of collagen fiber morphology therefore represents a promising approach for identifying structural biomarkers capable of discriminating normal from tumor tissue. Second harmonic generation (SHG) microscopy enables label-free, high-resolution imaging of collagen fibers and is well suited for quantitative morphological analysis [3]. In this work, we developed an integrated computational workflow for the automatic segmentation and morphological characterization of collagen fibers from SHG images of breast tissue. Binary masks generated by ImageJ-based thresholding were used to train a multitask U-Net convolutional neural network [4,5], which simultaneously performs fiber segmentation and pixel-wise thickness estimation. The model trained on SHG–ImageJ mask pairs achieved high segmentation accuracy (Dice = 0.91, IoU = 0.85). Morphological parameters were extracted using CT-FIRE software [5], validated against manual measurements (errors < 2%). Comparison of normal (n = 18) and tumoral (n = 18) breast tissue images showed that fiber width and density were significantly greater in tumor tissue (mean width: 4.28 vs 3.92 px; mean density: 4.67% vs 3.63%; Mann–Whitney U, $p < 0.001$), while length, straightness, and orientation did not differ significantly. Density heatmaps revealed spatially heterogeneous collagen distribution in tumoral samples. These results confirm fiber width and density as structural biomarkers for tissue classification, demonstrating the effectiveness of an automated, reproducible deep learning pipeline for ECM characterization.

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Keywords: collagen, SHG microscopy, U-Net, ECM, breast cancer, morphological biomarkers.

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Modulation of Fibroblast-to-Myofibroblast Transition and Extracellular Matrix Remodeling under Oxidative Stress: Potential Protective Effects of Ergothioneine and Equisetum arvense

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Successful wound healing relies on the coordinated activation of fibroblasts, extracellular matrix (ECM) remodelling, and tissue regeneration. During the proliferative phase of tissue repair, fibroblasts migrate into the wound bed and undergo a phenotypic transition toward contractile myofibroblasts, a process characterised by the expression of α-smooth muscle actin (α-SMA) and primarily regulated by transforming growth factor-β1 (TGF-β1) [1,2]. These activated cells play a crucial role in extracellular matrix deposition, remodelling, and wound contraction [1]. Oxidative stress is recognised as a major factor impairing tissue repair. Excessive accumulation of reactive oxygen species (ROS) disrupts fibroblast function, alters ECM homeostasis, and may interfere with fibroblast-to-myofibroblast transition, ultimately compromising wound healing efficiency [2]. Ergothioneine (EGT) and Equisetum arvense have been reported to exert antioxidant and cytoprotective effects [4,5]. However, their potential role in modulating fibroblast plasticity and extracellular matrix remodelling under oxidative stress conditions remains poorly understood. This study aims to investigate whether EGT and Equisetum arvense preserve fibroblast functionality, regulate fibroblast-to-myofibroblast transition, and maintain extracellular matrix remodelling capacity following oxidative stress induction in immortalized mouse embryonic fibroblasts (MEFs). To evaluate extracellular matrix remodelling and fibroblast plasticity, quantitative molecular analyses and immunofluorescence assays are performed. Particular attention is devoted to the expression of ECM-related markers and proteins involved in myofibroblast differentiation, as well as to the assessment of morphological changes associated with cytoskeletal reorganisation and fibroblast activation. We hypothesise that oxidative stress impairs fibroblast migratory activity and disrupts ECM homeostasis, whereas treatment with EGT or Equisetum arvense preserves fibroblast morphology, supports physiological matrix remodelling, and promotes the maintenance of a balanced reparative phenotype. These effects may contribute to the preservation of tissue repair mechanisms under oxidative stress conditions. This study provides insight into the relationship between oxidative stress, fibroblast plasticity, and extracellular matrix remodelling, highlighting the potential of naturally derived compounds as modulators of cellular responses involved in tissue regeneration.

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Keywords: Fibroblast-to-myofibroblast transition, extracellular matrix remodeling, wound healing, oxidative stress, ergothioneine, Equisetum arvense, α-SMA, TGF-β1, tissue regeneration.

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Cryopreservation preserves the morpho-structural and mechanical integrity of human decellularized tracheas and supports a reduced immunogenic profile

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Tracheal substitutes must support revascularization and regeneration while resisting necrosis and infection. Decellularized tracheas are promising, but degradation limits their shelf life, necessitating tissue banking. Few studies have assessed the impact of storage, particularly cryopreservation, on tissue quality, hindering clinical translation. This study, a collaboration between the Section of Human Anatomy of the University of Padova and Fondazione Banca dei Tessuti del Veneto - FBTV, compares decellularized (DecellT) and decellularized + cryopreserved (DecellT+CryoT) tracheas, evaluating a novel FBTV decellularization protocol (used on trachea for the first time) and the impact of cryopreservation on tissue characteristics. DecellT and DecellT+CryoT tracheas from human donors were here compared to native controls. Residual DNA and nuclei were evaluated via DAPI staining and DNA quantification. Tissue and extracellular matrix architecture were examined using Hematoxylin and Eosin, Alcian Blue, Masson's Trichrome, and Weigert-Van Gieson stains. Glycosaminoglycans and elastic fibers were quantified using quantitative and semi-quantitative methods. Immunostaining for Human Leukocyte Antigen–DR (HLA-DR) assessed residual immunogenicity, while scanning electron microscopy (SEM) and compression tests evaluated ultrastructure and mechanical properties. The experimental results showed that decellularization significantly reduced DNA content to less than 50 ng/mg, which was maintained post-cryopreservation. Unlike native controls, treated tracheas retained only the basal lamina, losing the respiratory epithelium. Submucosal architecture was preserved across groups but lacked nuclei in treated samples. Alcian Blue and Masson's trichrome confirmed excellent GAG and collagen preservation. However, Weigert-Van Gieson staining and morphometric analysis showed decreased elastic fiber integrity and content. Only a few HLA-DR-positive elements remained after treatment. SEM confirmed epithelial removal with preserved basal lamina and slightly less compact adventitial collagen. Treated cartilage showed empty lacunae. No significant differences in stiffness were observed between groups. Combining decellularization with cryopreservation preserves tracheal architecture and enhances the reduction of HLA-DR–immunopositive elements compared to decellularization alone, confirming the clinical viability of these grafts for tissue banking.

Keywords: Cryopreservation, decellularization, mechanical behaviour, reduced immunogenicity, trachea.

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Microstructure and extracellular matrix suspension modulate bone marrow-derived mesenchymal stem cell proliferation for osteochondral regeneration

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Osteochondral defects remain a major challenge in orthopedic surgery due to the complex structure and function of articular cartilage. To address this issue, this study developed and characterized bioactive porous scaffolds based on oxidized polyvinyl alcohol (OxPVA), both with and without human cartilage-derived decellularized extracellular matrix (dECM), to serve as platforms for HM1-SV40 cell adhesion and proliferation. OxPVA scaffolds were fabricated via a particle-leaching technique using varying gelatin concentrations (10%, 15%, and 25% w/w). Ultrastructural features were examined using Scanning Electron Microscopy (SEM), while a morphometric analysis assessed pore number, size, and total porosity. Pore interconnectivity was evaluated through Fluorescence Recovery after Photobleaching (FRAP). To boost bioactivity, dECM (25% w/w) was incorporated into the OxPVA matrix. Consequently, the expression of genes involved in collagen synthesis and chondrogenic differentiation/remodeling was analyzed via quantitative PCR, alongside the evaluation of SOX9, ACAN, and COMP protein expression. In vivo biocompatibility of the composite scaffolds was validated through subcutaneous implantation in Sprague–Dawley rats. For the bone component, 3D-printed polylactic acid (PLA) scaffolds with distinct geometries (40%, 53%, and 67% porosity; 600–1400 μm pore size) were fabricated and evaluated in vitro. Results indicated that lower gelatin concentrations yielded numerous superficial pores, whereas higher concentrations produced larger, coalescing pores. Notably, HM1-SV40 cells demonstrated superior adhesion on scaffolds prepared with 25% gelatin. The OxPVA+dECM composites displayed a homogeneous matrix distribution that further enhanced cell interaction, despite a reduction in mean pore size compared to dECM-free scaffolds. Furthermore, the 25% gelatin OxPVA+dECM formulations provided a highly favorable microenvironment that supported chondrogenic differentiation and cartilage matrix deposition. In vivo, the implants elicited no inflammatory response. Regarding the bone layer, all PLA scaffolds maintained excellent cell viability; SEM imaging confirmed full-thickness cell distribution throughout the structures, indicating complete colonization. While further studies are required to evaluate stem cell differentiation, these bioactive OxPVA and 3D-printed PLA scaffolds hold significant promise for osteochondral regeneration.

Keywords: Cartilage, polyvinyl alcohol, decellularized matrix, porosity, mesenchymal stem cells.

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Morpho-Structural Architecture and Biomechanical Behavior of the Ileum and Colon

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Managing intestinal stomas remains a significant clinical challenge due to frequent peristomal complications and poor physiological compatibility between commercial devices and host tissues. This study characterizes the morpho-structure and biomechanics of the human ileum and colon to support the rational design of a novel biomedical device for temporary stoma closure. Ileum and colon segments from 49 patients (63±11 years) were analyzed (Ethics Committee Approval, CESC Code: AOP3212). Tissue architecture, innervation and vascularization were assessed through histology (H&E, Azan-Mallory) and immunohistochemistry (S100/CD31). Extracellular matrix (ECM) components (glycosaminoglycans and total collagen) were quantified through biochemical assays. Mechanical properties were assessed via uniaxial tensile and cyclic compression tests at varying strain rates, with data processed via Matlab to determine elastic moduli, tensile strength, and energy dissipation. The colon exhibited a significantly higher submucosal collagen density than the ileum (50.45% vs 33.99%, $p<0.0001$), correlating with its superior mechanical performance. Biomechanical analysis highlighted marked tissue anisotropy: the longitudinal colon was the stiffest in the linear region, while the circumferential colon showed the highest energy absorption (toughness: 0.460±0.369 MPa). The presence of the taenia coli significantly increased both tensile strength and stiffness. Cyclic compression tests confirmed the viscoelastic nature of both tissues, with the colon showing higher maximum stress and stiffness than the ileum, consistent with its thicker circular muscle layer and denser ECM network. This study establishes fundamental correlations between intestinal morpho-structure and mechanical behavior. These findings provide a quantitative framework essential for developing biomimetic temporary stoma devices that physiologically match the native properties of the human intestinal wall.

Keywords: intestinal stoma, morpho-structure, biomechanics.

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Refining Ethmoidal Foramina Topography Through Digital Relative Indexes: A CT-Based Study

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Ethmoidal foramina (EFs) are the inlet of important channels that connect the orbit with cranial, nasal and paranasal cavities. They host arteries that sometime feed intracranial vascular anomalies or are the source of refractive epistaxis. Because of their importance in such clinical settings, determining their topography is certainly a priority. Over the years, at least 18 studies have measured the distance of EFs from anterior landmarks (mostly the anterior lacrimal crest) generating, however, results that appear discouragingly inconsistent [1]. For instance, the anterior EF distance from anterior bony landmarks has been reported as ranging from 17 to 28 mm. A greater degree of standardization, therefore, would be welcome and could be useful for neuronavigation in orbital surgery.

A retrospective study was carried out on computed tomographies (CTs) performed for pathologies not altering the structural integrity of the medial orbital wall. After reorienting CT spatial planes, a series of measures were recorded along perpendicular planes intersecting the orbital axis or the EFs to produce two indexes for both the anterior and posterior EFs: average and median digital relative depth index (dRDI) for anterior and posterior EF positions in an anteroposterior direction, and average digital relative height index (dRHI) for anterior and posterior EF positions along vertical axes. A dRDI watershed index (dRDIwi) was also devised for the correct assignment of EFs to the anterior and posterior types.

The dRDIwi defined very well EF belonging to anterior or posterior types. This was particulary useful when orbits contained just one EF or more than 2 EFs. Whereas the average dRHI did not appear to be a very effective tool for vertical standardization of EFs, the median and average dRDIs were predictive of EF localization in the anteroposterior direction with a tolerance of about 2 mm in 50% of cases. Indeed, the dRDI, normalizing EFs position relative to length of the orbital axis, was effective to narrowing the standard deviation (SD) of the EF distance from an anterior landmark. Whereas the SD of the distance from the anterior orbital opening was ±2.9 mm for the anterior EF and ±2.8 mm for the posterior EF, calculation of the SD for the dRDIs from the same set of data allowed to narrow distance to ±2.2 mm for the anterior EF and ±2.1 mm for the posterior EF.

In conclusion, we demonstrated that the plain distance of the EF from any anterior landmark is doomed to produce a greater dispersion of data (and, accordingly a greater uncertainty of the EF position) than the dRDI. The effectiveness of median and average dRDIs to locate anterior and posterior EFs along the orbital wall make these indexes good candidate parameters for neuronavigation in orbital surgery.

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Keywords: Orbit, ethmoidal foramina, neuronavigation.

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Juvenile nasopharyngeal angiofibroma: analysis of 12 cases

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Adolescent males are the main victims of juvenile nasopharyngeal angiofibroma (JNA), an uncommon, benign tumor that is extremely vascular and locally aggressive. Because of its invasive growth and proximity to important anatomical systems, JNA presents major therapeutic hurdles despite its benign classification. Understanding the molecular and histological processes behind juvenile nasopharyngeal angiofibroma (JNA) is the goal of the study. 12 Nasopharyngeal Angiofibroma (8 males and 4 females, mean age 16.2, range 14–18 years) and 4 control samples (normal tissue samples contained no visible tumor cells; three males and one female) were retrieved from archival paraffin embedded blocks with a sufficient sample size. The expression of TGF-β1, VEGF-A, and TNF-α has been studied. The technique chosen for the immunohistochemical study of the samples was microdensitometry. By analyzing tissue samples, it investigates the presence of Transforming Growth Factor Beta (TGF-β), Vascular Endothelial Growth Factor (VEGF), Tumor Necrosis Factor Alpha (TNF-α) in angiogenesis, fibrosis, and inflammation—three critical processes in the development of malignancies. Overexpression of these growth factors was found in tumor tissues by immunohistochemical analysis, indicating their role in vascularization and tumor progression. These findings suggest potential targets for treatment, such as anti-angiogenic drugs, to improve management strategies beyond surgical resection, which remains the primary therapeutic strategy. Future research should focus on developing novel therapy strategies that use molecular inhibitors to improve clinical outcomes for JNA patients.

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Keywords: juvenile nasopharyngeal angiofibroma, Transforming Growth Factor Beta (TGF-*β*), vascular endothelial growth factor (VEGF), Tumor Necrosis Factor Alpha (TNF-*α*).

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Morphological Insights into Bortezomib-induced Peripheral Neurotoxicity Using hiPSC-Derived Schwann Cell Precursors and Sensory Neurons

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Chemotherapy-induced peripheral neurotoxicity (CIPN) remains a severe dose-limiting side effect of several anticancer agents, such as the proteasome inhibitor bortezomib (BTZ). While *in vitro* platforms offer a controlled environment to dissect specific neurotoxic pathways, their clinical predictability is often limited by a lack of human-centric architectural relevance. To bridge this translational gap, current neurobiology is shifting towards more advanced, human-based models. In this context, a detailed morphological and structural characterization of these platforms is an absolute priority to ensure they can accurately replicate the complex neuro-glial alterations typical of human CIPN pathology [1].

To achieve this, we performed a comprehensive morpho-functional evaluation of human induced Pluripotent Stem Cell (iPSC)-derived sensory neurons (RealDRG™) and compared them against primary cultured mouse Dorsal Root Ganglion (DRG) neurons upon exposure to different doses of BTZ [2,3]. Meanwhile, we conducted a preliminary characterization of human iPSC-derived Schwann Cell Precursors (SCPs). Cellular morphology together with the expression of specific population markers were characterized in detail using immunofluorescence. To capture early architectural changes and neurite alterations in real time, label-free live imaging was performed using holotomography microscopy (Nanolive SA, Tolochenaz, Switzerland). This morphological assessment was complemented by multiple functional assays evaluating proteasome inhibition, cell viability, mitochondrial morphology and network analysis as well as axonal degeneration.

Our results showed successful differentiation toward a broader peripheral neuron phenotype with several specific markers for neuronal subpopulations in RealDRG™ neurons, together with specific cellular markers expression in SCPs. Proteasome inhibition and cell viability assays showed similar dose/response curves between RealDRG™ and their murine counterpart, while label-free microscopy enabled the observation of early morphological alterations due to BTZ-induced neurotoxicity. Additionally, immunofluorescence studies revealed an early impact of BTZ on axonal degeneration that was successfully replicated. Mitochondrial morphology and network analysis also revealed good correspondence in the BTZ-induced dysfunctions among all models.

Taken together, these findings elucidate the potential of these cellular models and strongly suggest that the translational gap in CIPN research is being bridged by our successful alignment of human sensory neurons with already characterized models. Finally, the early results provided on human-derived SCPs ultimately aim to pave the way for the establishment of a co-culture model that better mimics the physiological landscape.

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Keywords: iPSCs, Schwann Cell Precursors, human-derived sensory neurons, Nanolive, chemotherapy, neurotoxicity, mitochondria.

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Artificial Intelligence in Transmission and Scanning Electron Microscopy: Applications, Challenges, and New Frontiers in Ultrastructural Analysis

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Electron microscopy (EM) has revolutionized the morphological sciences, progressively providing new levels of magnification and resolution for exploring biological and non-biological specimens [1]. Specifically, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have enabled high-resolution visualization of ultrastructural features. In recent years, artificial intelligence (AI) has been integrated into EM workflows, offering new approaches for image analysis and data interpretation. The aim of this study is to map the current evidence regarding the application of AI techniques in TEM and SEM analysis, highlighting their main advantages and challenges. The literature search was performed through Scopus, Web of Science, and PubMed, selecting studies published between September 2019 and January 2026. The search strategy combined terms related to "artificial intelligence," "machine learning," "deep learning," "transmission electron microscopy," "scanning electron microscopy," and "cell" or "tissue" or "ultrastructure" or "biological specimen". The review process followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for *Scoping Reviews* (PRISMA-ScR) guidelines. After screening and eligibility assessment, 15 studies were included, reporting extensive use of AI-based methods in TEM and SEM, with improved performance across image segmentation, quantitative and morphometric analysis, and disease classification tasks. Furthermore, AI methods showed improved efficiency and reproducibility in image processing workflows, although variability in dataset size, annotation quality, and model generalizability remains a limitation across studies [2]. In conclusion, AI integration into EM revealed strong potential to enhance image analysis capabilities, although further standardization and validation across different experimental settings will be necessary to sustain broader clinical and research adoption.

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Keywords: morphology, transmission electron microscopy, scanning electron microscopy, ultrastructure, artificial intelligence.

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Ultrastructural Profile of Human Periodontal Ligament Fibroblasts Exposed to Bovine Pericardium Membranes: Insights into Cellular Responses to Bioresorbable Collagen Membranes

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Recent advances in regenerative dentistry have been fostered by the development of biomaterials capable of guiding and promoting dental tissue regeneration. The main chemical, mechanical and biological features, with a specific emphasis on osteoinductivity and osteoconductivity, make them suitable to interact with living tissue [1]. A significant contribution to the advancement of regenerative dentistry has been provided by the development of resorbable membranes, derived from animal collagen, such as bovine ones. The aim of this study is to investigate the ultrastructural profile of human periodontal ligament fibroblasts (HPLFs) cultured in standard conditions and exposed to bovine pericardium membranes of different thicknesses (0.2 mm and 0.4 mm) for 24, 72 hours and 7 days. The HPLFs were processed according to standard protocols for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM observations detected thickening of the HPLFs, with enlarged polygonal shape and developed filipodia and lamellipodia [2]. TEM investigation further revealed ultrastructural changes in HPLFs' profile, when exposed to bovine pericardium membranes for 24, 72 hours and 7 days: among them, the presence of a large nucleus, prominent nucleoli, clustered rod-like shaped mitochondria, richly developed endoplasmic reticulum (ER) and Golgi apparatus, and extensive tapering cytoplasmic projections [1]. HPLFs in contact with bovine pericardium membranes of varying thicknesses exhibited distinct ultrastructural changes, detected by SEM and TEM, consistent with the active functional and mature stage of HPLFs. These ultrastructural details offer valuable insights into the cellular architecture of HPLFs, which may help clarify microenvironmental changes during the regeneration process.

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Keywords: morphology, biomaterials, collagen membranes, human periodontal ligament fibroblasts, regenerative dentistry, scanning electron microscopy, transmission electron microscopy.

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CPSF1 Is a Novel Transcriptional Target of MYC in Prostate Cancer

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In PC patients, CPSF1 (Cleavage and Polyadenylation Specificity Factor 1) expression correlates with unfavorable prognosis (1), suggesting that its upregulation may contribute to tumor progression and therapy resistance. The oncogenic transcription factor MYC is frequently overexpressed in advanced PC and promotes tumor progression by globally regulating gene expression programs, including genes involved in RNA processing (2). Since both MYC and CPSF1 are upregulated in PC (1,3), we investigated whether their expression levels correlate in patients. Using the cBioPortal platform, we revealed a significant positive association between MYC and CPSF1 expression, which was confirmed by stratifying patients according to MYC levels. Analysis of ENCODE ChIP-seq datasets identified a MYC-binding peak upstream of the CPSF1 transcription start site containing an E-box motif (CACGCG) embedded within a CpG island, a genomic context frequently associated with MYC occupancy at active promoters (4). ChIP assays confirmed MYC recruitment to the CPSF1 promoter in LNCaP cells, while MYC depletion significantly reduced CPSF1 mRNA and protein expression, identifying CPSF1 as a novel MYC transcriptional target.

Enzalutamide is widely used in the treatment of advanced PC, however resistance is acquired within a short time interval (5). Consistent with our preliminary observations (7), enzalutamide-resistant HT-LNCaP cells displayed increased CPSF1 expression. Given the established role of MYC in the acquisition of therapy resistance (6,7), we investigated whether CPSF1 upregulation in resistant cells was associated with altered MYC expression. Western blot analyses revealed increased MYC protein levels in HT-LNCaP cells, whereas mRNA levels remained unchanged. Two c-MYC protein isoforms, p67 (c-Myc1) and p64 (c-Myc2), are generated from the same mRNA through alternative translation initiation at distinct start codons, CUG and AUG, respectively (8). Interestingly, selection of alternative promoter yields MYC transcript variants differing at the N-terminal sequence. Using isoform-specific primers, we observed increased expression of MYC isoform-213 in HT-LNCaP cells, which encodes the oncogenic p64 (c-Myc2) isoform. Since p67 appears to have strong tumour suppressor properties compare to p64 (8), the p64:p67 ratio switching may contribute to therapy resistance.

Collectively, these results identify CPSF1 as a MYC transcriptional target. Future studies will determine whether Myc isoform-213 displays altered mRNA stability compared to the canonical MYC isoform. In addition, we will investigate whether this isoform contributes to CPSF1 upregulation and to the establishment or maintenance of enzalutamide resistance in PC cells.

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Keywords: PC, MYC, CPSF1.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Anthropometric indicators of growth and nutritional status in three -year-old Macedonian children

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Growth monitoring and promotion of optional growth are essential components of primary health care for children. Serial measurements of weight, height/length, for all children, and measurements of circular and transversal parameters compared with growth of a large sample population help to confirm a child's healthy growth and development [1]. It also allows early identification of potential nutritional or health problems and enables prompt action before a child's health is seriously compromised. The aim of the study is evaluation of sex-specific differences in anthropometrical parameters as indicators of growth [2]. The study included 200 healthy 3 years old preschool children from Macedonian nationality. Thirteen anthropometric parameters were measured, defining longitudinal, circular and transversal dimensionality of the skeleton using standard technique and instruments for measurement. The following indices were selected and calculated: weight-for –age; height-for-age and BMI. Skin –folds (triceps, scapula and thigh) were also measured [3]. Sex-specific differences for almost all anthropometric parameters were detected, but they were not significant. Girls showed higher values than boys regarding height, weight but for BMI were significant in boys. Values at the 50th percentile in girls were 16.8 kg for BW, 102 cm for BH and 16.5 kg/m^2. The values of these parameters in boys were 18 kg for BW, 96.93 cm for BH and 17.5 for kg/m^2 for BMI. The values for skin fold for triceps were higher in girls (13.1 ± 3.5) instead of boys (12.2 ± 3.3). The results obtained can be used for criteria for assessment and detecting deviations in growth and nutritional status in preschool children.

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Keywords: anthropometry, growth, nutritional status, preschool children.

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Morphological and Molecular Effects of Di-n-Butyl Phthalate on Adipocyte Differentiation in 3T3-L1 Cells

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Phthalates are widespread endocrine-disrupting chemicals (EDCs) linked to metabolic alterations and adipose tissue dysfunction. Among these compounds, Di-n-butyl phthalate (DBP) has been suggested as a possible obesogen, although its involvement in adipogenesis is still not fully understood [1]. This study explored the impact of DBP on adipocyte differentiation in 3T3-L1 cells. Differentiation was induced using a conventional adipogenic cocktail containing insulin, 3-isobutyl-1-methylxanthine (IBMX), and dexamethasone (DEXA). DBP was tested at concentrations from 10^{-6} M to 10^{-9} M under four different experimental settings to assess its capacity to activate or modulate glucocorticoid signalling. DBP was applied either as DEXA-substitute or together with it (DBP+DEXA), during the induction phase (days 0–2) or throughout the whole differentiation process (days 0–8). The MTT assay demonstrated that none of the tested concentrations caused cytotoxicity. Adipogenesis was assessed through Oil Red O staining and Western blot analysis of the adipogenic markers C/EBPα and PPARγ [2]. Oil Red O staining showed modified lipid accumulation patterns in all DBP-treated groups compared with the differentiated control, indicating that DBP affects adipogenesis regardless of DBP concentration [3]. When administered without DEXA, DBP increased the expression of both adipogenic markers, with the most pronounced effect observed at 10^{-7} M in both treatment periods. Conversely, in both temporal conditions, the combined treatment DBP+DEXA reduced the expression of these markers, especially at lower concentrations. Overall, DBP displayed a dual action: when administered alone, it exerted a DEXA-like effect by promoting adipogenesis, whereas in combination with DEXA it counteracted its activity, exhibiting an antagonistic effect and highlighting a context-dependent modulation of glucocorticoid signaling.

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Keywords: EDCs, DBP, Obesity, metabolism disfunction.

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The neutrophil megakaryocyte emperipolesis blockage by P-selectin genetic ablation prevents the bone marrow fibrosis in the Gata1^{low} mouse model for myelofibrosis.

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Myelofibrosis (MF) is a rare chronic hematological disorder, within the family of myeloproliferative neoplasms. The MF patients present clinical abnormalities such as anemia, and thrombosis, as well as alterations in the bone marrow (BM) microenvironment, an increased number of megakaryocytes (MKs), most of which are found in emperipolesis with neutrophils. In MF, the MKs emperipolesis is induced by an altered MK secretome, containing increased levels of pro-inflammatory cytokines, proteins, and growth factors such as interleukin-8 (IL-8) and P-selectin (P-sel). These, allow the altered cell-to-cell interactions and cause the transforming growth factor-β (TGF-β) to be released into the BM microenvironment. This fibrogenic cytokine contributes to BM fibrosis and disease progression. Emperipolesis has already been identified as a pathobiological event that contributes to MF and it is widely recognized in the most advanced stages of the disease. In this study, we evaluated the role of P-sel in BM alterations associated with emperipolesis in the Gata1low mouse model of MF. Our data show that emperipolesis is driven by P-sel. Genetic ablation of P-sel rescued the BM microenvironment, by decreasing fibrosis, suggesting that pharmacological targeting of P-sel could contribute to reduce the BM dysfunction and disease progression.

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Keywords: P-selectin, emperipolesis, myelofibrosis, megakaryocytes, Gata1^{low}.

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Morphometric Analysis of GFP- and Iba1-Positive Cells Reveals Neuroinflammatory Activation in the Superior Colliculus in Experimental Parkinsonism

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Parkinson's disease (PD) is increasingly recognized as a multisystem neurodegenerative disorder characterized not only by the loss of dopaminergic neurons in the substantia nigra but also by widespread neuroinflammatory processes affecting extra-nigral brain regions. The superior colliculus (SC), a midbrain structure involved in sensorimotor integration and closely interconnected with dopaminergic pathways, has received limited attention in the context of PD pathology. The present study aimed to investigate neurodegenerative and neuroinflammatory alterations in the SC of an MPTP-induced rat model of Parkinsonism, with particular emphasis on the quantitative assessment of glial activation.

Adult male Sprague-Dawley rats were treated with MPTP and SC sections were analyzed using histological, immunohistochemical, and morphometric approaches. Nissl staining and NeuN immunolabeling revealed neuronal loss and disruption of the normal laminar organization of the SC following dopaminergic injury. Neuroinflammatory changes were evaluated through GFP and Iba1 immunoreactivity, demonstrating marked glial activation in MPTP-treated animals. To objectively characterize these alterations, a quantitative morphometric analysis of GFP-positive and Iba1-positive cells was performed. Activated cells exhibited significant morphological remodeling, including enlargement of the cell body and structural changes consistent with reactive glial phenotypes. These findings provided quantitative evidence supporting the presence of an ongoing neuroinflammatory response within the SC. Furthermore, increased expression of the inflammasome-related markers NLRP3, Caspase-1, and IL-1β confirmed the activation of pro-inflammatory signaling pathways.

Collectively, these results demonstrate that the superior colliculus undergoes substantial neurodegenerative and neuroinflammatory remodeling following MPTP-induced dopaminergic degeneration. The morphometric characterization of GFP-positive and Iba1-positive cells highlights the extent of glial activation and further supports the involvement of the SC in the extra-nigral pathology of PD. These findings provide additional insight into the mechanisms underlying disease progression and identify neuroinflammation within the superior colliculus as a potential target for future therapeutic strategies.

Keywords: Parkinson's disease; superior colliculus; neuroinflammation; morphometric analysis; Iba1; GFP; glial activation; inflammasome.

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Exploring the molecular basis of Non-small Cell Lung Cancer: the role of Phospholipase C enzymes and the inhibition of c-Myc

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Lung Cancer (LC) is the leading cause of cancer morbidity and mortality worldwide. Despite considerable progress in LC treatment, the prognosis remains poor. Hence, the complex biology of LC is still to be investigated in order to understand its molecular aspects and to further improve treatments and survival of patients. Recently, the potential role of Phospholipase C (PLC) enzymes in tumor proliferation has been proposed [1]. Also, c-Myc oncogene was presented as a potential therapeutic target in LC, since it modulates protein synthesis, DNA replication, and metabolism [2]. Our study examined the involvement of PLCs and Myc in four LC cellular models. First, mRNA expression of all PLC isoforms and Myc was estimated by real-time qPCR, and protein levels were observed by Western Blot (WB). Then, a stable transfection of H358 and H460 cells with a novel construct for Omomyc peptide or a treatment with MYCi975 was performed. Omomyc simply blocks Myc activity by displacement [3], whereas MYCi975 can also phosphorylate Myc and therefore induce its degradation [4]. Cell cycle analysis and wound healing assay were conducted to observe the modulation of proliferation and migration. Subsequently, qPCR and WB were performed to monitor the expression of signaling proteins. In this experimental model, many PLCs were deregulated and PLCB1 was highly expressed in cell lines. In addition, we observed that both Omomyc and MYCi975 counteract c-Myc activity by decreasing cell viability and modulating cell cycle and signal transduction. In fact, our results demonstrated the contribution of signaling cascades related to cell proliferation and survival, including PLCs. The outcomes of this study illustrated the involvement of PLC enzymes in LC, suggesting their implication in cellular mechanisms supporting cancer and their connection to c-Myc activity. Moreover, the inhibition of c-Myc significantly reduced cell growth and migration, therefore representing a promising strategy in LC treatment.

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Keywords: Non-Small Cell Lung Cancer, Phospholipase C, Myc.

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Microplastic Exposure Influences the Response to HER2-positive Breast Cancer Therapies

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Human exposure to microplastics is increasingly recognized as a public health concern [1]. Oral ingestion is considered a major exposure route, given the widespread presence of microplastics in food, beverages, and drinking water [2]. Following ingestion, these particles can cross the intestinal barrier, enter the circulation, and accumulate in multiple organs. The potential impact of microplastic exposure on cancer development and therapeutic response has received limited attention and has been explored only in a few malignancies, such as gastric and ovarian cancers [3]. However, the role of microplastics in breast cancer remains largely unexplored, despite its status as the most frequently diagnosed cancer in women and one of the leading causes of cancer-related death worldwide. Approximately 20% of breast carcinomas overexpress the HER2 (human epidermal growth factor receptor 2) receptor, a condition associated with aggressive disease and poor prognosis [4]. Trastuzumab has substantially improved clinical outcomes in patients with HER2-positive breast cancer; nevertheless, its long-term effectiveness is often limited by the emergence of resistance mechanisms. The present examined the impact of long-term oral exposure to microplastics on breast cancer development and on tumor sensitivity to anticancer treatment, specifically assessing their effects on trastuzumab responsiveness. FVB-Δ16HER2 mice, which spontaneously develop HER2-positive mammary tumors [5], were used. Polystyrene (PS) microplastics (5 μm diameter) were administered at a dose of 1.2 mg/kg/day beginning at 8 weeks of age, when animals were still tumor-free, and continued until 28–30 weeks of age. Tumor latency, growth, and multiplicity were monitored to evaluate the effects of plastic exposure on tumor initiation and progression. Trastuzumab was administered once weekly for five consecutive weeks (8 mg/kg per mouse in 0.1 mL by intraperitoneal injection). While chronic exposure to 5 μm PS microplastics did not significantly alter tumor onset or growth in FVB-Δ16HER2 mice, it was associated with a late increase in tumor multiplicity. Consistent with its known antitumor activity, trastuzumab markedly delayed tumor onset and reduced both tumor growth and multiplicity. Notably, chronic microplastic exposure substantially attenuated these therapeutic effects, indicating that 5 μm PS microplastics may promote resistance to trastuzumab treatment. At the same time 5 μm PS microplastics interfere with the effects of trastuzumab on metastasis progression. These findings suggest that chronic exposure to PS may affect trastuzumab responsiveness and metastatic progression in HER2-positive breast cancer. Further studies are needed to clarify the mechanisms involved and their impact on breast cancer progression and treatment response.

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Keywords: microplastics, FVB-Δ16HER2 mice, trastuzumab, lung metastasis.

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Immunoistochemical and Ultrastructural Characterization of Liver Alterations in PCOS and Role of Carnitine Supplementation

Silvia Zaccardi[1], Domenica Cocciolone[1], Carla Tatone[1], Giovanna Di Emidio[1], Mariacarla De Rubeis[2], Guido Macchiarelli[1], Maria Grazia Palmerini[1]

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Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting women in reproductive age, associated to infertility, insulin resistance, obesity, and cardiovascular problems. PCOS is a polygenic, multifactorial, systemic, inflammatory, and dysregulated steroid state [1]. Its etiology is still under discussion, indeed, oxidative and glycative stress have been proposed to play a central role in its pathogenesis. L-carnitine (LC) supplementation has emerged as a promising complementary therapeutic approach, together with its endogenous esters acetyl-L-carnitine (ALC) and propionyl-L-carnitine (PLC) [2]. PCOS affects mainly the reproductive organs but androgen excess may be responsible for an increased risk of liver fibrosis, steatosis, and metabolic dysfunction. We investigated immunoistochemical and ultrastructural alterations in the liver of a PCOS mouse model. PCOS was induced in CD-1 mice by subcutaneous administration of dehydroepiandrosterone (DHEA; 6 mg/100 g body weight) for 20 days. Two additional groups received oral supplementation with either LC-ALC (DHEA/LC-ALC group) or LC-ALC-PLC (DHEA/LC-ALC-PLC group); untreated mice served as controls. Liver samples were subjected to histological, immunohistochemical and ultrastructural analyses to evaluate morphology, fibrosis, inflammation, oxidative and glycative stress, apoptosis, glycogen distribution and ultrastructural organization. In the previous analysis, histological examinations (H&E and Azan-Mallory staining) revealed hepatocyte swelling, cellular hypertrophy and fibrosis in mice treated with DHEA. The increase in hepatic inflammation was confirmed by a greater IL-1β immunoreactivity, whilst the elevated expression of MG-AGE and HNE indicated glycative and oxidative damage, respectively. Both carnitine-based formulations improved liver morphology and reduced markers of inflammatory, glycative and oxidative stress, particularly the formulation containing LC-ALC. ApopTag analysis demonstrated increased apoptotic activity in the liver of mice with PCOS, whilst treatment with LC-ALC markedly reduced the presence of apoptotic cells. PAS staining revealed cytoplasmic accumulation of hepatic glycogen in the animals treated with DHEA, with the most marked recovery observed in the LC-ALC group. Meanwhile, preliminary transmission electron microscopy (TEM) analyses showed mitochondrial alterations and an enlargement of the Disse space in DHEA-treated mice compared to controls, supporting the presence of early ultrastructural liver damage associated with PCOS. Ultrastructural analyses of the other groups are currently underway. Overall, these results indicate that L-carnitine supplementation exerts protective effects against PCOS-associated liver damage and suggest that treatment with LC-ALC may be effective in mitigating liver apoptosis and metabolic alterations.

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Keywords: PCOS, L-Carnitine, Liver Damage, Oxidative Stress, Apoptosis, Ultrastructure.

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Morphological and ultrastructural characterisation of human liver clots

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The liver clot, also termed currant jelly clot, is an uncommon postoperative complication of oral surgery presenting as a dark red, gelatinous, protruding mass; detailed histological and ultrastructural data are scarce and largely confined to single cases. The morphology of seven liver clots retrieved after tooth extraction from seven consecutive patients with no known systemic disease was characterised (a boy aged 7 years, a girl aged 8 years and five adults aged 52 to 72 years); paediatric extractions involved deciduous teeth removed for orthodontic indications. Specimens were processed in parallel by light microscopy and field-emission scanning electron microscopy. Light microscopy showed biconvex, non-adherent, erythrocyte-rich coagula with scant eosinophilic fibrin-like material, no morphologically evident platelet-rich aggregates on haematoxylin and eosin, and a thin peripheral fibrin-like rim (20 to 40 μm). Scanning electron microscopy revealed a disorganised fibrin network with thin filaments (100 to 200 nm), wide interfibrillar spacing (1 to 3 μm), heterogeneous erythrocyte degradation and adherent deposits putatively attributable to salivary debris and/or microbial material. The pattern is compatible with a structurally immature, erythrocyte-dominant coagulum in which clot stabilisation appears incomplete. Reproducibility of the pattern across adult and paediatric patients without known systemic disease is consistent with a significant contribution of local oral factors. Recognising the liver clot as a morphological correlate of incomplete clot maturation supports conservative management and assists differentiation from reactive vascular lesions.

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Keywords: Liver clot, Currant jelly clot, Postoperative haemorrhage, Fibrin, Tooth extraction, Scanning electron microscopy.

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Functional and Structural Brain Plasticity Across Animal Models of Neuropathic Pain

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Neuropathic pain (NP) is a severe and disabling condition affecting millions worldwide, characterized by allodynia, dysesthesia, and hyperalgesia [1]. The transition from acute to chronic pain is a devastating process involving complex central nervous system (CNS) adaptations that remain poorly understood [2]. This study aimed to characterize the longitudinal evolution of structural and functional plasticity in key supraspinal networks during chronic pain development [3]. We employed two validated rat models of neuropathic pain: Sciatic Nerve Ligature (SNL) and Bortezomib-induced peripheral neuropathy (BIPN). Female Wistar rats were divided into SNL and BIPN groups, along with respective controls. Behavioral tests, including mechanical withdrawal thresholds and thermal withdrawal latencies, were measured weekly. Free-moving behaviours were assessed in an open field arena. To evaluate functional plasticity, Multielectrode Arrays (MEAs) were surgically implanted in the medial Prefrontal Cortex (mPFC), primary somatosensory cortex (S1), ventroposterolateral thalamus (VPL), and periaqueductal gray (PAG). Both spontaneous and pain-evoked neuronal activities were recorded longitudinally. Additionally, brain tissue was processed with Golgi staining to investigate structural remodeling. Dendritic arborization and directionality were subsequently analysed by circular statistic. The results indicated that both SNL and BIPN models showed mechanical allodynia, thermal hyperalgesia and gait impairments. Moreover, NP rats showed increased neuronal firing in pain-related brain regions both at rest and during tactile and thermal stimulation. The neurite analysis at the Golgi staining revealed a significant rebranching of dendrites in NP models with a significant decrease in upward neurites (90 and -90 degrees) in favor of lateral directions (-45, 0 and 45 degrees). These findings indicate distinct, time-dependent modifications in supraspinal firing patterns and dendritic morphology across pain etiologies. These results provide novel insights into the neuroplastic substrates driving pain chronification, potentially highlighting new targets for therapeutic intervention in chronic pain. *This work was funded by Italian Ministry of University and Research, PRIN PNRR project ID P2022C5P3F.*

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Keywords: Neuropathic pain, brain plasticity, rat models, multielectrode array, Golgi staining.

IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

Brain-adipose tissue crosstalk: Leptin indirectly stimulates oxytocin release by the supraoptic nucleus of the hypothalamus

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Oxytocin (Oxt) is a hormone produced by the Paraventricular (PVN) and Supraoptic (SON) nuclei of the hypothalamus, known to partly mediate the central anorexigenic effect of the adipokine leptin (Lep) [1] and to promote adipose tissue (AT) lipolysis [2]. Our aim is to characterize the neuroendocrinological path- way underlying the interaction between Lep and Oxt neurons, combining structural, morphological, and functional approaches to understand whether Oxt neurons directly or indirectly respond to Lep by inducing Oxt release into plasma circulation. We hence intra- peritoneally injected saline or Lep (0.5 μg/mg) in 8 weeks-old C57BL/6 mice and found that Lep activates c-Fos mainly in SON-Oxt neurons. Importantly, Lep administration resulted in increased Oxt plasma levels in a sex-dependent manner, with an earlier Oxt secre- tion in males compared to females, respectively 1.5- and 6-hours post-injection. Furthermore, Lep also in- creased the expression levels of Oxt receptor in the visceral white AT, suggesting that Lep induces Oxt release in circulation to target AT. Ex-vivo calcium (Ca2+) imaging revealed that Lep is not able to direct- ly stimulate Ca2+ signalling in SON, finding that, to- gether with the lack of Lep receptor in Oxt neurons by RNA-Scope, suggests an indirect action of Lep on these neurons. Confocal microscopy on C57BL/6J-Tg (Pomc-EGFP)1Low/J mice revealed that proopiomelanocortin neurons, one of the main neuronal populations targeted by Lep [3], abundantly innervate SON-Oxt neurons and form glutamatergic synaptic-like contacts in Oxt-immunoreactive projections. The latter observation was further confirmed by the presence of asymmetric, putatively glutamatergic, synapses on Oxt dendrites by immuno-electron microscopy. In conclusion, we provided a novel morpho-functional basis for a Lep-dependent, sexually dimorphic Oxt secretory pathway, potentially involved in the regulation of the energy balance.

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Keywords: Oxt, SON, plasma, Lep, POMC, adipose tissue.

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2025

SIAI Società Italiana di Anatomia e Istologia

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI DELLA SOCIETÀ ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI) TENUTASI VENERDÌ 19 SETTEMBRE 2025 ALLE ORE 17:00 PRESSO IL COMPLESSO DIDATTICO DEL CORSO DI LAUREA IN MEDICINA E CHIRURGIA DEL POLO DI RAVENNA DELL' UNIVERSITÀ DEGLI STUDI DI BOLOGNA

In data 19 Settembre 2025, alle ore 17:00, in seconda convocazione, ha avuto luogo, presso il Complesso Didattico del Corso di Laurea in Medicina e Chirurgia del Polo di Ravenna dell'Università degli Studi di Bologna, l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia per discutere il seguente Ordine del Giorno:

1) Comunicazioni del Presidente.
2) Relazione del Tesoriere sul rendiconto finanziario dell'anno 2024 e sulla previsione finanziaria per l'anno 2026. Relazione dei Revisori dei Conti.
3) Attività dei Collegi di Anatomia Umana e di Istologia ed Embriologia Umana. Relazioni dei Presidenti o dei loro Delegati.
4) Aggiornamento sull'Italian Journal of Anatomy and Embryology.
5) Aggiornamento sul sito web della SIAI.
6) Premio alla Carriera.
7) Assegnazione Premi Ricercatori under 40.
8) Assegnazione Premi Congressuali.
9) Prossimi Congressi Nazionali SIAI: proposte temi di relazione.
10) Borsa di Studio SIAI intitolata al Prof. Giovanni Mazzotti.
11) Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
12) Commemorazione Soci Scomparsi.
13) Varie ed eventuali

Presiede la riunione il Presidente della SIAI, Prof. Lucio Ildebrando Maria Cocco; funge da Segretario Verbalizzante la Prof.ssa Gigliola Sica.

Il Presidente dichiara aperta l'Assemblea e procede alla discussione dell'Ordine del Giorno.

1) Comunicazioni del Presidente

Il Presidente comunica che:

- le Discipline Morfologiche sono definitivamente fuori dal semestre filtro per l'accesso a CdL in Medicina e Chirurgia;
- la legge per il riordino/abolizione dell'attuale ASN è in fase avanzata nelle aule parlamentari e che durante la riunione del Collegio dei Docenti di Anatomia Umana il Prof. Gaudio e il Prof. Gianfrilli riferiranno sullo stato dell'arte;
- la Socia Prof.ssa Roberta Di Pietro è stata eletta Presidente della Federazione Internazionale delle Società di Istochimica;
- il Socio Prof. Domenico Ribatti è stato nominato nell' Editorial Board della Rivista Anatomical Record.

2) Relazione del Tesoriere sul rendiconto finanziario dell'anno 2024 e sulla previsione finanziaria per l'anno 2026. Relazione dei Revisori dei Conti.

Il Presidente invita il Tesoriere uscente, Prof. G. Papaccio, a prendere la parola e ad illustrare il Bilancio Consuntivo dell'anno 2024 (**Allegato N.1**).

Il Prof. Papaccio fa presente che il suddetto Bilancio è stato inviato a tutti gli associati e che contiene tutte le indicazioni ed una piccola relazione. Illustra le voci presenti e rileva che spesso capita che alcuni dei vincitori del Premio Migliore Comunicazione Orale non richiedano in tempo utile la somma, forse perché assenti l'ultimo giorno. Pertanto, dal Bilancio Consuntivo si evince che, oltre alla somma erogata alla vincitrice dell'anno 2024, è stata versata anche quella per la vincitrice dell'anno 2023. Inoltre, fa notare che i Premi Poster, che consistono in iscrizioni ai Congressi degli anni successivi, non vengono richiesti in quanto capita spesso che i vincitori poi non si rechino ai Congressi successivi. Infine, il Prof. Papaccio fa presente che la Società è in piena solvibilità ed in ottime condizioni finanziarie e spera che lo sarà ancora in futuro. Il Presidente invita, poi, il nuovo Tesoriere, Prof. Virginia Tirino, a presentare la Previsione Finanziaria dell'anno 2026 (**Allegato N.2**). La Prof. Tirino espone le entrate e le uscite previste per l'anno 2026 per un totale di € 42.800,00 ciascuna, risultando in un bilancio in pareggio. La relazione di accompagnamento, aggiornata al mese di Agosto 2025, evidenzia una situazione finanziaria stabile con 468 Soci totali ed entrate effettive pari a € 73.777,29 superiori alle previsioni iniziali per il 2025, grazie anche a una diminuzione dei Soci morosi. La maggior

parte (280 Soci), infatti, è in regola con i pagamenti fino al 2025. Alcuni Soci (in totale 53) devono quote arretrate per più annualità. I Soci con la posizione più critica (con 3 o 4 anni di quote non pagate) sono proposti per la decadenza, come previsto dall'art. 15 dello Statuto. Il Tesoriere invita i Soci a versare le quote a partire dal mese di Gennaio. I nuovi Soci devono versare la quota solo dopo la ratifica in Assemblea e coloro che non sono regola per due anni consecutivi decadono automaticamente, come previsto dall'articolo 15 dello Statuto. Infine, conclude che l'Associazione conferma la propria solidità economica, con entrate e uscite in equilibrio e un progressivo miglioramento della gestione delle quote sociali. Questo permette di guardare con fiducia al 2026 e di continuare a sostenere le finalità statutarie con particolare attenzione alle nuove generazioni. A seguire viene letta dal Presidente la Relazione dei Revisori i Conti, che approvano i suddetti Bilanci (**Allegato N.3**). Poi, comunica i nominativi dei Soci decaduti in base all'art. 15 dello Statuto (**Allegato N.4**).

3) **Attività dei Collegi di Anatomia Umana e di Istologia ed Embriologia Umana. Relazioni dei Presidenti o dei loro Delegati.**

Il Presidente dà la parola alla Prof. Angela Di Baldassarre che riferisce le proposte relative all'attività del Collegio. In particolare, comunica che:

- è stata proposta l'organizzazione di attività di formazione sulla didattica innovativa in ambito morfologico, attraverso una serie di 3–5 webinar distribuiti nel corso dell'anno. È in corso una verifica della disponibilità dei relatori. Tra le criticità emerse, la difficoltà a raggiungere i ricercatori; a tal fine si è ipotizzato di utilizzare la mailing list della Società. È stato inoltre considerato il rilascio di un attestato ai partecipanti.
- è stato presentato il Corso "Anatomia Settoria e Virtuale: dalla Dissezione alle Tecnologie Digitali".
- si è discusso della ripresa dei gruppi di lavoro per l'organizzazione dei Sillabi, con riferimento ai corsi di Scienze motorie, Farmacia e CTF, Biologia e Biotecnologie, Professioni sanitarie.
- è stata affrontata la questione della riorganizzazione amministrativa. È stata contattata un'agenzia specializzata nella gestione di società scientifiche. È previsto l'utilizzo di un desk online con un costo di circa 500 euro, mentre i costi per iscrizione e tesseramento sarebbero circa doppi. Tale passaggio è ritenuto importante anche per la creazione di una mailing list distinta tra tesserati e ricercatori, utile alla diffusione degli eventi delle singole sedi, quali corsi di microscopia, seminari e corsi di anatomia settoriale.

A seguire il Prof. Papaccio riferisce sull'attività del Collegio dei Docenti di Istologia ed Embriologia Umana.

Egli fa presente che la Società CDIE ora conta 212 Soci e che organizza un Congresso annuale che nell'anno 2025 si è svolto a Napoli con ampia partecipazione, "invited speakers" stranieri ed elevato livello scientifico, frutto del grande impegno degli Istologi. Nell'anno 2026 il Congresso si terrà a Siena.

Nell'anno 2025 l'Assemblea ha effettuato una survey sull'importanza dell'insegnamento dell'Istologia con ottimi risultati a livello nazionale. Le Giunte si sono occupate dei vari problemi inerenti alla disciplina e dei molti cambiamenti che sono sempre proposti, forse troppi. Tutto quanto illustrato è stato accompagnato da diapositive esplicative. Il Presidente si congratula per l'attività svolta.

4) Aggiornamento sull'Italian Journal of Anatomy and Embryology.

Il Presidente chiede al Prof. Ferdinando Paternostro, Direttore Responsabile dell'Italian Journal of Anatomy and Embryology, di informare delle novità editoriali. Egli comunica che:

- è stato pubblicato il **Numero 1** dell'Italian Journal of Anatomy and Embryology per l'anno in corso;
- è già disponibile il **Supplemento n. 1**, contenente gli abstracts del Congresso**;**
- entro la fine dell'anno sarà pubblicato anche il **Numero 2** della rivista;
- nel corso dell'anno uscirà un **secondo supplemento**, curato dalla **Prof.ssa Quartu**, dedicato alla **terminologia neuroanatomica**;
- infine, i **Professori De Caro, Porzionato e Macchi** coordineranno un **numero monografico** dedicato ai rapporti tra **Anatomia Umana e Medicina Legale.**

5) Aggiornamento sul sito web della SIAI.

il Presidente informa che il sito Web della Società viene costantemente aggiornato con le ultime News, Attività, Eventi, Congressi. Purtroppo, non è stata ancora predisposta la versione in inglese.

6) Assegnazione Premio alla Carriera.

Il Presidente riferisce che il Consiglio Direttivo in data 18.09.2025 ha approvato il Verbale della Commissione per l'assegnazione del Premio alla Carriera composta dai Proff. Michelangelo Cordenonsi, Paolo Onori e Sandra Zecchi (**Allegato N.5**). Il Premio per l'anno 2025 viene attribuito alla Prof. Oriana Trubiani.

La Prof. O. Trubiani riceve la Targa e ringrazia tutto il Direttivo.

7) Assegnazione Premi Ricercatori under 40.

Il Presidente informa che il Consiglio Direttivo SIAI in data 18.09.2025 ha approvato il Verbale del Comitato Scientifico (**Allegato N.6**) per l'assegnazione dei due premi Ricercatori under 40. I premi vengono assegnati al **Dott. Iacopo Junio Valerio Branca**, Università degli Studi di Firenze, e alla **Dott.ssa Larisa Ryskalin,** Università degli Studi di Pisa. I vincitori ricevono la pergamena e ringraziano. Il premio in denaro sarà loro accreditato dal Tesoriere tramite bonifico bancario.

8) Assegnazione Premi Congressuali.

Il Presidente comunica che, a seguito di interlocuzioni via e-mail, il Direttivo ha deciso di premiare una Comunicazione per ognuna delle cinque Sessioni Giovani e che la selezione sia affidata ai Moderatori di ognuna di esse.

Quindi, i Premi relativi alle Comunicazioni delle cinque Sessioni sono stati così assegnati:

Giovani 1

Dopaminergic differentiation from the novel pluripotent cell line hGMSCs-derived iPS: a new tool for personalized regenerative medicine

Ylenia Della Rocca, Antonella Mazzone, Paolo Guglielmi, Simone Carradori, Maurizio Piattelli, Guya Diletta Marconi, Oriana Trubiani, Jacopo Pizzicannella, Francesca Diomede

Giovani 2

Unravelling axonal damage mechanisms in peripheral nerves: the role of sodium-calcium (NCX) exchanger

Paola Alberti, Chiara Invernizzi, Margherita Kraus, Alessio Malacrida, Virginia Rodriguez-Menendez, Elisa Ballarini, Sara Di Girolamo

Giovani 3

Preclinical evaluation of a small molecule inhibitor of WDR5 in facioscapulohumeral muscular dystrophy (FSHD)

Emanuele Mocciaro, Frida Karakashi, Brian Wilson, Ahmed Aman, Jai Ramnauth, David Uehling, Rima Al-Awar, Claudia Moscheni, Davide Gabellini

Giovani 4

DYRK3 regola la motilità e l'invasione delle cellule tumorali tramite fosforilazione di Liprin-α1

Martina Ramella, Daniele Brambilla, Sara Surini, Diletta Tonoli, Lucrezia Maria Ribolla, Ivan de Curtis

Giovani 5

Face-off: exploring how sex shapes facial variation in italian adults

Riccardo Solazzo, Annalisa Cappella, Alice Alderighi, Daniele Gibelli, Claudia Dolci, Chiarella Sforza

Relativamente ai Premi Poster, Il Presidente ricorda che il Consiglio Direttivo anche per la valutazione dei migliori Poster aveva nominato come Membri della Commissione i Moderatori della relativa Sessione. Inoltre, comunica che, a far data da quest'anno, i vincitori, oltre ad avere l'iscrizione gratuita al Congresso SIAI successivo, riceveranno un attestato ufficiale.

I Premi Poster sono stati così assegnati:

1.Poster - *Detection of neurodegenerative and neuroinflammatory biosignatures characterizing mild cognitive impairment condition*

Alessandra Pistilli, Anna Maria Stabile, Gabriele Di Sante, Mariangela Ruggirello, Desirée Bartolini, Mario Rende

2.Poster - *Novel insights into the molecular mechanisms of LGMDD2: role of TNPO3 in experimental cell and Zebrafish models*

Maria Teresa Rodia, Martina Fazzina, Roberta Costa, Maria Teresa Altieri, Giuseppe Sabbioni, Elisabetta D'Aversa, Giulia Breveglieri, Elia Gatto, Cristiano Bertolucci, Sara Lombardi, Matteo Bergonzoni, Raffaella Casadei, Spartaco Santi, Valentina Papa, Stefano Ratti, Giovanna Cenacchi, Monica Borgatti, Flavia Frabetti.

9) Prossimi Congressi Nazionali SIAI: proposte temi di relazione.

Il Presidente comunica che per il 2026 il Prof. Guido Macchiarelli ha confermato la disponibilità ad organizzare il 79° Congresso SIAI nella sede dell'Università degli Studi dell'Aquila, in sinergia con il programma di L'Aquila Capitale italiana della Cultura 2026.

10) Borsa di Studio SIAI intitolata al Prof. Giovanni Mazzotti.

Il Presidente dà lettura del Bando per la Borsa di Studio SIAI 2026, intitolata al Prof. Giovanni Mazzotti, già approvato dal Direttivo nella seduta del 7 Giugno u.s. (**Allegato N.7**).

Viene, poi, presentata una breve sintesi sull'attività svolta dalla Dott.ssa Mariangela Di Vincenzo, vincitrice della Borsa di Studio 2025 intitolata ai Proff. Carlo Enrico Grossi e Alessandro Moretta e usufruita presso la Division of Extracellular Matrix Biology and Regenerative Medicine, Prof. Cossu, Regno Unito.

La Dott.ssa Di Vincenzo non ha ancora completato la sua attività e sarà lieta di presentare una dettagliata relazione scientifica quando il suo lavoro sarà giunto a conclusione.

11) Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

Il Presidente presenta l'elenco dei nominativi di coloro che hanno presentato la Domanda di Ammissione alla SIAI in qualità di Soci Ordinari durante l'anno e che sono stati approvati dai vari Direttivi durante il 2025:

BASSI ANDREA
CAMPOLO FEDERICA
CAPULLI MATTIA
CHIARELLA EMANUELA
DE ANGELIS FLAVIO
GATTA VALENTINA
GIOVANNINI CATIA
LA ROSA PIERGIORGIO
LETO STEFANO
LODOLO ELISA
LOMBARDO GIORGIA PIA
LUCA TONIA
MARRAUDINO MARILENA
MOCCIARO EMANUELE
NICITA FABIANA
OXILIA GREGORIO
PONTERIO GIULIA
RAMELLA MARTINA
REHMAN AYESHA
RUCCI NADIA
SARTI GIORGIA
TASSONE ANNALISA
TITO CLAUDIA
ZARA SUSI
ZUCCHI ALICE

L'Assemblea approva i nominativi che saranno inseriti nell'elenco dei Soci a partire dal 1° Gennaio 2026.

12) Commemorazione Soci Scomparsi.

Il Prof. Carlo Dell'Orbo viene commemorato dal Prof.ssa Protasoni.
Inoltre, Il Presidente comunica di aver ricevuto prima dell'inizio della seduta la triste notizia della scomparsa in data odierna del Prof. Giovanni Tredici. Esprimendo il cordoglio dell'intera Assemblea, comunica che la commemorazione del Prof. Tredici sarà tenuta dal Prof. Guido Cavaletti in seno al prossimo Congresso che si terrà a L'Aquila nel Settembre 2026.

13) Varie ed eventuali.

Nulla da discutere.

Essendo esaurito l'OdG, il Presidente dichiara chiusa la seduta alle ore 18:30. Il presente verbale viene, letto approvato e sottoscritto seduta stante.

F.to Il Presidente
Lucio Ildebrando Maria Cocco

F.to Il Segretario Verbalizzante
Gigliola Sica

SIAI Società Italiana di Anatomia e Istologia

Bilancio consuntivo anno 2024 ALLEGATO N.1

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Quote sociali incassate nel corso dell'anno 2024 (n° 360), comprese le quote arretrate, le quote incassate non al netto ed in attesa di integrazioni e le quote non riconducibili allo stato di alcun Socio	**€ 28.800,00**	**Elenco spese per attività statutarie**	
Incasso Contributi per stampa articoli (n.34) su IJAE	**€ 9.988,72**		
		Pagamento Quote di Iscrizione al Congresso SIAI 2024 n.2 Premi migliore comunicazione anno 2023 Angelica Perna – Mariagemma Nasoni	€ 462,46
		n. 2 Premi Ricercatori under 40, anno 2024 Carolina Pellegrini – Celeste Caruso Bavisotto	€ 4.000,00
		Rimborso spese sostenute durante espletamento borsa di studio conferita ad Irene Neri nell'anno 2023	€ 130,00
		Rimborso Spese partecipanti al congresso internazionale di Anatomia Varna anno 2024 sig.ri Manna e Cerreto	€ 1.359,85
		Contributo IFAA anno 2024	€ 300,00
		Spese Bancarie (mantenimento conto corrente bancario, spese bollo e commissioni bancarie ecc.), anno 2024	€ 1.060,02
		Premio migliore comunicazione anno 2023 a Silvia Masciarelli riscossa nell'anno 2024 ed anno 2024 ad Boschetti Elisa	€ 2.000,00
		Contributo liberale società GISN anno 2024	€ 500,00
		Contributo Congresso Genova, anno 2024	€ 5.000,00
		Quota associativa EFEM, anno 2024	€ 411,20
		Spese sito web (FILARETE), anno 2024	€ 175,69
		Pagamento FUP per IJAE anno 2024	€ 10.361,50
		Elenco spese di funzionamento	
		Spese per il funzionamento del Consiglio Direttivo, anno 2024	€ 1.818,93
TOTALE DELLE ENTRATE	**€ 38.788,72**	**TOTALE DELLE USCITE**	**€ 27.579,65**

AVANZO DELL'ESERCIZIO FINANZIARIO 2024	**€ 11.209,07**		

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Saldo Conto Corrente Bancario al 31/12/2023	**€ 41.930,10**		
TOTALE SALDO FINANZIARIO AL 31/12/2023	**€ 41.930,10**		
AVANZO DELL'ESERCIZIO FINANZIARIO 2024	**€ 11.209,07**		
SALDO FINANZIARIO AL 31/12/2024	€ 53.139,17		
STANZIAMENTI IMPEGNATI AL 31/12/2024			**Euro**
Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2024			€ 6.000,00
TOTALE IMPEGNO DI SPESA			**6.000,00**
SALDO DISPONIBILE al 31.12.2024	**€ 47.139,17**		

SIAI Società Italiana di Anatomia e Istologia

Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2024

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/12/ 2024 è pari ad **€ 53.139,17**
A tale importo devono essere sottratti **€ 6.000,00** impegnati nel bilancio previsionale del 2024, ma non ancora effettivamente utilizzati alla data del 31/12/2024, per le seguenti voci di spesa:

- **Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2024: € 6.000,00;**

Pertanto l'anno 2024 si chiude con un **saldo disponibile** di **€ 47.139,17**

Durante l'anno 2024, le quote associative incassate sono state 360 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corrette, per un totale di **€ 28.800,00**, che sommate sia all'avanzo (incasso) delle quote liberali (dei contributi) per la stampa degli articoli pubblicati sull'IJAE pari ad **€ 9.988,72** sia al saldo finanziario al 31/12/2023 pari ad **€ 41.930,10**, hanno dato la disponibilità di **€ 80.718,82.**
Le entrate hanno permesso di coprire sia le spese previste che non previste, includendo i fondi impegnati e non erogati.
La rispondenza dei Soci in merito alla regolarizzazione dei pagamenti delle quote associative è stata buona tenuto anche conto che la maggioranza dei soci sono in regola con i versamenti delle quote arretrate tutte raccolte durante gli anni precedenti. Rimane ancora un piccolo numero di Soci che debbono regolarizzare la loro posizione, che è stabilmente bassa grazie alla regolarizzazione delle quote. Il Tesoriere sottolinea che il pagamento delle quote da parte dei Soci deve continuare ad essere puntuale, ad inizio di ciascun anno solare, in modo da consentire alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative.
La solidità dei conti della Società permette alla stessa di poter operare con notevoli margini di tranquiliità e di poter raggiungerre ancor maggiormente gli scopi sociali, specie nei confronti dei giovani di qualità scientifica, verso i quali è auspicabile investire maggiormente.
Nel lasciare definitivamente la Tesoreria si sottolinea che il rigore ha avuto effetti benefici e che va mantenuto insieme a quella che sarà la modulazione degli interventi che il Direttivo andrà a mettere in atto per il raggiungimento dello scopo sociale.

Il Tesoriere
Prof. Gianpaolo Papaccio

Firmato digitalmente da Gianpaolo Papaccio
Data: 03.09.2025 14:21:12 CEST

ALLEGATO N. 2

Previsione finanziaria anno 2026

SOCI NEL 2024:	**465**
SOCI NEL 2025:	**468**
SOCI ORDINARI 2025:	**449***
*compresi n. 34 nuovi Soci ratificati all' Assemblea di settembre 2024	
SOCI DIMISSIONARI/CANCELLATI/DECEDUTI 2025:	**31**
SOCI EMERITI/ONORARI:	**19**

Quote Sociali anno 2025	449	€	35.920,00
Quote Sociali arretrate 2022 –2025		€	880,00
Contributi liberali per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology		€	6.000,00
Totale Entrate		**€**	**42.800.00**

USCITE

Contributo al 79° Convegno Nazionale 2026, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	18.000,00
Accantonamento per premi poster dell'anno 2026 e per premio alla migliore comunicazione orale assegnati nell'anno 2026	€	3.000,00
Accantonamento per premi SIAI (n. 2 Premi Ricercatori under 40), anno 2026	€	4.000,00
Contributo alla Firenze University Press per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology, anno 2026	€	6.000,00
Spese per sito web della Società, anno 2026	€	1.000,00
Contributo GISN anno 2026	€	500,00

Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2026	€	500,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2026	€	500,00
Spese varie (bancarie, necrologi, etc.), anno 2026	€	1.300,00
Spese impreviste, anno 2026	€	2.000,00
Totale spese per attività statutarie	***€***	***36.800,00***
Spese per il funzionamento della Segreteria, Tesoreria, Presidenza e Consiglio Direttivo	€	6.000,00
Totale spese di funzionamento	***€***	***6.000,00***
Totale Uscite	€	**42.800,00**

SIAI Società Italiana di Anatomia e Istologia

Relazione di accompagnamento alla previsione finanziaria per l'anno 2026

La chiusura del bilancio consuntivo del 2024 con un saldo disponibile di **€ 67.893,51** ha permesso al Tesoriere di sostenere alcune spese indicate nella Previsione Finanziaria del 2025.

Al 31 agosto-2025, sono state incassate 346 quote sociali comprensive di quelle relative all'anno in corso e arretrate (dal 1° settembre 2024 al 31 agosto 2025).

Al 31 agosto 2025, il totale delle entrate è pari a **€ 73.777,29** e comprende le quote riscosse finora.

Il piano previsionale del 2025 prevedeva entrate pari a **€ 41.800,00**, dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate. In particolare, in tale previsione, è stata indicata questa cifra sulla base dell'esperienza degli anni precedenti, tenuto anche conto del fatto che, grazie al monitoraggio costante ed alla decadenza dei soci non in regola per più di 2 anni consecutivi, vi è sostanziale stabilità finanziaria.

La Società conta attualmente 468 Soci, di cui 449 Soci Ordinari e 19 Soci Emeriti o Onorari (esonerati dal pagamento della quota Sociale). Nel corso del 2024 ad oggi (31 agosto 2025), n. 31 Soci sono stati cancellati poiché decaduti o hanno espresso la volontà di rassegnare le dimissioni dalla Società.

Allo stato attuale, dei 449 Soci Ordinari che sono tenuti a pagare la quota associativa:

- 3 Soci sono in regola fino al 2026;
- 280 Soci sono in regola fino al 2025;
- 112 Soci sono in regola fino al 2024, devono la quota 2025;
- 41 Soci sono in regola fino al 2023, devono le quote 2024 e 2025;
- 12 Soci sono in regola fino al 2022, devono le quote 2023, 2024 e 2025**;
- 1 Socio è in regola fino al 2021, devono le quote 2022, 2023, 2024 e 2025**.

** Sulla base dell'art. 15 dello Statuto, questi soci sono proposti per la decadenza alla Assemblea.

Il Tesoriere fa presente che la parità di bilancio potrà essere raggiunta con spese contenute e che le previsioni non si discostano dalla realtà.

Il Tesoriere fa inoltre presente che, essendo di molto diminuiti i Soci morosi, l'ammontare delle quote arretrate si è considerevolmente ristretto, per cui la Società è in grado di raggiungere gli scopi sociali ed aumentare le liberalità in favore dei giovani.

Il Tesoriere
Prof. Virginia Tirino

VIRGINIA TIRINO
01.09.2025
14:23:28
GMT+02:00

ALLEGATO N.3

Ravenna, 19.09.2025

Il giorno 19.09.2025 alle ore 13.15, presso il Polo Didattico della Facoltà di Medicina e Chirurgia dell'Università di Bologna, Sede di Ravenna, viale Randi 5, Ravenna, i Revisori dei Conti dei Bilanci della SIAI, nelle persone dei proff. Guido Carpino, Matilde Y. Follo e Maria Zingariello, si sono riuniti telematicamente per esaminare i documenti ricevuti in data 01.09.2025 e 04.09.2025 via mail. Dopo attento esame, i proff. Carpino, Follo e Zingariello hanno ritenuto approvare senza riserve i documenti così come inviati.

La riunione termina alle ore 13.45.

Prof. Guido Carpino

Prof.ssa Matilde Y. Follo

Prof.ssa Maria Zingariello

ALLEGATO N.4

N.	Elenco Soci decaduti 19 Settembre 2025
1	Crescimanno Caterina
2	Conconi Mariateresa
3	Girolamo Francesco
4	Guizzardi Stefano
5	Marini Mirca
6	Moretti Matteo
7	Noli Barbara
8	Piras Franca
9	Tamma Roberto

ALLEGATO N. 5

2 settembre 2025

Al Presidente della Societa' Italiana di Anatomia ed Istologia (SIAI)

In data 2 settembre 2025 alle ore 15:30, per via telematica, si è riunita la Commissione istituita dal Consiglio Direttivo della SIAI per l'attribuzione del premio alla carriera, nominata dal Consiglio Direttivo del 7 giugno 2025.

La Commissione è composta dalla prof.ssa Sandra Zecchi Orlandini e dai professori Michelangelo Cordenonsi e Paolo Onori. I membri della Commissione hanno ricevuto la documentazione in data 26 agosto dal prof. Lucio Ildebrando Cocco, Presidente della Società. Tale documentazione consiste in 2 documenti:

a) Proposta di conferimento del premio alla prof.ssa Oriana Trubiani, già ordinario di Istologia ed Embriologia Umana nell'Ateneo di Chieti-Pescara, da parte dei colleghi prof. Gianpaolo Papaccio e prof.ssa Monica Mattioli Belmonte;
b) CV della prof.ssa Oriana Trubiani.

A seguito di un'attenta valutazione del CV della candidata, la Commissione ha verificato la presenza dei requisiti previsti dal regolamento riconoscendo il rilevante valore scientifico e didattico del suo contributo nel campo delle discipline morfologiche. La prof.ssa Trubiani ha inoltre ricoperto ruoli istituzionali di rilievo e ha partecipato con impegno e dedizione alle attività organizzative della SIAI.

Considerando il suo rilevante operato, la Commissione propone all'unanimità di conferire alla prof.ssa Oriana Trubiani il "*Premio alla Carriera SIAI*".

La Commissione conclude i lavori alle ore 16:30 e redatto il presente verbale lo trasmette al Presidente della SIAI.

Letto, approvato e sottoscritto seduta stante

Sandra Zecchi Orlandini

Michelangelo Cordenonsi

Paolo Onori

ALLEGATO N. 6

VERBALE DELLE RIUNIONI DEL COMITATO SCIENTIFICO PER L'ATTRIBUZIONE DI NUMERO 2 PREMI AI RICERCATORI "UNDER 40"

Il Comitato Scientifico, nelle persone di:

Prof.ssa Emanuela Marcenaro (in qualità di Presidente)
Prof.ssa Bianca Maria Scicchitano (in qualità di Componente)
Prof.ssa Matilde Yung Follo (in qualità di Componente)
Prof. Antonio De Luca (in qualità di Componente)
Prof. Guido Carpino (in qualità di Segretario)

si è riunito telematicamente (via TEAMS) venerdì 1° agosto 2025 per valutare le Candidature per l'assegnazione dei 2 Premi ai ricercatori "under 40".

Sono pervenute n. 3 Candidature nelle persone di:

Dott. Jacopo Junio Valerio Branca (presentato dal Prof. Massimo Gulisano)
Dott.ssa Maria Vittoria Marvi (presentata dal Prof. Stefano Ratti)
Dott.ssa Larisa Ryskalin (presentata dal Prof. Marco Gesi)

Il Comitato ha dapprima valutato la posizione dei Candidati rispetto al pagamento delle quote di iscrizione alla SIAI e ha rilevato che tutti i Candidati sono in regola.

Successivamente, il Comitato ha valutato attentamente i curricula dei Candidati, le loro pubblicazioni selezionate, gli indici bibliometrici specificati nel Bando, le attività in qualità di PI in progetti di ricerca nazionali e/o internazionali che prevedano la revisione tra pari; gli eventuali brevetti accettati almeno in EUROPA (PCT); ed eventuali altri titoli di qualità e non di quantità.

Alla fine di tale valutazione, il Comitato propone di assegnare all'unanimità i 2 Premi a:

Dott. Jacopo Junio Valerio Branca, la cui attività di ricerca è incentrata sulla caratterizzazione morfologica dell'apparato muscolo-scheletrico e sull'analisi dei sistemi di regolazione neuromuscolari, sia in condizioni fisiologiche che patologiche.

Dott.ssa Larisa Ryskalin, la cui ricerca si focalizza principalmente sui meccanismi di regolazione neuromuscolare, con particolare attenzione ai processi degenerativi correlati.

Addì, 01 agosto 2025

Prof.ssa Emanuela Marcenaro Marcenaro

Prof.ssa Bianca Maria Scicchitano

Prof.ssa Matilde Yung Follo

Prof. Antonio De Luca Antonio De Luca

Prof. Guido Carpino Guido Carpino

ALLEGATO N.7

BANDO PER BORSA DI STUDIO DELLA SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI)

Articolo 1
Tema dell'iniziativa
La **Società Italiana di Anatomia ed Istologia (SIAI)** ha istituito **nel 2022** una **Borsa di Studio** del **valore di Euro 10.000,00 di durata semestrale, da svolgersi presso prestigiose Istituzioni estere. La SIAI garantirà inoltre una indennità di viaggio di importo non superiore ad Euro 1.000,00 a fronte della esibizione dei titoli di viaggio. La prima edizione della Borsa è stata intitolata al Prof. Francesco Antonio Manzoli, la seconda al Prof. Giovanni Orlandini, la terza ai Proff. Carlo Enrico Grossi e Alessandro Moretta. La quarta, a far data dal 1° Febbraio 2026, sarà intitolata al Prof. Giovanni Mazzotti.**
Possono partecipare candidati che posseggano i requisiti esposti nell'**Articolo 2**.
Le tematiche di ricerca, che faranno parte del progetto presentato da ciascun candidato, dovranno essere coerenti con quelle delle discipline che costituiscono la Società Scientifica (Anatomia e Istologia ed Embriologia Umana). **Qualora vengano prodotte pubblicazioni scientifiche nell'ambito del progetto, sarà obbligatorio citare la SIAI nei ringraziamenti.**

Articolo 2
Requisiti per la partecipazione
Possono partecipare alla selezione:
- i dottorandi di ricerca iscritti a Dottorati e con Tutor iscritto alla SIAI;
-gli assegnisti di ricerca;
- i dottori di ricerca con titolo conseguito in Dottorati e con Tutor iscritto alla SIAI;
-i borsisti;
-i ricercatori di tipologia RTD-A, RTT.

I candidati debbono inoltre possedere i seguenti requisiti:
-essere iscritti alla SIAI ed essere in regola con i pagamenti della quota sociale sino all'anno di emissione del bando;
-in alternativa, essere stati presentati come nuovi Soci SIAI e approvati dall'Assemblea Generale dei Soci;
-avere una età inferiore ai 40 anni al momento della scadenza del bando (**15 Novembre 2025**).
-frequentare una struttura universitaria sotto la responsabilità di un Socio SIAI.

Ciascun candidato deve inoltrare formale lettera di richiesta, con allegato CV in formato europeo, corredato **dalle pubblicazioni.**

La **richiesta** deve contenere le seguenti informazioni:

- cognome e nome del candidato;
- paese di residenza;
- cittadinanza;
- data di nascita;
- indirizzo e-mail;

- numero di telefono;
- un dettagliato programma di ricerca che si intende effettuare utilizzando anche (e non soltanto) le risorse di cui alla Borsa;
- **lettera formale di accettazione** da parte della Istituzione **ospitante.**

Articolo 3
Esclusione
-Non possono partecipare **al bando** familiari entro il IV grado dei membri del **Consiglio Direttivo** della SIAI;
-I candidati che non posseggano i requisiti esposti nell'Articolo 2.

Articolo 4
Assegnazione della Borsa di Studio
La Borsa di Studio sarà assegnata a seguito di un **procedimento** di selezione effettuato dal **Comitato Scientifico della SIAI**.
Il Comitato selezionerà e classificherà le candidature pervenute via mail all'indirizzo segreteria.siai@unicatt.it indicando nell'oggetto "Candidatura Borsa Studio" e allegando tutta la documentazione richiesta entro il **15 Novembre 2025.**
Saranno accettate unicamente le candidature inviate alla suddetta mail ed entro la data suindicata.

Articolo 5
Tempistiche di assegnazione della Borsa di Studio e modalità di comunicazione
Il Comitato Scientifico si impegna a comunicare il nominativo del candidato selezionato entro e non oltre il **31.12.2025** sul sito www.siaionline.it e tramite indirizzo di posta elettronica (indicato nella candidatura) alla persona selezionata.
Qualora il vincitore risultasse impossibilitato ad accettare la Borsa di Studio, essa verrà assegnata ad altro candidato secondo l'ordine di classifica stabilito dal **Comitato Scientifico**.

Articolo 6
Relazione finale sull'attività svolta
Il vincitore della Borsa di Studio è tenuto a presentare entro e non oltre tre mesi dal termine della missione di ricerca, sia una dettagliata relazione delle attività svolte sia un consuntivo delle spese di viaggio sostenute.
Qualora le ricerche svolte siano pubblicate su riviste scientifiche, l'articolo dovrà riportare nei ringraziamenti la seguente dicitura: "Lavoro svolto con il contributo della Società Italiana di Anatomia e Istologia (SIAI) attribuito al Dr. (nome del borsista) quale vincitore della Borsa di Studio SIAI intitolata al Prof. Giovanni Mazzotti".

IJAE
Italian Journal of Anatomy and Embryology
Vol. 130, N. 1 – 2026 Supplement