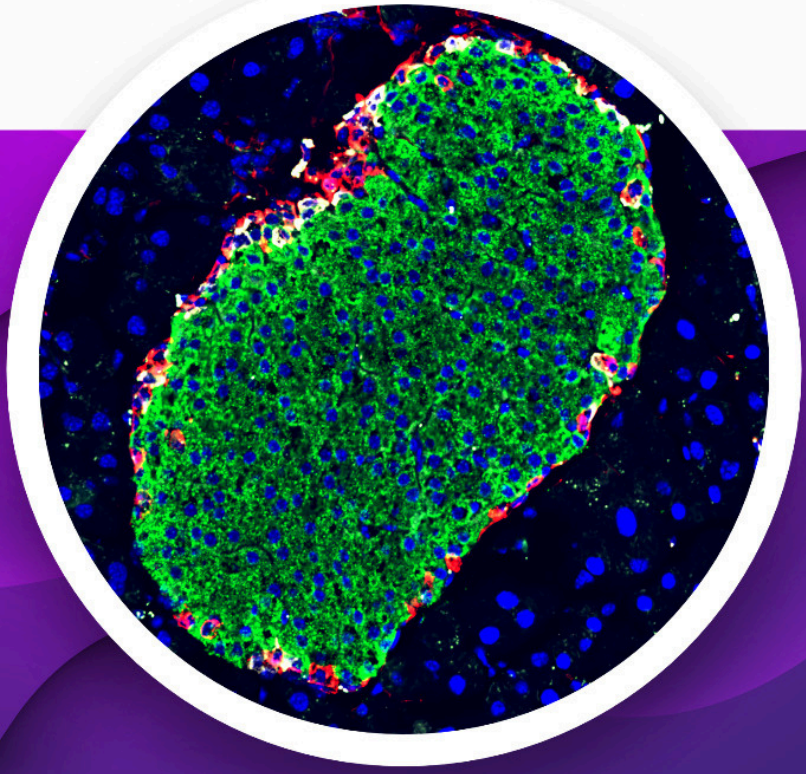
ACADEMIA ROMÂNĂ

BULLETIN OF THE

ROMANIAN SOCIETY FOR CELL BIOLOGY

42nd Annual Scientific Session of the Romanian Society for Cell Biology



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INTRODUCTION

Acad. Maya Simionescu

Motto:
"Together we find ideas... and good ideas change the world"

I am quite pleased to present this edition of our Society's Bulletin, which contains the summaries of the presentations held at the 42nd Annual International Scientific Session of the Romanian Society for Cell Biology (RSCB). The conference takes place between November 20-21, 2025 at the Institute of Cellular Biology and Pathology "Nicolae Simionescu" (ICBP-NS), Bucharest, under the auspices of the Romanian Academy.

You will find gathered in this collection of summaries the ideas, work, and results of numerous researchers in the field, from the country and from abroad, whose efforts have become original scientific works that push the limits of knowledge and contribute to our understanding of the biological world.

Nowadays, cellular and molecular biology are undergoing rapid transformations. The abstracts in this volume capture the essence of this dynamic landscape, presenting research that covers a broad spectrum of biological subdisciplines.

My full appreciation to the researchers behind these abstracts. I know that it is not easy to do high-performance research in general and in our field in particular. And yet these researchers have proven that it is possible!

I hope that this book of abstracts will be a source of ideas, will inspires curiosity and will contributes to the ongoing dialogue that propels cellular and molecular biology research.

Every year, on this conference, we remember with reverence our great ancestors, courageous scientists, who, in 1982, joined the vision of the founder

of the RSCB - Prof. Nicolae Simionescu - and inaugurated the "Romanian Society for Cell Biology". Since then, until today, the researchers from the whole country have contributed to maintaining a true scientific life of the Society.

An example is shown in this Book of Abstracts of the 2025 RSCB Annual Scientific Session.

Appreciation and thanks to the Arad Branch of the RSCB, to Prof. Anca Hermenean and her collaborators, who kindly helped and contributed to the successful editing of this volume.

Acad. Maya Simionescu
President of the Romanian Society for Cell Biology

PROGRAM

INTERNATIONAL CONFERENCE
42nd ANNUAL SCIENTIFIC SESSION
OF THE ROMANIAN SOCIETY FOR CELL BIOLOGY

A hybrid event organized online and onsite
under the aegis of the Romanian Academy
by
Institute of Cellular Biology and Pathology "Nicolae Simionescu"
Bucharest branch of Romanian Society for Cell Biology (RSCB)
and
Section of Biological Sciences of the Romanian Academy

Dedicated to the Researcher's and Designer's Day

20-21 November 2025

ORGANIZING AND SCIENTIFIC COMMITTEE

Acad. Maya SIMIONESCU, President of RSCB
Dr. Anca V. GAFENCU, President of Bucharest RSCB Branch
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Prof. Tudor C. BADEA, President of RSCB Brașov Branch
Assoc. Prof. Vlad-Alexandru TOMA, President of RSCB Cluj Branch
Prof. Anca HERMENEAN, President of RSCB Arad Branch

DAY ONE

THURSDAY, 20 NOVEMBER 2025

9:30 - 10:15 Opening session

- **Acad.** ***Maya SIMIONESCU*****,** President, RSCB
- **Acad.** ***Marius ANDRUH*****,** Vice-president of the Romanian Academy
- **Book launch event:** ***NICOLAE SIMIONESCU*** **–** ARHITECTUL ȘCOLII ROMÂNEȘTI DE BIOLOGIE CELULARĂ, Ion Longin Popescu

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Chair: Acad. ***Maya SIMIONESCU***

Keynote lecture

- **Prof**. ***Kyriakos KYPREOS***, ICBP-NS, Bucharest and University of Patras, Greece - Unmet Cardiovascular Risk beyond LDL-C: Insights into Contributing Factors

10:45 - 11:00 Coffee break

11:00 - 12:00 Scientific Session 2

Chairs: Prof. ***Carmen E. COTRUTZ*** and **Assoc. Prof.** ***Vlad-Alexandru TOMA***

Round Table - Pathophysiology and Molecular Interactions

- ***Darius D. RUS***, Babes-Bolyai University, Cluj-Napoca - Non-Enzymatic Sugar Association Leads to Structural and Functional Modification in Haemoproteins
- ***Marta ANATOLIE***, Institute of Animal Physiology and Genetics, Czech Academy of Science, Libechov, Czech Republic - From Endomitosis to Aberrant Chromosome Pairing: Cellular Mechanisms Driving Clonal Reproduction and Male Sterility in Fish Hybrid Complexes
- ***Doinița TEMELIE-OLINICI***, "Grigore T. Popa" University of Medicine and Pharmacy Iași - The Role of IL-6 in Activating the "Molecular Scar" of Psoriasis
- ***Kriti PATHAK***, Promega GmbH - NanoLuc® Luciferase: A New Era of Gene and Protein Reporter Technologies

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13:00 - 14:30 Scientific Session 3

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Young Scientists Communications

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- ***Marius MULȚESCU***, Ph.D. student, ICBP-NS, Bucharest - Human Apolipoprotein A-II Modulates the Mitochondrial Respiration in Endothelial Cells
- ***Mihaela G. TURTOI***, Postdoc, ICBP-NS - Nanodelivery of Bioactive Natural Products to Inhibit Endothelial-to-Mesenchymal Transition: A Novel Therapeutic Strategy for Atherosclerosis
- ***Ruxandra ANTON***, Ph.D. student, ICBP-NS, Bucharest - Targeted Nanoparticles for Hsp90 Inhibitors Delivery to Fibrotic Cardiac Tissue
- ***Diana V. UȚĂ***, Ph.D. student, ICBP-NS, Bucharest - Preliminary Data Evaluating the TLR4 Inhibition as a Strategy to Mitigate Diabetic Foot Syndrome
- ***Paul-Ștefan PANAITESCU***, Ph.D. student, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca - Effect of Gut Microbiota-Derived Metabolite TMAO on Neurodegeneration, Inflammation, Oxidative Stress and Motor Function in Animal Models of Parkinson's Disease
- ***Răzvan-Geo ANTEMIE***, Ph.D. student, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca - In Vitro Evaluation of the Antioxidant Activity of Polyphenol Quinazolin-4(3h)-One and Catechol Hydrazinyl-Thiazole Derivatives on a Selection of Cell Lines
- ***Ioana REDNIC***, Ph.D. student, University of Medicine and Pharmacy of Craiova - Antioxidant and Neuroprotective Perspectives of Some Edible Fruit's Extracts Based on a Comparative Phytochemical Profiling and Bioactivity
- ***Bianca SOCEANU***, Ph.D. student, "Grigore T. Popa" University of Medicine and Pharmacy Iași - The Role of Bioactive Molecular Components of Human Milk in Regulating Cellular Processes

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- ***Elena M. LAMBĂ*** et al., ICBP-NS, Bucharest - Sweet Habits: Shaping Pancreatic β-cell Adaptation
- ***Mădălina DUMITRESCU*** et al., ICBP-NS, Bucharest - Expression of Apolipoprotein E4 by Adenovirus-Mediated Gene Transfer to ApoE Deficient Mice Modulates the Properties of HDL in a Dose Dependent Way
- ***Ioana M. FENYO*** et al., ICBP-NS, Bucharest - Apolipoprotein A1 is involved in glucose homeostasis
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- ***Claudia-Andreea MOLDOVEANU*** et al., Babes-Bolyai University, Cluj-Napoca - Matrix Metalloproteinases as Early Biomarkers in Parkinson's Disease
- ***Mihaela BALAȘ*** et al., Faculty of Biology, University of Bucharest - Exploring the Biological Effects of $EuTiO_2$ Nanoscintillator-Mediated Radio-Photodynamic Therapy in Breast Cancer Cells

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- **Sonia VLAICU**, University of Maryland School of Medicine, Baltimore, USA - The Role of Response Gene to Complement-32 in Atherogenesis
- **Alexandru TATOMIR**, University of Maryland School of Medicine, Baltimore, USA - RGC-32 as a new factor regulating reactive astrocytosis in neuroinflammation

- **Horea RUS**, University of Maryland School of Medicine, Baltimore, USA - RGC-32 is a Biomarker of Disease Activity and Treatment Response in Multiple Sclerosis

DAY TWO

FRIDAY, 21 NOVEMBER 2025

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- ***Sergiu-Marian VATAMANU*** et al., ICBP-NS, Bucharest - Establishment and Characterization of Human Gingival Fibroblast Cultures
- ***Ana-Maria ROŞCA*** et al., ICBP-NS, Bucharest - Composites Based on Collagen, Chondroitin Sulfate, and Sage Oil with Potential Use in Dentistry
- ***Daniela-Mădălina GHEȚU*** et al., ICBP-NS, Bucharest - Phenoxazine Counteracts High-Glucose-Induced Alterations of Human Mitochondrial Genes: Results on Cardiac Organoids

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10:30 - 11:45 Scientific Session 8

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- ***Anca-Lelia RIZA*** and ***Mihai G. NETEA***, University of Medicine and Pharmacy of Craiova - Functional Genomics in Sepsis

- ***Daniel PIRICI***, University of Medicine and Pharmacy of Craiova - Water channel aquaporin 4 modulates amyloid deposits and cognitive performance in a transgenic animal model for Alzheimer's disease
- ***Adrian FRANTEA***, University of Medicine and Pharmacy of Craiova - Dual Transcriptome –Proteome Analysis Reveals Distinct Immune Endotypes in Sepsis
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Keynote lecture
- **Prof. *Anca D. DOBRIAN***, Department of Biomedical and Translational Sciences, Eastern Virginia Medical School at Old Dominion University, Norfolk, VA USA- Extracellular Vesicles: from cellular sentinels to mediators of the biology of adversity

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- ***Cătălina MARINESCU-COLAN*** et al., ICBP-NS, Bucharest - From Cancer to Infarcted Hearts: GLIPR1 as a Detrimental Regulator in Activated Cells
- ***Gabriela TANKO*** et al., ICBP-NS, Bucharest - An R Shiny-Based Platform for Automated Quantitative Analysis of Insulin Granule on Electron Micrographs
- ***Alexandra VÎLCU*** et al., ICBP-NS, Bucharest - Exploring the Role of Mitochondrial Dysfunction in Cardiac Hypertrophy in an Experimental Model of Atherosclerotic Cardiovascular Disease; Evaluation of the Therapeutic Potential of MTP-131

12:50 - 13:00 Coffee break

13:00 - 13:30 General Assembly of RSCB members

- 2025 Reports of RSCB Branches
- 2025 Financial Report for RSCB
- Proposals for 2026 Scientific Meeting of RSCB
- Varia Topics
- Final Conclusions and Remarks: Presidents of RSCB Branches

Unmet Cardiovascular Risk beyond LDL-C: Insights into Contributing Factors

Kyriakos E. KYPREOS

Institute of Cellular Biology and Pathology "Nicolae Simionescu" of the Romanian Academy, Bucharest, Romania
Pharmacology Laboratory Department of Medicine, University of Patras, Rio Achaias, Greece

Numerous clinical and epidemiological studies identified elevated plasma low density lipoprotein cholesterol (LDL-C) as a critical factor contributing to the development and progression of atherosclerotic cardiovascular disease (ASCVD). As a result, different pharmacological agents aiming at reducing LDL-C levels by various mechanisms have contributed significantly to the reduction of morbidity and mortality associated with atherosclerosis. However, despite the clear benefit, clinical studies involving LDL-C lowering agents revealed the existence of residual cardiovascular risk even at very low LDL-C levels. Estimates vary across studies, but it is generally accepted that at least one in three patients attaining LDL-C targets remain at elevated risk.

Beyond LDL-C, additional factors—including high-density lipoprotein (HDL), apolipoprotein B (APOB)-containing triglyceride-rich lipoproteins (TRLs), and systemic inflammation—contribute to residual risk. The relationship between HDL and residual risk exhibits a U-shaped curve, largely influenced by particle subtypes within the HDL density spectrum. Although elevated triglycerides have been associated with atherosclerosis in some studies, interventions lowering triglyceride levels have not consistently conferred atheroprotection, suggesting that APOB particle number and size, rather than triglyceride content per se, are key determinants of atherogenesis. The contribution of lipoprotein(a) [Lp(a)] to residual cardiovascular risk remains to be definitively established. Surprisingly, anti-inflammatory treatment reducing IL-1β reduced ASCVD-related mortality independently of plasma LDL-C levels, highlighting the importance of inflammation in the disease.

Collectively, atherosclerosis is a multifactorial chronic disease, and effective risk mitigation necessitates a comprehensive and mechanistically informed approach. A precise understanding of the most relevant targets and intervention strategies is critical to fully abrogate ASCVD risk.

Extracellular Vesicles: from Cellular Sentinels to Mediators of the Biology of Adversity

Anca D. DOBRIAN, ChE MS PhD FAHA

Professor, Department of Biomedical and Translational Sciences,
Eastern Virginia Medical School at Old Dominion University, Norfolk, VA USA

Extracellular vesicles (EV) act as integrators of metabolic, psychological and social stress that transform endothelial plasticity from an adaptive mechanism into a driver of cardiovascular pathology. Central to this transformation is the recognition that EVs function as intercellular communication vehicles, encoding the physiological state of parent cells within their molecular cargo and transmitting these signals to distant vascular beds.

The endothelium, positioned at the interface between blood and tissue, serves both as a primary producer and principal recipient of circulating EVs. Under homeostatic conditions, endothelial EVs carry cargo such as stabilizing miRNAs and pro-angiogenic proteins that support vascular repair, maintain barrier integrity through VE-cadherin preservation, and facilitate adaptive responses to injury. This represents intercellular communication as a protective mechanism, with EVs serving as molecular couriers that coordinate vascular homeostasis across anatomically distant sites.

Chronic exposure to inflammatory or metabolic stress fundamentally corrupts this communication network. When endothelial cells undergo endothelial-to-mesenchymal transition (EndMT) in response to sustained inflammation, they produce EVs with altered molecular signatures enriched in pathogenic miRNAs and TWIST1-regulated cargo. These stress-conditioned EVs become vehicles of dysfunction: they impair angiogenic responses in coronary endothelium, disrupt barrier integrity, suppress proliferation, and perpetuate inflammatory signaling in recipient cells. Our studies demonstrate that autologous EVs from inflamed endothelium reduce barrier function in healthy endothelial

monolayers, while EVs from non-inflamed cells protect against injury, illustrating how the same communication pathway can either maintain health or propagate disease depending on the message encoded.

Our translational work reveals that social determinants of health such as neighborhood socioeconomic deprivation and social isolation correlate with circulating EVs carrying distinct miRNA signatures that support endothelial dysfunction. Women experiencing chronic psychosocial stress produce EVs that reflect sustained neuroendocrine activation and inflammatory signaling. When these EVs encounter vascular endothelium, they transmit the molecular signatures of social adversity, compromising barrier function and accelerating atherosclerotic processes. Physical activity modulates but cannot fully reverse these pathogenic EV signatures, suggesting that biological embedding of chronic stress operates through sustained alterations in intercellular communication.

The pathogenic reach of endothelial EVs extends beyond cardiovascular disease. Obesity-associated inflammation drives adipose endothelial cells into EndMT, generating EVs that promote epithelial-to-mesenchymal transition in prostate cancer cells, enhancing invasion and metastatic potential. This reveals EVs as systemic messengers that transmit inflammatory and metabolic stress signals across tissue compartments, linking apparently disparate pathologies through shared communication pathways.

Non-Enzymatic Sugar Association Leads to Structural and Functional Modification in Haemoproteins

Darius Daniel RUS[1], Claudia-Andreea MOLDOVEANU[1,2], Vlad COJOCARU[1,3], Alina FILIP[4], Stefania IANCU[5], Nino MARQUÉ [2,6], Vlad-Alexandru TOMA [7,2*]

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Increasing evidence suggest that protein glycation (non-enzymatic post translational modification) could have important roles in the etiology, installation and development of numerous pathologies, including metabolic diseases, cardiovascular and neurodegenerative illnesses[1]. Here, we uncover a plethora of structural and incipient functional modifications of proteins subjected to *in vitro* glycation.

Cytochrome C and monomeric haemoglobin (PDB accession numbers 5TY3, 1ECA) incubated with 40 mM sugar solutions (glucose, galactose, fructose and ribose) at 37 ^{o}C for 3 days showed distinct bands following SDS-PAGE, and Soret band shifts when analyzed by UV-vis spectroscopy. This pointed to specific structural changes following glycation. Thus, we further investigated structural modifications using Raman, FTIR, and fluorescence spectroscopy, and compared this to human tetrameric glycated haemoglobin standards (Roche Diagnostics USA) used in clinical diagnostics. Following these investigations, substantial evidence points to

haem specific and peptide conformation alterations, but also sugar-protein associations, confirming structural changes due to glycation of these proteins. We also carried out kinetic analysis to unveil the impact of glycation on the functionality of haemoproteins by capitalizing on their peroxidase activity.

We conclude that small proteins, specifically haemoproteins, are viable targets for glycation. CytC seems to be the most sensitive to glycation, this could be due to its less compact structure but also to the numerically elevated Arg/Lys (target) residues. Thus, glycation is partly dictated by accessibility and presence of glycatable targets. Structural and functional modifications could contribute to the instalment and development of various diseases.

This research received support from the AOSR-TE-2024-2025 research grant and the PN-IV-P7-7.1-PED-2024-0585 project.

Keywords: Glycation; Haemoproteins

References

Fournet, M., Bonté, F., & Desmoulière, A. (2018). Glycation damage: a possible hub for major pathophysiological disorders and aging. Aging and disease, 9(5), 880.

From Endomitosis to Aberrant Chromosome Pairing: Cellular Mechanisms Driving Clonal Reproduction and Male Sterility in Fish Hybrid Complexes

Marta ANATOLIE [1,2], Dmitrij DEDUKH[1], Karel JANKO[1]

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Background: Sexual reproduction through meiosis, producing recombinant gametes, is a fundamental and widespread feature of eukaryotes. Although the underlying molecular mechanisms are highly conserved, they are often disrupted in hybrids. While hybridization typically leads to sterility, some taxa overcome this through cellular mechanisms that enable asexual reproduction. Freshwater fishes of the *Cobitis taenia* hybrid complex provide an excellent model for studying hybrid sterility, clonal reproduction, and the evolutionary significance of clonal lineages.

Hypothesis: Male sterility likely arises from genomic incompatibilities during gametogenesis, whereas fertility in hybrid females is restored either through premeiotic genome duplication or by suppression of the first meiotic division. Aim: We investigate the meiotic pathways of diploid and triploid hybrid males and females in comparison with their parental species, focusing on the cellular mechanisms that drive asexual reproduction and hybrid sterility. Methodology: We applied a comprehensive cytogenetic toolbox combined with molecular genetics and immunofluorescence to examine different stages of gametogenesis in naturally established clonal lineages and laboratory F_1 hybrids.

Results: Sterility in male hybrids arises from aberrant chromosomal pairing and incomplete synaptonemal complex formation, leading to meiotic arrest. Increasing ploidy, as observed in triploid males, can partially improve chromosomal pairing but does not restore fertility. In

diploid and triploid female hybrids, only a small fraction of gonial cells undergo premeiotic genome duplication. While non-duplicated cells are arrested due to pairing failures, fertility in duplicated cells is restored through **sister-chromatid pairing** rather than homologous chromosome pairing. This mechanism enables the proper assembly of synaptonemal complexes and completion of meiosis, allowing the production of viable clonal gametes.

Conclusion: Hybridization produces strongly sex-specific outcomes in the *Cobitis* taenia complex. In males, chromosomal incompatibilities and incomplete synaptonemal complexes lead to sterility, whereas premeiotic genome duplication in diploid and triploid females restores proper chromosomal pairing and allows meiosis to be completed, enabling clonal gamete production.

Funding: This research received support from the project PN-IV-PCB-RO-MD-2024-0238 no. 20PCBROMD since 01/09/2025 and by the Czech Science Foundation Project with No. 21-25185S.

The Role of IL-6 in Activating the "Molecular Scar" of Psoriasis

Doinița TEMELIE-OLINICI[1], Walther BILD[1,2], Cristina SAPANIUC[3], Dan VÂȚĂ[3], Laura GHEUCĂ-SOLOVĂSTRU[3]

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"Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania
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of Medicine and Pharmacy, Iasi, Romania

The biomolecular conceptualization of the etiopathogenesis of psoriasis, one of the most common chronic inflammatory dermatoses mediated by T lymphocytes, reveals the synergistic action of the main cytokine axes such as IL-23/Th17/IL-17, TNFα/NF-κB, and IL-6/STAT3 on persistent inflammation and keratinocyte proliferation. In the presence of various triggers, TLR receptors are activated, inducing the expansion of Th17 and Tγδ clones and stimulating the direct production of IL-17, as well as indirectly, mediated by IL-23 and IL-6.

The main objective of the study was to evaluate the role of IL-6 in the activation of the "molecular scar" of psoriasis by determining the serum level of IL-6 in patients with psoriasis vulgaris undergoing chronic biological treatment and correlating it with the severity of the disease and the type of biological agent.

The study included 42 patients with moderate-to-severe psoriasis vulgaris, of whom 22 (52.4%) were treated with anti-TNFα and 20 (47.6%) with anti-IL-17, monitored at the Dermatology Clinic of the "Sf. Spiridon" Emergency Clinical Hospital in Iași. Serum IL-6 levels were determined by the ECLIA (electrochemiluminescence) method on an automatic clinical immunology analyzer. Data analysis was performed by comparative evaluation of IL-6 distribution between groups, using non-

parametric tests for inter-group differences and evaluation of biological correlations with psoriasis severity.

Serum IL-6 levels increased progressively with psoriasis severity: mild forms – 3.3 pg/mL, moderate forms – 64.7 pg/mL, severe forms – 75.5 pg/mL, critical forms – 182.9 pg/mL. Patients treated with anti-TNFα had higher mean values compared to those treated with anti-IL-17, suggesting the persistence of IL-6/STAT3 axis activity despite biological therapy.

The results confirm the central role of IL-6 in perpetuating chronic inflammation and maintaining "molecular scarring" in psoriasis. The persistence of elevated levels, more pronounced in anti-TNFα patients, indicates that the IL-6/STAT3 axis remains active and represents a potential therapeutic target for complete molecular remission.

Keywords*:* IL-6; IL-17; TNFα; IL-23; molecular scarring; cytokines; psoriasis vulgaris

The Role of Response Gene to Complement-32 in Atherogenesis

Sonia I. VLAICU [1,2], Alexandru TATOMIR[2], M. FOSBRINK[2], C.A. TEGLA[2], C. CUDRICI[2], Tudor C. BADEA[3], F. NICULESCU[4], Violeta RUS[4], Horea RUS[2,5]

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[5] Veterans Administration, Maryland Health Care System, Baltimore, MD, USA

A valuable arsenal in the innate immune response, the complement system contributes to both the initiation and progression of the atherosclerotic process. A cell cycle regulatory protein induced by sublytic C5b-9, Response Gene to Complement-32 (RGC-32) is involved in immunity, oncogenesis, neurological diseases (multiple sclerosis), obesity, diabetes mellitus and arterial atherosclerotic disease. Our research focused on identifying RGC-32 expression in the atherosclerotic arterial wall and the mechanisms by which RGC-32 contributes to endothelial cell (EC) proliferation and migration and TGF-β-dependent smooth muscle cell (SMC) proliferation and differentiation in atherosclerotic arterial plaque. RGC-32 is expressed in both EC and media cells of the human atherosclerotic aortic wall, where it is found alongside SMC, other immune-inflammatory cells, and C5b-9 deposits. Immunohistochemistry data show that RGC-32 expression in human aortic atherosclerotic plaques increases with the progression of atherosclerotic lesions in the intima and media.

In vitro experiments on human aortic ECs (HAECs) have highlighted that a significant number of genes regulated by RGC-32 are involved in

HAEC proliferation and migration, by controlling RhoA and ROCK1 expression and consequently actin cytoskeletal organization and intercellular adhesion in HAECs. Studies on aortic CMNs (CMNA) have confirmed the functional "dyad" valence of RGC-32 in the case of CMNA: RGC-32 contributes on the one hand to the activation of the cell cycle induced by sublytic C5b-9 and also to the production of TGF-β-dependent extracellular matrix, and on the other hand it substantially influences the process of CMNA differentiation ("phenotypic switching") - by modulating the expression of myocardin, SM22 and α-SMA and that of type I collagen. In conclusion, the complex role of RGC-32 in EC proliferation and migration and in cell cycle activation and phenotypic switching of CMN in the atherosclerotic arterial wall configures a solid potential for targeted therapeutic interventions in arterial atherosclerotic disease for this complement-induced molecule.

RGC-32 as a New Factor Regulating Reactive Astrocytosis in Neuroinflammation

Alexandru TATOMIR[1,2], Jacob CUEVAS[1], Tudor C. BADEA[3], Dafin F. MURESANU[2], Violeta RUS[4], Horea RUS[1,5]

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Response gene to complement (RGC)-32 is a gene originally identified in rat oligodendrocytes stimulated with sublytic C5b-9 and later found to regulate the cell cycle and processes such as fibrosis, angiogenesis, tumorigenesis and epithelial-to-mesenchymal transition. Astrocytes are crucial for CNS homeostasis and can become reactive in response to damage, influencing inflammation and repair. In multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), reactive astrocytes can both worsen and limit disease progression.

Previous research showed that RGC-32 acts downstream of TGF-β, influencing extracellular matrix production and immune responses. Lack of RGC-32 in mice reduces EAE severity and alters astrocyte morphology, suggesting a role in astrocyte differentiation.

Our work investigates how RGC-32 regulates reactive astrocytes and astrogliogenesis using RGC-32 knockout (KO) mice. Results show that lack of RGC-32 renders astrocytes unable to become fully reactive during acute EAE, remaining in an immature, radial glia-like state. RGC-32 is required for TGF-β-driven transcriptional changes that promote gliosis

and tissue remodeling and for generating new astrocytes from progenitors.

Overall, RGC-32 is proposed as a key regulator of astrocyte reactivity and development, representing a potential therapeutic target in MS and related CNS disorders.

RGC-32 is a Biomarker of Disease Activity and Treatment Response in Multiple Sclerosis

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Regular assessment of disease activity in relapsing-remitting multiple sclerosis (MS) is necessary to optimize clinical outcomes. Biomarkers can be a valuable tool for measuring disease activity in multiple sclerosis (MS) if they reflect the pathological processes underlying the pathogenesis of MS. In this pilot study, we combined several biomarkers previously analyzed in MS patients into an MS disease activity score (called MSDA) to evaluate their ability to predict relapses and response to glatiramer acetate (GA) treatment. Levels of RGC-32, FasL, IL-21, SIRT1, phosphorylated SIRT1 (p-SIRT1), and JNK1 p54 were used to generate cutoff values for each biomarker. Any value below the threshold for RGC-32, FasL SIRT1, or p-SIRT1 or above the threshold for IL-21 or JNK1 p54 was assigned a value of +1, indicating relapse or lack of response to GA. Any value above the cutoff for RGC-32, FasL, SIRT1, p-SIRT1 or below that for IL-21 or JNK1 p54 was given a value of -1, indicating clinical stability or response to GA. An MSDA score above +1 indicated relapse or lack of response to treatment. An MSDA score below -1 indicated clinical stability or response to treatment. Our results showed that MSDA scores generated using either four or six biomarkers had higher sensitivity and specificity

and were significantly correlated with the expanded disability status scale. Although these results suggest that the MSDA test may be useful for monitoring therapeutic response to biologic agents and assessing clinically challenging situations, the present findings need to be confirmed in larger studies.

Seeing the Invisible: Imaging Neutrophil-Derived ROS in Tumor and Inflammatory Tissues

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Neutrophils serve as the primary cellular responders to pathogenic threats and inflammatory stimuli, playing a critical role in both immune defense and disease pathogenesis. During inflammation, neutrophils generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are essential for pathogen elimination but can also contribute to tissue damage in chronic inflammatory diseases and tumors. Neutrophil extracellular trap (NET) formation—a specialized cell death mechanism releasing DNA-protein complexes—represents a key mechanism of ROS-dependent neutrophil effector function that is difficult to visualize in tissue contexts using conventional imaging approaches.

To overcome limitations in detecting and localizing neutrophil-derived ROS in tissues, we have developed advanced near-infrared (NIR) fluorescent probes that exploit superior cellular penetration, reduced background autofluorescence, and improved signal-to-noise ratios. Our approach encompasses several complementary strategies:

ROS and Neutrophil Elastase Imaging: FRET-based and non-FRET-based squaraine-peptide conjugates specifically detect neutrophil elastase (NE) activity within NETs, enabling real-time monitoring of NETosis in inflammatory models and human clinical samples. These small-molecular-weight probes achieve subcellular resolution while maintaining compatibility with flow cytometry for detecting circulating NETs, mouse tissues nder cardiac inflammation.

Unbiased ROS Tracking: We have identified non-biased red fluorescent markers (BODIPY derivatives) that accurately track ROS-producing pro-drugs without inducing artifactual subcellular localization patterns common with

traditional dyes. This approach preserves the intrinsic cellular targeting and pharmacological properties of therapeutic agents.

RNS Detection in Tissues*:* Modular fluorogenic probes selectively detect nitric oxide (NO) and peroxynitrite in living cells and inflamed tissues, revealing previously hidden sites of neutrophil activation in disease models including gouty arthritis.

These integrated imaging strategies enable direct visualization of neutrophil-derived ROS in situ, uncovering previously undetectable inflammatory mechanisms in both tumoral and inflammatory microenvironments. This approach provides new insights into neutrophil biology and opens avenues for developing targeted diagnostics and therapeutics to modulate excessive immune responses.

Keywords*:* neutrophils, reactive oxygen species, neutrophil extracellular traps, NIR fluorescence, tissue imaging

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Aquaporin 4 Facilitation as a Treatment Option to Reduce Amyloid Burden in a PSAPP Mouse Model of Alzheimer's Disease

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Introduction*:* Preclinical studies have implicated the Aquaporin 4 (AQP4) water channel in the accumulation and inadequate clearance of amyloid-β (Aβ) in Alzheimer's Disease (AD), however, a head-to-head comparison of AQP4 facilitator/inhibitor has not been yet performed.

Methods*:* Two-months-old APPPS1 mice were treated daily for 28 days with either TGN-020-AQP4-inhibitor or the TGN-073-facilitator (200mg/kg), with vehicle-treated APPPS1 and wild type C57BL/6J mice as controls, and with extensive neuropathological/behavioral characterization.

Results*:* AQP4-inhibitor-treated mice showed a robust increase in total Aβ, while the facilitator led to a massive reduction in brain Aβ. AQP4-facilitator-treated mice also showed reduction in Aβ40 and Aβ40/Aβ42 ratio, while the inhibitor-treated animals showed an increase of both Aβ40 and Aβ42. Furthermore, AQP4-faciltator led to significant reduction in anxiety and increased memory performance.

Conclusion*:* Our data strongly suggest that AQP4 modulation is a powerful tool for facilitating Aβ clearance in AD and supports the development of AQP4-targeted therapies in the treatment of AD.

Dual Transcriptome–Proteome Analysis Reveals Distinct Immune Endotypes in Sepsis

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Background. Sepsis remains a global health crisis, driving about 49 million cases and 11 million deaths each year. Persistent failures of trials testing immunotherapies highlight the need to stratify patients into discrete immune endotypes and tailor treatment accordingly. Herein, we mapped the systemic immune landscape of sepsis, profiled site-specific transcriptomic signatures, paying special attention to *Clostridium*-driven disease, and integrated blood transcriptomics with targeted inflammatory proteomics to identify novel multi-omic endotypes.

Methods. Within the Functional Genomics in Severe Infections (FUSE) project, we enrolled 125 adults with Sepsis-2–defined sepsis and 299 healthy volunteers. Ninety-two plasma inflammatory proteins were quantified; unsupervised hierarchical clustering of differentially expressed analytes yielded two endotypes, "high-" and "low-inflammatory." Peripheral-blood mononuclear cells from the same participants underwent bulk RNA-seq. Differential expression was assessed with DESeq2, and enriched pathways

were identified by over-representation analysis. Transcriptomic profiles were then compared across infection sites (pneumonia, urinary tract, and gastrointestinal), with a focused sub-analysis of culture-confirmed *Clostridium* cases.

Results. Sepsis prompted widespread gene-expression remodeling: pathways mediating phagocytosis and antimicrobial-peptide synthesis were markedly up-regulated, whereas NK-cell cytotoxic programs and adaptive-immune pathways linked to T-cell signaling were defective. Proteomics-defined endotypes a high-inflammatory endotype carrying a heavier cytokine burden and more severe organ dysfunction. Within this high-inflammatory endotype, the IFN-γ–responsive chemokines CXCL9 and CXCL10 were among the most up-regulated transcripts and displayed high cytokine concentrations in plasma. Transcriptomic patterns were remarkably consistent across pneumonia, urinary-tract and gastrointestinal-origin sepsis, indicating that disease severity, not infection site, dominates the host response. Finally, culture-confirmed *Clostridium* cases displayed an additional transcriptomic signature that set them apart from other gastrointestinal infections.

Conclusions. In sepsis, immune dysregulation is driven chiefly by disease severity rather than by the infection's origin or pathogen. The pronounced activation of the IFNγ–CXCL9/CXCL10 axis in the high-inflammatory endotype therefore stands out as a compelling target for precision, host-directed treatment.

Genotype and Phenotype Correlations in Breast Cancer – A Single Center Experience

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Background/Objectives: Conditions associated with *BRCA1/2* pathogenic (PVs) or likely pathogenic variants (LPVs) are often severe. Early detection of carrier status is ideal, as it provides options for personalized case management and can determine the optimal risk-reducing strategies for the carriers of such variants.

Materials *and Methods*: The study involved 58 patients with a personal and familial history of breast cancer (BC) who underwent genetic testing at the Regional Centre for Medical Genetics Dolj over a three-year period. An immunohistochemical panel (HER2, ER, PR, and Ki-67) was used to define the molecular subtypes of breast tumors. The AmpliSeq for Illumina *BRCA* Panel was used to evaluate germline variants in the *BRCA1* and *BRCA2* genes in patients with BC. The $\chi 2$ test and Fisher's exact test were used to compare the different parameters studied.

Results*:* Our findings revealed that 15.5% of the patients carried either *BRCA1* or *BRCA2* PVs or LPVs. *BRCA1* carriers manifested a more aggressive tumor phenotype whereas *BRCA2* carriers had rather low-grade tumors.

Conclusions*:* The study revealed that PVs in both BRCA genes have a significant frequency among BC patients in our region, and *BRCA1* carriers tend to develop more aggressive tumors than carriers of *BRCA2* PVs and patients with no germline PVs in either of the two genes. These observations could provide new epidemiologic data for this disease in our region and contribute further to the development of national screening strategies.

Overexpression of Endogenous Apolipoprotein A-I and Paraoxonase 1 Genes in the Liver of apoE $^{-/-}$ Mice by CRISPR/dCas9; an Approach to Impede Atherosclerosis Progression

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Introduction: A promising therapeutic approach to counteract the progression of atherosclerosis, the main cause of cardiovascular diseases (CVD), is to enhance both the quality and quantity of athero-protective high-density lipoproteins (HDL). The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene-editing system has gained significant attention for therapeutic use because of its remarkable efficiency and specificity.

Objective: To increase HDL quality and quantity by overexpressing the endogenous apolipoprotein A-I (apoA-I) or paraoxonase 1 (PON1) genes in the liver of mice, by using the CRISPR/deactivated Cas9 (CRISPR/dCas9) system.

Methods: CRISPR/dCas9 plasmids were used to transcriptionally activate the endogenous apoA-I/PON1 genes in apolipoprotein E deficient mice (apoE$^{-/-}$) fed with a regular diet.

Results: ApoA-I/PON1 genes were successfully overexpressed in the liver of apoE$^{-/-}$ mice by using CRISPR/dCas9 plasmids and in-vivo-jetPEI®. The results showed an increase in the levels of ApoA-I and PON1 in the sera of mice and a reduction of the area of the lipid deposits in the thoracic aorta. We prove that the anti-atherosclerotic effects of

overexpressed apoA-I or PON1 in the liver leads to the increase of cholesterol levels in HDL and its excretion in the liver and gallbladder, which occurs by the upregulation of hepatic scavenger receptor class B1 (SCARB1), 7α-hydroxylase (CYP7A1), and ATP-binding cassette sub-family G member 8 (ABCG8) transporter, and to an increase in the antioxidant potential (measured as TBARS and PON1 activity) in the circulation.

Conclusions: We successfully activated the endogenous hepatic apoA-I and PON1 genes by using the CRISPR/dCas9 system in apoE$^{-/-}$ mice. We demonstrate that the liver-secreted apoA-I or PON1 impede atheroma advancement. This approach initiates the way for the future use of CRISPR/dCas9 transcriptional activation of endogenous genes for CVD treatment.

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Human Apolipoprotein A-II Modulates the Mitochondrial Respiration in Endothelial Cells

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Introduction. Apolipoprotein A-II (apoA-II) is the second most abundant protein component of high-density lipoproteins, but its function remains poorly understood. Its levels have been associated with elevated cardiovascular risk and it has also been proposed as biomarker for several types of cancers. In this study, we investigated the impact of apoA-II on mitochondrial function in cultured murine endothelial cells.

Methods. Six-month-old C57BL/6 mice were fed a Western-type diet for two weeks and subsequently injected via the tail vein with a recombinant adenovirus (AdV) encoding human apoA-II or AdV with empty vector, as a control. Five days post-transduction, blood was collected and serum was isolated. Murine endothelial cells (C166 cell line, ATCC) were incubated with 10% serum from apoA-II-expressing mice or from control mice for 3, 17, or 24 hours in DMEM. Mitochondrial respiration was assessed using the RP2 (SUIT-002) protocol on the O2k high-resolution respirometer (Oroboros Instruments).

Results and Discussion. Short-term incubation (3 hours) with serum from apoA-II-expressing mice significantly increased oxygen consumption in C166 endothelial cells, as compared with serum from control mice. This effect is noticed across several mitochondrial complexes, such as complex I (161.79 vs. 88 $pmol \cdot s^{-1} \cdot Mx^{-1}$), complex II (after rotenone inhibition; 111.47 vs. 53.16 $pmol \cdot s^{-1} \cdot Mx^{-1}$), and complex IV (305.71 vs. 192.69 $pmol \cdot s^{-1} \cdot Mx^{-1}$). Additionally, fatty acid β-oxidation was

enhanced in the apoA-II-treated group (67.18 vs. 43.72 pmol·s^{-1}·Mx^{-1}). These effects persisted for 17 hours, albeit with reduced magnitude, but were no longer evident after 24 hours. In conclusion, human apoA-II enhances mitochondrial respiration and β-oxidation in murine endothelial cells in a time-dependent manner. The underlying mechanisms driving these effects are under investigation.

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Nanodelivery of Bioactive Natural Products to Inhibit Endothelial-to-Mesenchymal Transition: A Novel Therapeutic Strategy for Atherosclerosis

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Recent studies have highlighted endothelial-to-mesenchymal transition (EndMT) as a key contributor to the pathogenesis of atherosclerosis (AT). Bioactive compounds such as S-allyl-cysteine sulfoxide (alliin) and S-allyl-cysteine (SAC) have shown notable antioxidant and anti-inflammatory properties, yet their rapid metabolism and poor bioavailability *in vivo* limit their therapeutic potential. To overcome these challenges, our strategy involves encapsulating these compounds within lipid-based nanoparticles (NPs) to enhance stability and targeted delivery to endothelial cells (ECs) undergoing EndMT, thereby offering a novel approach for treating AT.

Three types of anionic NPs: All-NPs, SAC-NPs, and All/SAC-NPs were synthesized and physicochemically characterized using the extrusion method and the Zeta Sizer Malvern. The incorporation of alliin and/ or SAC was determined by UHPLC, with all formulations achieving a loading efficiency of ~ 60%. NPs exhibited an average size of ~125 nm and a Zeta potential between -19 – -29 mV. Cytotoxicity was assessed in EAhy.926 cells using the XTT method, confirming their cytocompatibility. Fluorescence microscopy revealed progressive uptake of All-NPs by ECs. In an experimental EndMT model, EAhy.926 cells were exposed to 50 ng/mL TGF-β1 for 8 days to induce mesenchymal transition. Treatment with alliin led to a restoration of CD31 expression and a marked reduction in α-SMA levels compared to untreated EndMT cells. These phenotypic

changes were confirmed by Western blot and immunofluorescence analysis, indicating a partial reversal of the EndMT process.

In conclusion, we successfully incorporated alliin, SAC, and their combination into cytocompatible anionic NPs. Alliin restored the endothelial phenotype by enhancing CD31 expression and reducing α-SMA levels. Incorporating a combination of alliin and SAC into lipid nanoparticles is our future approach for targeting EndMT and treating atherosclerosis.

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Targeted Nanoparticles for Hsp90 Inhibitors Delivery to Fibrotic Cardiac Tissue

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Background: Cardiac fibrosis involves pathological remodeling of myocardial tissue and excessive deposition of extracellular matrix (ECM), impairing heart function and leading to heart failure.

Hypothesis: Targeted delivery of HSP90 inhibitors may reduce cardiac fibrosis by disrupting TGF-β signaling and reducing ECM accumulation.

Aim: To develop heart-targeted nanoparticles for localized delivery of the HSP90 inhibitor, aiming to suppress pro-fibrotic signaling pathways.

Methodology: Nanoparticles (NPs) were synthesized by first preparing an oil phase composed of a chloroform solution comprising L-α-Phosphatidylcholine (9.6 mM) and 5% soybean oil. After solvent evaporation, an aqueous phase containing water, glycerol, and the HSP90 inhibitor 17-DMAG (a geldanamycin derivative) was added. Nanoemulsions were then formed through sonication. Targeted nanoparticles (T-NPs) included DSPE-maleimide-PEG (0.4 mM) to enable conjugation of fibrosis-homing peptides that recognize ECM components. Biomimetic nanoparticles (Bio-NPs) were coated with neutrophil plasma membranes to mimic immune cell surfaces and enhance tissue targeting. We assessed the antifibrotic effects of 17-DMAG in vitro using primary cardiac fibroblasts treated with free or nanoparticle-encapsulated drug. Cardiac fibrosis was induced in mice via angiotensin II infusion over two weeks. Biodistribution of fluorescently labeled nanoparticles was investigated 24 hours post-intravenous injection using the Li-Cor Pearl imaging system.

Results: Fibronectin-targeted NPs significantly reduced ECM markers (periostin, collagen III) in fibroblasts, confirming antifibrotic efficacy. α-SMA expression remained unchanged, consistent with its variable relevance in fibrosis. In vivo, fibronectin-targeted NPs preferentially accumulated in the heart, supporting their potential for site-specific delivery. In contrast, Bio-NPs predominantly localized to the liver, highlighting distinct organ tropisms between the two nanoparticle formulations.

Conclusion: Fibronectin-targeted nanoparticles delivering 17-DMAG reduced extracellular matrix markers in cardiac fibroblasts, demonstrating potent antifibrotic activity. Their preferential accumulation in the heart supports site-specific delivery and reinforces the therapeutic potential of targeted nanocarriers for localized antifibrotic intervention in cardiac fibrosis.

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Preliminary Data Evaluating the TLR4 Inhibition as a Strategy to Mitigate Diabetic Foot Syndrome

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Background. Peripheral artery disease (PAD) is a major complication of diabetes which, in conjunction with lower limb skin ulcerations and neuropathy, leads to the diabetic foot syndrome (DFS). *Hypothesis***.** Conventional treatment strategies do not effectively resolve the increased tendency to develop peripheral arterial disease associated with diabetes. Inflammatory status may lead to disease progression that could be mitigated by active inhibition of TLR4 signaling pathway. *Aim*. We evaluated the proteomic alterations of murine lower-limb ulceration tissue harvested from mice with diabetes and chronic ischemia complications. The influence on the DFS progression was evaluated by TLR4-targeted inhibition in an experimental diabetic-ischemic animal model.

Methodology. We used 4 groups of C57Bl/J6 mice with experimental diabetes induced by 5 daily doses of streptozotocin (40 mg/kg body): 1) a control diabetic group (D, n=3); 2) a group with induced plantar wound (DW, n=3); 3) a group with lower left ischemic limb, made by excision of a part of the femoral artery and induced plantar wound (DIW, n=4) and 4) an ischemic mouse group with the previously described wounds which received 4 doses of TLR4 inhibitor (TAK-242) treatment (3mg/kg) injected intraperitoneally in mice (DIWT, n=5) on days 16, 18, 23, 25. The ischemic and wound procedures were performed after hyperglycemia confirmation. Mice were euthanized 14 days later and blood and plantar tissue were collected for further analyses, such as biochemical assays, RT-qPCR, Western blot and liquid chromatography mass spectrometry-based (LC-MS) proteomics.

Results. We confirmed the onset of diabetes by measuring the serum glucose (≥240 mg/dL), and the surgery procedure generated an inflammatory lesion in progress. The LC-MS bioinformatic analysis indicated 2667 statistically differentially abundant proteins, with a factor of ≥1.2 when comparison to the D group was performed. Our data revealed 961 upregulated and 929 downregulated proteins in DIWT vs DIW. 19 proteins were associated with enrichment of the *Inflammatory Response*, with 5 of them involved in the *Acute Inflammatory Response*. Six identified proteins were associated with enrichment of *Wound Healing* pathway. Notably, these proteins were favorably regulated by TLR4 inhibition. Further validating RT-qPCR and Western blot experiments are in progress.

Conclusion. Proteins involved in the *Inflammatory Response*, *Acute Inflammatory Response* and *Wound Healing* offer new insights into the understanding of the inflammatory mechanism involved in DFS progression. These molecules may possess a significant potential to define the stages of DSF, and help design a suitable therapeutic strategy.

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Effect of Gut Microbiota-Derived Metabolite TMAO on Neurodegeneration, Inflammation, Oxidative Stress and Motor Function in Mice Models of Parkinson's Disease

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The gut-brain axis is well established as a two-way communication between the brain and the gut microbiota through routes as the immune system, neurotransmiter metabolism, the vagus and the enteric nervous system. These links are influenced by gut microbiota-derived metabolites, such as trimethylamine-N-oxide (TMAO). This metabolite has grown in proeminence in recent neurodegenerative animal model studies. It results from the microbial choline metabolism and subsequent hepatic oxidation and recent studies have proven an impact on neurodegeneration, inflammation, oxidative stress and motor function in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal models of Parkinson's Disease. Our study was aimed towards trying to replicate these results also in a lower, atherosclerotic dose of TMAO. Our findings indicate that some of the previously described impacts on neurodegeneration, inflammation,

oxidative stress and motor function can also be found at an atherosclerotic dose of TMAO.

In Vitro Evaluation of the Antioxidant Activity of Polyphenol Quinazolin-4(3H)-one and Catechol Hydrazinyl-Thiazole Derivatives on a Selection of Cell Lines

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Introduction: As human life expectancy rises, so does the prevalence of age-related conditions, including retinal degenerative diseases. The latter are a significant cause of blindness among older individuals, with age-related macular degeneration (AMD) being one of the most common. Currently, the non-neovascular form of AMD has limited therapeutic options, primarily consisting of antioxidant supplements.

Aim: To synthesize, characterize, and evaluate the antioxidant capacity of two structurally analogous compounds based on thiazole, catechol, and β-ionone, as well as two others based on quinazolinon-aceto-hydrazides and phenolic aldehydes. These compounds have shown significant biological antioxidant and anti-inflammatory activities on their own, and by effectively addressing oxidative stress, a well-known contributor to retinal diseases, they may have potential for treating retinal degeneration.

Materials and Methods: The compounds were obtained either by derivatizing 4-quinazolinon-2-mercapto-acetohydrazides and condensing them with phenolic aromatic aldehydes or through the Hantzsch heterocyclization of thiosemicarbazones. Retinal cytotoxicity was tested *in vitro* using a human retinal pigment epithelial cell line (ARPE-19), while potential systemic effects were screened using the BJ and A549 cell lines. The antioxidant activity of the hybrid compounds was evaluated through various *in vitro* assays, including radical scavenging and electron transfer assays, as well as an H_2O_2-induced oxidative stress model using ARPE-19 cells.

Results: When retinal cells were treated with various concentrations, toxic effects from the catechol group appeared sooner than those from the quinazolinone derivatives, with an unexpected pro-proliferative effect at lower doses, suggesting a potential retinoprotective profile. The doses that caused systemic toxicity varied, but all compounds demonstrated strong antioxidant properties, especially in the cellular oxidative stress model.

Conclusion: The results highlight the higher antioxidant activity of these hybrid compounds when compared to ascorbic acid and Trolox, making them promising candidates for further testing in more complex *in vitro* and *in vivo* models of retinal degeneration.

Antioxidant and Neuroprotective Perspectives of Some Edible Fruit's Extracts Based on a Comparative Phytochemical Profiling and Bioactivity

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Background/Objectives*:* Neuroinflammatory processes in the brain play a crucial role in the development of neurodegenerative diseases, mainly to an overproduction of reactive oxygen species. Oxidative stress seems to have an important role in brain aging and in the pathogenesis of neurodegenerative diseases. Polyphenols are key dietary compounds with roles in reducing oxidative stress and neurodegeneration relying on their ability to cross the blood-brain barrier and directly scavenge reactive species and chelate transition metal ions. There is a growing interest in the potential of natural polyphenols in various fruits to improve brain functions.

This study investigated the potential antioxidant and neuroprotective properties of some edible fruits - apricots (*Prunus armeniaca*), plums (*Prunus domestica*), and figs (*Ficus carica*) in relation to the phytochemical composition of their ethanol extracts.

Material and Methods: Antioxidant potential was assessed by ABTS and DPPH assays, while neuroprotective activity was evaluated through the inhibition of acetylcholinesterase (AChE). Phenolic profiles were characterized by UHPLC and HPTLC, and compound–activity relationships were examined using correlation analysis.

Results: Plums extract exhibited the strongest antioxidant capacity (IC_{50} = 1.733 ± 0.079 mg/g for ABTS and IC_{50} = 1.593 ± 0.069 mg/g for DPPH), linked to its high chlorogenic and caffeic acid content. This extract revealed the best anti-AChE effect also. Apricot ethanolic extract was characterized by notable gallic, syringic, and chlorogenic acids, supporting moderate neuroprotective activity. Fig extract showed weaker

radical scavenging ability but provided a balanced profile of protocatechuic, caffeic, and syringic acids. Correlation analysis revealed specific compound–activity associations, including syringic and vanillic acids with DPPH scavenging, p-coumaric acid with total phenolic content, and gallic/ferulic acids with AChE inhibition.

Conclusion*:* In summary, these species exhibit distinct but complementary phytochemical and biofunctional profiles. Their combined use may support the formulation of functional foods with synergistic antioxidant and neuroprotective benefits.

The Role of Bioactive Molecular Components of Human Milk in Regulating Cellular Processes

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Background. Human Milk (HM) is a dynamic biological network that goes beyond its nutritional role, profoundly influencing the epigenetic, immunological, and metabolic programming of the infant.

Hypothesis/Aim. This synthetic review elucidates the cellular and molecular mechanisms by which Human Milk Oligosaccharides (HMOs), Protein Trophic Factors (Lactoferrin), and Micro Ribonucleic Acids (miRNAs) modulate intestinal homeostasis, immune competence, and nascent neurological development.

Material and Methods. This review integrates recent evidence concerning the molecular mechanisms and cellular processes regulated by HMOs, Lactoferrin, and miRNAs, focusing on their distinct roles in early development and tissue regeneration.

Results. Bioactive components of HM modulate early cellular processes. HMOs are essential for intestinal development, acting as a selective metabolic substrate for the microbiota and as soluble receptor analogues to block pathogen adhesion, enhancing barrier resilience. Lactoferrin (LF), a trophic factor, stimulates the proliferation of intestinal crypt cells via the Wnt signaling pathway , with a suggested synergism with Osteopontin in activating the Brg1/Notch1/Hes1 pathway. MiRNA**s** are protected within Exosomes which are absorbed and distributed systemically. They function as post-transcriptional regulators of gene expression, with specific miRNAs being essential in intestinal maturation and the suppression of NF-kappa B signaling.

Conclusion. The cellular programming induced by human breast milk is the result of a complex molecular signaling network. The synergy among HMOs (microbial modeling), Lactoferrin (epithelial support), and

miRNAs (gene regulation) is fundamental for optimizing clinical translation and developing nutritional strategies.

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Preliminary Dynamics of β-Cell Adjustment to Reduced Mass and Diet-Related Stress

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Background. Diabetes is characterized by β-cell dysfunction and death, making it crucial to understand how remaining β-cells adapt and compensate under metabolic stress to maintain glucose homeostasis.

Aim. We investigate how an additional stress of reduced β-cell mass changes the early metabolic and molecular adaptation of diet-induced burden.

Methods. We used the NSG RIP-DTR mouse model, that expresses the human diphtheria toxin receptor (*DTR*) driven by the rat insulin 2 promoter (*RIP*) in an immunosuppressed background. We ablated ~50% of β-cells through DT administration and exposed the mice to a high-fat diet (60% calories from fat, HFD) plus high glucose water (20%, HGW) for 4 weeks. To capture the dynamics of metabolic adaptation, we analyzed physiological and metabolic parameters at 2 and 4 weeks. In addition, we isolated islets and performed transcriptomic analysis at the same time points.

Results. DT-treated animals had increased levels of serum insulin at 2 weeks, while at 4 weeks, secreted insulin reversed to levels similar with control. In addition, DT-treated mice showed impaired glucose tolerance when challenged with HFD+HGW. Transcriptional profiling of pancreatic islets revealed distinct temporal patterns of gene expression. The early response, at 2 weeks, was characterized by increased β-cell differentiation and stress responses pathways. However, by 4 weeks, the response was shifted towards adaptive mechanisms, with enriched biological processes including pathways related to protein folding and glucose homeostasis.

Conclusion. These findings emphasis on the increased β-cells plasticity and their capacity to adapt to a combination of various stressors.

These adaptative mechanisms can be exploited to prevent β-cell dysfunction and to design better therapeutic strategies in diabetes.

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Sweet Habits: Shaping Pancreatic β-cell Adaptation

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Introduction*:* Chronic high glucose intake disturbs metabolic homeostasis, placing increased pressure on pancreatic insulin secretion. Pancreatic β-cells exhibit remarkable plasticity, adjusting their functional state to meet elevated insulin demands. This study aimed to characterize β-cell stress adaptation mechanisms following sustained high-glucose exposure in an inflammatory-prone experimental model. **Methodology**: Our experimental approach included female non-obese diabetic (NOD) mice, aged 4 weeks, which were exposed to either high-glucose water (HGW) or standard water (NW) across variable time intervals. Pancreatic tissues were subjected to fluorescence microscopic evaluation, while isolated islets underwent transcriptomic analysis, both following acute HGW exposure (3 and 4 weeks). **Results***:* Prolonged HGW exposure (24 weeks) resulted in a significantly reduced diabetes onset and diabetes incidence. By immunofluorescence, we were able to identify a modification of the secretory capacity, presented by the dynamic expression of both proinsulin and insulin. Transcriptomic profiling of islets, through Gene Ontology (GO) enrichment analysis, from acute HGW exposed mice evidenced substantial alteration in metabolic pathways, as compared to NW. Analyzing the pathways enriched, we identified potential markers that could play significant roles in mitochondrial lipid-stress response. This indicated a non-proteotoxic mechanism of adaptation that operates independently of the ER stress and unfolded protein response (UPR). **Conclusion***:* Our results suggest the existence of an adaptation mechanism that enables pancreatic β-cells sustain insulin

production in response to metabolic stress. The activation of metabolic pathways generated the necessary energy to preserve β-cells function, potentially delaying the diabetes onset.

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Expression of Apolipoprotein E4 by Adenovirus-Mediated Gene Transfer to ApoE Deficient Mice Modulates the Properties of HDL in a Dose Dependent Way

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Apolipoprotein E (ApoE) plays a central role in lipid and lipoprotein metabolism. Its presence on triglyceride-rich lipoproteins (TRLs) regulates both the lipolysis of triglycerides and the subsequent clearance of lipoprotein remnants via the LDL receptor. In plasma, ApoE exhibits a dual function: at physiological concentrations it promotes efficient remnant clearance, whereas at elevated levels it inhibits lipoprotein lipase (LPL) activity, resulting in TRL accumulation in circulation. Moreover, ApoE also contributes to HDL-like particles formation through interactions with ATP-binding cassette transporter A1 (ABCA1) and lecithin: cholesterol acyltransferase (LCAT). These ApoE-containing HDL particles facilitate modest cholesterol efflux while displaying strong antioxidant properties. In humans, there are three natural ApoE isoforms, ApoE2, ApoE3 and ApoE4, with ApoE4 being the second most prevalent. Our aim is to elucidate the conditions under which ApoE4 promotes HDL particle biogenesis and confers cardioprotective effects. To this end, we employed adenoviral mediated transfer of *Apoe4* to ApoE-deficient mice via tail-vein using different concentrations. Five days following infection, plasma and tissue specimens were harvested from infected mice. The

efficiency of adenoviral transduction was assessed by measuring hepatic ApoE4 gene expression and plasma ApoE4 protein levels. Plasma cholesterol distribution was analysed by fast protein liquid chromatography (FPLC), while HDL subpopulation profiles were determined by Lipoprint. ApoE4 and ApoA1 protein expression were quantified within HDL fractions. Our data support that the atheroprotective properties of ApoE4-HDL are largely dependent on the levels of ApoE4 in plasma. *Acknowledgements:* study supported by GAR Project No. 82 (Contract No. 754/23.11.2023), funded by the Donor Recurring Fund, which is at the disposal of the Romanian Academy and managed by the "PATRIMONIU" Foundation GAR2023 and PNRR/2022/C9/MCID/I8, contract no. 760060/23.05.2023 (#258-STROMA).

Apolipoprotein A1 is Involved in Glucose Homeostasis

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Apolipoprotein A1 (apoA1) is the main apolipoprotein of classical high-density lipoproteins (HDL), playing a pivotal role in HDL synthesis, functionality and plasma concentration. Besides its importance in reverse cholesterol transport, apoA1 has anti-inflammatory, anti-atherogenic and anti-thrombotic properties, thus attributing a cardioprotective character to HDL. Mendelian randomization studies suggested a causative relationship between HDL-C levels and diabetes. Indeed, recent evidence supports a bidirectional correlation between hyperglycemia and HDL, resulting in a vicious circle with detrimental effects on glucose homeostasis. Thus, hyperglycemia reduces circulating HDL-C levels and contributes to HDL dysfunction by modifying its proteins and lipids. In return, low levels and altered HDL affect the function of organs important for glucose homeostasis, such as the pancreas and skeletal muscle. To get better insight on the involvement of apoA1 in glucose homeostatic mechanisms, we used adenovirus mediated gene transfer of human *Apoa1* in apoAI KO mice and we investigated their response to glucose or insulin challenge. On the basis of data obtained during glucose tolerance and insulin sensitivity tests, we established that apoA1 has an important positive role in key glucose homeostatic mechanisms. *Acknowledgements*: study supported by GAR Project No. 83 (Contract No. 755/23.11.2023), funded by the Donor Recurring Fund, which is at the disposal of the

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Peroxidase Activity of Hemoglobin Subjected to Glycative Stress by Measuring Peroxidase Activity and Molecular Changes in Hemoproteins in a High-Sugar Concentration Microenvironment

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Introduction*:* Glycation is a non-enzymatic reaction between reducing sugars and amino groups of proteins, leading to the formation of advanced glycation end products (AGEs) that alter protein structure and function (Singh et al., 2001). Hemoglobin is particularly susceptible to glycation, which affects its oxidative and peroxidase activities (Thornalley et al., 1999). These molecular modifications play a key role in oxidative stress and diabetic complications (Brownlee, 2001). Our goal is to extend the glycation profiles of different proteins to determine and compare the consequences of this reaction depending on the type of protein affected. For this, we worked on two proteins with different sizes: human Tetrameric Hemoglobin (64 kDa) as well as monomeric Hemoglobin (16 kDa). We wanted to study the consequences of glycation on hemoglobin functions. We were interested in its peroxidase activity.

Material and Methods*:* This reaction, which can take place in the plasma, was therefore reproduced in vitro: 6 samples were made for each hemoglobin. Four of them contained 60 mM sugars, respectively glucose, ribose, fructose, galactose. The other 2 are the negative and positive controls, and the latter contained methylglyoxal with a concentration that ensures glycation within 6 hours after incubation. We used PBS as a

solvent and the hemoglobin concentrations were 50 μM for the tetrameric and 40-125 μM for the monomer. The samples were incubated at 37°C for 5 days. SDS-PAGE was used to evaluate the new glycation end-products with/without enzyme digestion and also to evaluate structural changes in hemoglobins. To measure the peroxidase activity, we conducted kinetic monitoring by UV-vis spectrophotometry.

Results*:* Glycation does not significantly affect the peroxidase activity of monomeric hemoglobin. However, it greatly affects that of tetrameric Hemoglobin and differentially according to sugar. Glycation and its functional consequences depend on the type of hemoglobin as well as the type of sugar that glycate them. **Conclusion***:* Glycation affect the oxidative function of hemoglobin (in terms of K_{MM}, V_{max} and k_{cat}) and lead to different pattern of protein-sugar interaction as compared to other results, by increased CBB affinity after glycation without molecular weight increasing.

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Keywords*:* glycation, peroxidase activity, hemoglobin

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Matrix Metalloproteinases as Early Biomarkers in Parkinson's Disease

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Introduction*:* Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by the gradual loss of dopaminergic neurons, typically diagnosed only when significant neuronal damage has already occurred. Consequently, identifying reliable early biomarkers is crucial for timely diagnosis and intervention. Among potential candidates, matrix metalloproteinases (MMPs)—notably MMP-2 and MMP-9—have drawn increasing attention due to their established roles in neuroinflammatory and neurodegenerative mechanisms.

Material and method*:* This study aimed to elucidate the contribution of MMP-2 and MMP-9 to Parkinson's disease pathogenesis using a validated MPTP-induced mouse model exhibiting full survival. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), known for its selective toxicity toward dopaminergic neurons, was administered intraperitoneally to CD21 mice (n = 10/group) at a dose of 25 mg/kg body weight every two days over two weeks. Following the treatment phase, blood and brain samples were collected for biochemical and molecular analyses. The expression levels and enzymatic activities of MMP-2 and MMP-9 were evaluated using gelatin zymography and Western blotting. All experimental procedures were conducted in compliance with ethical

regulations approved by the National Authority for Veterinary Health and Food Safety (authorization no. 356/14.03.2023).

Results*:* The results demonstrated a marked upregulation and increased enzymatic activity of MMP-9 in the brains of MPTP-treated mice, whereas MMP-2 expression remained relatively stable compared to controls. Elevated MMP-9 levels point to its active participation in early neurodegenerative events, potentially through mechanisms involving blood–brain barrier disruption and extracellular matrix remodeling. These findings suggest that MMP-9 may serve as an early and minimally invasive biomarker for PD, detectable in biological fluids such as blood or cerebrospinal fluid.

Conclusion*:* MMP-9 emerges as a promising molecular target for early detection and monitoring of Parkinson's disease, warranting further validation in clinical studies to elucidate its diagnostic and therapeutic potential.

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Exploring the Biological Effects of $EuTiO_2$ Nanoscintillator-Mediated Radio-Photodynamic Therapy in Breast Cancer Cells

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X-ray-induced photodynamic therapy (X-PDT) represents a promising strategy for cancer treatment, offering deep-tissue penetration, tumor selectivity, and minimal invasiveness. In this study, we investigated the potential of scintillating europium-doped titanium dioxide (Eu:TiO_2) nanoparticles (NPs) to convert ionizing radiation into visible light, thereby activating the photosensitizer tetratosylate porphyrin (TMPyP4) and suppressing the growth of cancer cells.

Eu:TiO_2 NPs functionalized with TMPyP4 (Eu:TiO_2_$TMPyP_4$) were applied on normal breast epithelial cells (MCF-12A) and triple-negative breast cancer cells (MDA-MB-231) at various concentrations for 24 h, followed by irradiation with Bremsstrahlung X-rays generated by a high-power (10 PW) laser system at the ELI-NP facility. Cell viability and X-PDT efficiency were assessed by MTT, Live/Dead, LDH, ROS, and clonogenic assays. The Eu:TiO_2_$TMPyP_4$ nanocomplexes exhibited a slight cytotoxicity in non-irradiated conditions, whereas the free NPs and porphyrin were non-toxic. Upon irradiation, a significant increase in ROS generation was detected only in cancer cells, indicating their greater

susceptibility to oxidative stress compared to normal cells. Moreover, results of the clonogenic assay revealed a strong inhibition of cancer cell colony formation following treatment with the Eu:TiO_2_TMPyP4 nanocomplexes.

Overall, Eu:TiO_2-mediated X-PDT shows strong potential as a selective and efficient therapeutic platform for deep-seated breast tumors, supporting its further development as a safe and precise tool in the field of nanomedicine.

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Characterization and Biological Assessment of Sturgeon-Derived Collagen Porous Scaffolds for Soft Tissue Engineering

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Introduction*:* In the last years, the adoption of circular economy principles has driven research into utilizing fish processing byproducts, such as skin, scales, and bones, as alternative sources to traditional mammalian-derived materials for biomaterial development in tissue engineering.

Aim*:* To assess sturgeon-derived collagen porous scaffolds as feasible candidates for tissue engineering applications.

Materials and methods*:* Eight types of sturgeon-derived collagen scaffolds were produced with varying collagen concentrations, glutaraldehyde levels, and freeze-drying temperatures, then characterized for physicochemical and structural properties- water absorption, enzymatic degradation and microstructure. Human dermal fibroblasts were cultured on the scaffolds to assess cell proliferation for 5 days. Overall cell colonization over a three-week period was evaluated using Hematoxylin and Eosin (H&E) and Masson's Trichrome staining.

Results*:* From the 8 samples tested, 4 of them sustained at a higher level the fibroblasts proliferation, while the other half was at 70%. At 3 weeks post-seeding, cell colonization was evident by the presence of cells throughout the scaffolds, including both peripheral and internal regions, as revealed by H&E. Trichrome Masson staining indicated *de novo* collagen synthesis and deposition, more abundantly observed in the scaffolds that promoted enhanced cellular proliferation.

Conclusion*:* Our study identified the sturgeon-derived collagen scaffolds variants which promoted higher fibroblast proliferation and

collagen formation over time, opening the way for their potential use in tissue engineering applications.

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Establishment and Characterization of Human Gingival Fibroblast Cultures

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Introduction*:* Gingival fibroblasts (Fb) play a crucial role in maintaining connective tissue homeostasis, extracellular matrix (ECM) remodeling, and wound healing. Their isolation and characterization provide a fundamental basis for studies in periodontal regeneration and maxillofacial tissue engineering.

Aim*:* The present study's aim was to perform *in vitro* characterization of the human gingival Fb in order to evaluate novel biomaterials for maxillofacial reconstruction.

Materials and Methods*:* Human primary gingival Fb were isolated from biopsies (two subjects). Primary human dermal Fb were used as control. The expression of specific markers such as: vimentin, collagen type I, as well as molecules expressed by activated Fb, including α-SMA, PDGFR-α, FAP, was assessed by immunocytochemistry. Flow cytometry was used to assess marker expression. Protein expression of collagen was validated by Western blot. Contractility was evaluated by gel contraction assay.

Results*:* Immunofluorescence analysis revealed positive expression for vimentin, confirming the mesenchymal origins of the cells, alongside variable expressions of alpha-SMA, PDGFRa, and FAP, suggesting differences in activation states between Fb populations. Flow cytometry corroborated these findings, demonstrating patient-dependent differences in marker intensity and distribution. Western blot results confirmed the presence collagen. All cell populations were able to contract the collagen

gel suggesting their biomechanical ability to generate tension and reorganize collagen which is essential for tissue repair and wound healing

Conclusion*:* Human gingival Fb expressed all the characteristic markers, confirming homogenous cell populations, that can be used as a reliable tool for evaluation of innovative biomaterials for maxillofacial reconstruction.

Acknowledgements*:* The work was supported by the project PN-IV-P7-7.1-PED-2024-1446 and the Romanian Academy.

Composites Based on Collagen, Chondroitin Sulfate, and Sage Oil with Potential Use in Dentistry

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Background. The success of dental implants relies on the osseointegration of biocompatible materials, such as collagen. Still, its functional properties could be enhanced by including additional components that improve mechanical strength and promote cellular growth - chondroitin sulphate (CS), or confer anti-inflammatory benefits - sage essential oil (EO).

Aim. We describe the preparation and characterization of composites based on bovine collagen, CS, and sage EO, obtained by freeze-drying method, for dentistry use.

Materials and Methods. The composites were characterized by water uptake and *in vitro* collagenase-mediated degradation. To evaluate their biocompatibility, the cytotoxicity of extracts was first assessed, followed by investigation of the scaffold's ability to support cellular colonization through the long-term culture of MG63 osteoblast-like cells (four weeks), confirmed by histological analysis using Haematoxylin-Eosin and Masson's Trichrome staining.

Results. Water uptake analysis revealed that the incorporation of CS increased scaffold hydrophilicity, while adding sage EO decreased water uptake. Collagenase degradation tests demonstrated that scaffolds

containing sage EO exhibited enhanced stability. Cytotoxicity assays confirmed that all scaffolds were non-toxic. Histological evaluation revealed preserved integrity for up to 6 weeks and allowed cell colonization. Samples containing CS promoted cellular growth and extracellular matrix deposition, shown by Masson's Trichrome staining.

Conclusions. The synergistic combination of CS and sage EO into bovine collagen scaffolds enhances their biological and mechanical performance. While CS improves hydrophilicity and supports osteoblastic activity, sage EO contributes to structural stability through crosslinking and provides anti-inflammatory effects, offering a promising strategy for dentistry approaches.

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Phenoxazine Counteracts High-Glucose-Induced Alterations of Human Mitochondrial Genes: Results on Cardiac Organoids

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Background. Mitochondrial dysfunction is a hallmark of cardiovascular diseases and hyperglycemia suppresses mitochondrial energy metabolism. Thus, drugs to correct this impairment are highly needed. Self-assembling human cardiac organoids (HCOs) allow drug screening, facilitating the discovery of new candidates for the treatment of cardiac disorders. Hence, our **aim** was to evaluate the effects of phenoxazine (Phx) and iminostilbene (Imi) on high glucose (HG)-induced mitochondrial dysfunction using HCOs.

Methodology. Beating HCOs (15 days old) generated from human induced pluripotent stem cells were exposed to 33.3 mM glucose for 6 days in order to generate an experimental model that mimics the in vivo hyperglycemic conditions. HCOs were characterized by immunofluorescence and the effect of HG was assessed by quantification of beats per minute and transcriptomic analysis (RNA sequencing); the latter was also employed to evaluate the effects of the tested drugs.

Results. Immunofluorescence experiments revealed that HCOs were positive for TnnT2, CD31, vimentin, collagen type I and III, an indication for the presence of the major cardiac cell populations: cardiomyocytes, endothelial cells and fibroblasts. HG decreased the contractility of HCOs. Interestingly, exposure of HCOs to HG downregulated the genes implicated in: aerobic electron transport chain, ATP synthesis and coupled electron transport, oxidative phosphorylation, mitochondrial matrix and protein-containing complex. The data indicated important mitochondrial

impairment. Upon the treatment with Phx these gene ontology groups were upregulated. Moreover, the drug induced the downregulation of extracellular matrix related genes, suggesting an anti-fibrotic activity. In contrast, Imi downregulated the collagen-containing extracellular matrix related genes, but had no effect on mitochondrial associated genes.

Conclusion. Phx, but not Imi, modulates mitochondrial function by reversing the glucose-induced dysregulation of mitochondrial genes. These findings underscore Phx as a promising therapeutic candidate for cardiac dysfunction associated with mitochondrial impairment.

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MiR-210 Deficiency Enhances Pro-Inflammatory Activation and Disrupts M2 Macrophage Differentiation in Murine Models

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Background: Macrophages are well known as central mediators of immune regulation and tissue repair. MiR-210, a hypoxia-inducible small non-coding RNA positioned at the interface between hypoxia and inflammation has been reported to coordinate both metabolic and inflammatory adaptation to diverse stress factors in various settings. Given the interconnection of these two processes in tissue repair after injury, unravelling the role of miR-210 in directing macrophage polarization toward the pro-reparatory phenotype would be helpful in designing therapeutic interventions in chronic inflammation, immune dysfunction and tissue repair.

Objective: To investigate how miR-210 influences macrophage biology by examining its role in the metabolic adaptation and inflammatory signaling response under stress conditions, as well as in functional reprogramming during polarization toward the pro-reparatory M2 phenotype.

Methods: Bone marrow–derived macrophages from wild-type (WT) and miR-210 knockout (KO) mice were cultured under conditions favoring basal (M0) and pro-reparatory (M2) phenotypes. Transcriptomic (RNAseq), metabolic (Seahorse analysis), cytokine (ELISA), and phagocytosis assays were performed to characterize the impact of miR-210 loss on both cell phenotypes.

Results: MiR-210 deficiency resulted in reduced glycolytic activity and limited metabolic flexibility. Interestingly, KO macrophages showed increased phagocytic capacity in both M0 and M2 states. Transcriptomic analysis revealed distinct clustering of WT and KO macrophages and a more pronounced pro-inflammatory profile of KO M0 cells as compared to WT cells. Importantly, upon IL-4 stimulation, KO macrophages failed to acquire a fully polarized M2 phenotype, exhibiting delayed cell-cycle progression and reduced proliferation.

Conclusion: MiR-210 is essential for efficient macrophage polarization toward the pro-reparative state. Its absence leads to a persistent pro-inflammatory phenotype, potentially contributing to chronic inflammation and impaired tissue repair. Our data point towards the potential of fine-tuning miR-210 expression in macrophage to modulate immune responses, with potential implications for improving reparative outcomes.

From Cancer to Infarcted Hearts: GLIPR1 as Detrimental Regulator in Activated Cells

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Background: Cardiac fibroblasts have emerged as key regulators of post-MI remodeling, with GLIPR1 consistently upregulated in these cells following MI. Although the functional consequences of GLIPR1 upregulation in this context remain unclear, interestingly, mesenchymal stromal cell (MSC) therapy attenuates its expression in cardiac fibroblasts, suggesting that GLIPR1 may be detrimental to cardiac repair.

Purpose: To get insight into the functional role of GLIPR1 in post-MI cardiac remodeling by investigating the in vitro effects on cell proliferation and fibrotic genes expression and in vivo effects of GLIPR1 overexpression on cardiac function post-MI.

Methods: GLIPR1 effect on cell proliferation was investigated using U-87 MG-Luc2 glioblastoma cells engineered for knockdown or overexpression of GLIPR1, by following the increase in the bioluminescence signal over 72 hours after lentiviral transduction. Comparative analysis of fibrotic gene expression was performed on immortalized murine cardiac fibroblasts before and after lentiviral induced overexpression of GLIPR1. In vivo effects of GLIPR1 were addressed by assessing the cardiac function in a mouse model of MI that underwent MSC therapy with native or GLIPR1-overexpressing cells.

Results: GLIPR1 overexpression significantly enhanced proliferative capacity in U-87 MG-Luc2 cells compared to control or knockdown conditions. In cardiac fibroblasts, GLIPR1 induced a pro-fibrotic phenotype characterized by marked upregulation of TIMP3 and IGFBP2. MSCs overexpressing GLIPR1 demonstrated diminished therapeutic efficacy post-MI with impaired cardiac function compared to native cells.

Conclusions: GLIPR1 appears to act as a maladaptive regulator linking enhanced cellular proliferation, pro-fibrotic fibroblast activation, and impaired MSC-mediated cardiac remodeling following MI. These findings highlight GLIPR1 as a putative therapeutic target for improving cell-based interventions and outcomes in ischemic heart disease patients.

An R Shiny-Based Platform for Automated Quantitative Analysis of Insulin Granule on Electron Micrographs

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Background: Insulin granules remodel during β-cell stress adaptation or decline, yet their temporal dynamics remain unclear. Defining this trajectory is key to understanding β-cell resilience. **Aim:** To develop an R Shiny-based application that automates post-processing and statistical characterization of insulin granules from electron microscopy (EM) images.

Methods: Pancreatic tissue from mice subjected to diphtheria toxin (DT)-induced β-cell stress at 0, 1, 3, and 5 days post-administration (daDT) was analyzed by EM. Granule features (area, Feret diameter, mean intensity, and aspect ratio) were extracted using a Fiji-ilastik-Python pipeline. The R Shiny app performed IQR-based outlier filtering, skewness analysis, and distribution visualization (violin and density plots), with group comparisons by Kruskal-Wallis testing. Principal component analysis (PCA), Pearson correlations, and Loess regression were used for multivariate and relational analyses. **Results:** Fewer than 5% of granules were excluded by IQR filtering across all features, confirming dataset robustness. Normalized distributions showed consistent right skewness for granule area and Feret diameter, while mean intensity distributions progressively shifted toward lower density at later timepoints. Aspect ratio remained stable, indicating preserved granule shape. Skewness and violin plots revealed significant timepoint differences (Kruskal–Wallis, $p < 0.05$), highlighting a gradual enlargement and intensity decline from 0 → 5 daDT. PCA explained ~77% of total variance, with PC1 capturing the size–intensity trade-off. Centroid trajectories in PCA space followed a temporal sequence (0 → 1 → 3 → 5

daDT), consistent with progressive structural remodeling. Correlation analysis showed a significant negative association between granule area and intensity ($r \approx -0.3$, $p < 0.0001$), indicating that larger granules are less electron-dense. Loess-fitted area–intensity relationships confirmed a reproducible, time-dependent decline in intensity with increasing granule size.

Conclusion: This R Shiny-based tool enables reproducible, quantitative, and biologically interpretable analysis of β-cell ultrastructural dynamics, providing a scalable platform to investigate adaptive and stress-induced remodeling in diabetes research.

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Exploring the Role of Mitochondrial Dysfunction in Cardiac Hypertrophy in an Experimental Model of Atherosclerotic Cardiovascular Disease; Evaluation of the Therapeutic Potential of MTP-131

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Background: Cardiac hypertrophy is a relatively under-explored complication of atherosclerotic cardiovascular disease, characterized by profound molecular changes, with mitochondrial dysfunction being one of the key pathological features.

Aim: In this study, we investigated the potential beneficial effects of MTP-131, a tetrapeptide that has been shown to reduce ROS generation and stabilize mitochondrial structure in a hypertensive-hyperlipidemic (HH) experimental animal model that mimics human atherosclerosis.

Methodology: The experiments were performed on Golden Syrian hamsters that were divided into four groups: (1) Control (C): normal, healthy animals fed standard food; (2) HH: generated by feeding the animals atherogenic diets (standard chow supplemented with 3% cholesterol and 15% butter) and administering 8% NaCl via gavage for four months; (3) HH-MTP-P: followed the same protocol as HH, but received weekly subcutaneous MTP-131 injections starting two weeks after initiating the diet; and (4) HH-MTP-R: received atherogenic diets and NaCl via gavage for two months, then switched to a standard diet (1% NaCl) and received weekly MTP-131 injections for six weeks.

Results: Compared to the HH group, treatment with MTP-131 significantly improved mitochondrial dysfunction and attenuated cardiac hypertrophy. The following changes were observed for the treated groups: (1) a reduction in plasma levels of total cholesterol and triglycerides; (2) a significant decrease in plasma concentrations of TNF-α and TGF-β; (3) a

significant reduction in the levels of MIF, ANF, CTN1, CLS1, and vimentin in the left ventricle; (4) a reduced number of mitochondria and mitochondrial protein concentration; and (5) an attenuation of mitochondrial membrane potential.

Conclusion: The MTP-131-based treatment resulted in a significant modulation of markers involved in mitochondrial dysfunction and cardiac hypertrophy, leading to improved cardiac structure and function.

Keywords: cardiac hypertrophy, atherosclerosis, mitochondrial dysfunction, MTP-131.

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