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НАЦИОНАЛНА ПЪТНА КАРТА ЗА НАУЧНА ИНФРАСТРУКТУРА (2020-2027)

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ИЗДАВА БЪЛГАРСКА АСОЦИАЦИЯ ПО РЕПРОДУКТИВНА ЧОВЕШКА ЕМБРИОЛОГИЯ

ТОМ 14 КНИЖКА 1
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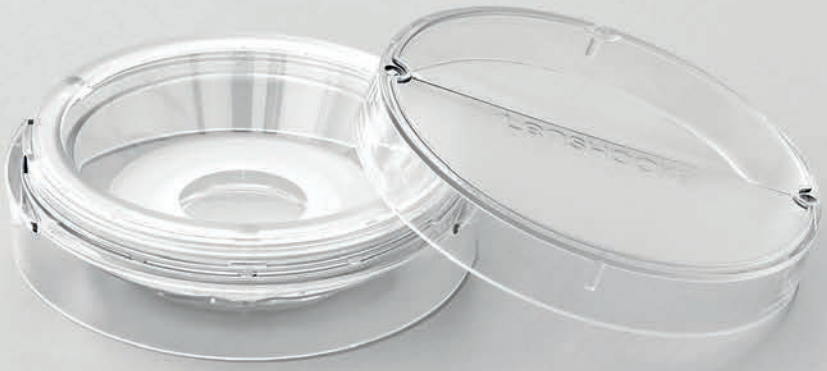

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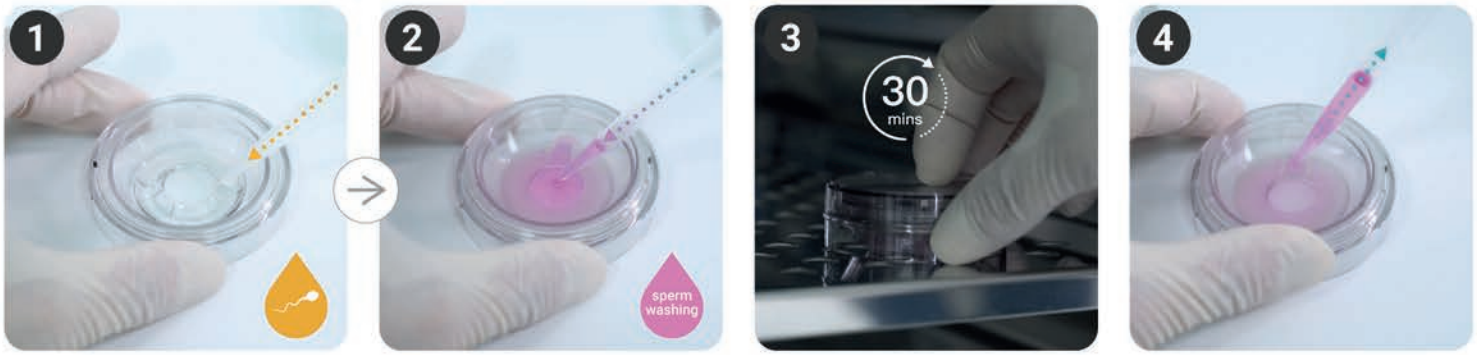

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СЪДЪРЖАНИЕ:

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Уважаеми колеги, скъпи приятели,

Всеки път, около Коледа и в навечерието на Новата година, ние очакваме и се надяваме Светът да се опомни и да започне да се подрежда в по-хармонична структура и да функционира по-благоприятно за всички обитатели на тази планета. Празниците и свързаните с тях традиции и ритуали са своеобразна психотерапия за измъченото ни човечество. Уви, празниците отминават, фойерверките угасват, а Светът става все по-объркан и непредвидим.

От тинята на алчността и властолюбието изникват странните фигури на хора със скромен морален и интелектуален капацитет, но надарени с почти безкрайни арогантност и хъс, и най-вече с усещането за праведна мисия. Застават начело на огромни индустриални компании, на държави и армии, на политически партии и движения. Определят нови понятия за добро и зло, нов дневен ред, нова ценностна система. Как ли би описал персонажите на сегашния свят Франсоа Рабле почти 500 години по-късно?

За древните гърци хаосът е празното пространство преди сътворението на Вселената. В днешно време, понятието има и по-друг смисъл – на бъркотията в материалния свят, която произтича от въздействието на случайни хора и събития, подчиняващи се, в най-добрия случай, на закона за запазване на ентропията. Важното е, че според философските традиции, хаосът е пререквизит и строителен материал за реда и сътворението. А дали наистина е така?

Това, което държите във вашите ръце е само поредния брой на сп. Ембриология, но в него са подредени ценни късове информация от изминалата годишна среща на EATRIS - България, която се проведе на 7-9 Ноември 2024 г. в София.

А в духа на променливия ни Свят, от 2025 г. Българска асоциация по репродуктивна човешка ембриология (БАРЧЕ) има нови Председател, Управителен съвет и Главен редактор на сп. Ембриология. Мога само да им пожелая – крепко здраве и успех във всичките им начинания.

Д-р Георги Николов,
Главен редактор

Dear colleagues and friends,

Every single time, around Christmas and the New Year's Eve, we anticipate and hope for the World to come to its senses and begin to arrange itself in a more harmonious way and function more auspiciously for all inhabitants on this planet. The holidays, with their traditions and rituals, are a kind of psychotherapy for our tormented humanity. Alas, the holidays pass, the fireworks come to rest, and the World becomes more and more dazed, confused and unpredictable.

From the mire of greed and lust for power emerge the strange figures of people with scant moral and intellectual capacity, but endowed with almost infinite arrogance and ambition, and above all with the sense of a righteous mission. They stand to lead huge industrial companies, states and armies, political parties and movements. They define new concepts of good and evil, a new agenda, a new value system. I can't help wondering, how would François Rabelais describe the characters of the current world almost 500 years later.

For the ancient Greeks, chaos was the empty space before the creation of the Universe. Nowadays, the concept has a different meaning – the mess in the material world, resulting from the influence of random people and events, obeying, at best, the law of conservation of entropy. The important thing is that, according to philosophical traditions, chaos is a prerequisite and building material for order and creation. But is it really so?

The 'structure' in your hands is merely the new issue of the Embryology journal and it contains valuable pieces of information from the annual meeting of EATRIS - Bulgaria, which was held on November 7-9, 2024 in Sofia.

And in the spirit of our changing world, from 2025 the Bulgarian Association of Reproductive Human Embryology (BARHE) has a new Chairman, Executive committee and Editor-in-Chief of Embryology. I can only wish them good health and success in all their endeavors.

Dr Gueorgui Nikolov, MD, PhD,
Editor in chief

МИНИСТЕРСТВО НА ОБРАЗОВАНИЕТО И НАУКАТА

eatris BULGARIA

FACULTY OF BIOLOGY
SOFIA UNIVERSITY

EATRIS – Bulgaria

Annual Conference 2024
7-9 November 2024
Grand Hotel Sofia, Bulgaria

International conference

ADVANCES IN TRANSLATIONAL MEDICINE

BOOK OF ABSTRACTS

APPLICATION OF NOVEL IR IMAGING APPROACHES TO VISUALIZE INFLAMMATION AT THE LEVEL OF CELLS, TISSUES, AND ORGANS.

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Infrared (IR) imaging presents a transformative approach to visualizing inflammation at cellular, tissue, and organ levels, revealing crucial aspects of inflammatory responses that are often hidden from conventional observation. Neutrophils, as primary responders to pathogen invasion, play a central role in immune defense by engaging in phagocytosis, producing reactive oxygen species (ROS), degranulation, and forming neutrophil extracellular traps (NETs).

NETs, composed of DNA fibers coated with neutrophil elastase (NE) and other antimicrobial proteins, are pivotal in trapping pathogens. However, NET formation can also exacerbate tissue damage in chronic inflammatory diseases. NE, a key component of NETs, is implicated in various inflammatory conditions, including chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, cystic fibrosis, sepsis, and COVID-19. Detecting NE activity within NETs offers insights into the dual role of neutrophils in both defense and pathogenesis. Additionally, ROS produced during NET formation regulates their release and intensifies oxidative stress, making NE and ROS crucial markers of NET-driven inflammation. Both are essential for understanding the complex dynamics of neutrophil activity and are critical targets for detection using advanced IR imaging technologies.

Here we summarize application of NIR probes and specific animal models to monitor systemic NET-driven inflammation. To meet the need for enhanced NE detection, we used synthesized squaraine-based fluorescent probes and peptides for both fluorescence resonance energy transfer (FRET)-based and non-FRET NIR imaging. These probes either bind specifically to NE or are cleaved by it, allowing real-time monitoring of NE activity. Their specificity, kinetics, and photostability were evaluated in vitro before application in in vivo models of chronic inflammation. Unbiased IR probes capable of detecting ROS further provided a non-targeted method to assess oxidative stress and cellular responses in a variety of inflammatory conditions. This integrated approach offers a deeper understanding of inflammation, paving the way for novel therapeutic strategies to control excessive immune responses.

Crucially, NIR imaging revealed unexpected sites of neutrophil activity associated with systemic inflammation, such as neutrophil activation in the heart in response to a high-fat, highcholesterol diet. This discovery underscores the significance of advanced IR imaging for uncovering hidden inflammatory processes and highlights its potential for guiding therapeutic interventions in systemic and localized inflammatory diseases.

ACKNOWLEDGMENTS

Creation of NE-spesific IR labels - Sai Kiran Mavileti, Shyam Pandey, Graduate School of Life Science and System Engineering, Kyushu Institute of Technology , Kitakyushu, Japan; Fluorescent probes NoBiasFluor Consortium - Viktor Chernii & Andriy Mokhir. Financial support - European Commission grants 872331 “NoBiasFluors”, and 101129095 “LungCare”, Romania’s National Recovery and Resilience Plan, PNRR-III-C9-2022-I8, CF 93/15.11.2022, Financing Contract no. 760063/23.05.2023. In vivo imaging facilities used in the current research were established under the grant of National Research Foundation of Ukraine 2020.02/0131.

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM HAPLO-SELECTED CORD BLOOD SAMPLES (CA21151)

Anna Veiga
Director of Barcelona Stem Cell Bank and Group leader at Pluripotent Stem Cell Therapy research group at the Regenerative Medicine Program (REGENBELL) at Institut d’Investigació Biomèdica de Bellvitge (IDIBELL).

There is a critical need worldwide for tissue for transplantation in patients with organ failure and with degenerative diseases with no treatments available. The generation of human induced pluripotent stem cells (hiPSC) offers a unique opportunity to obtain an unlimited supply of specialized cells. An alternative to the use of patient-specific hiPSC would be an hiPSC collection from healthy donors that could be expanded and differentiated to treat different patients.

COST (European Cooperation in Science and

Technology) is a funding organisation for research and innovation networks. COST connects research initiatives across Europe and enables researchers and innovators to grow their ideas in any science and technology field by sharing them with their peers.

HAPLO-iPS is a COST Action that aims to create a collaborative network to provide a framework for hiPSC generation of hiPSC homozygous for frequent HLA haplotypes, compatible with a significant percentage of the population to be used for cell therapy clinical trials, and to collect a data collection system for such lines and all the associated data. This network involves key stakeholders such as hiPSC generation/banking centers,
cord blood banks, manufacturing centers compliant with Good Manufacturing Practices (GMP), immunology experts, chemistry and manufacturing controls professionals, regulatory bodies, national agencies, and ethics experts. The approach to this challenge involves networking among stakeholders, sharing knowledge, standardizing methodologies, and developing an educational training program for researchers.

HAPLO-iPS also promotes the participation of researchers from less research-intensive countries, as a significant percentage of the members come from these countries. Participants from these countries will have access to research facilities, training courses, and mentoring programs for young researchers, contributing to spreading excellence and widening participation. Furthermore, key leadership positions in the Action Management are reserved for COST ITC members. The project is structured in 7 independent working groups interacting among them.

HAPLO-iPS offers new approaches that will foster the progress of haplo-selected hiPS generation through the development, implementation, and exploitation of a registry with all the information for the benefit of patients.

FROM BENCH TO BEDSIDE: PATIENT-ORIENTED RADIOPHARMACEUTICAL DEVELOPMENT IN NUCLEAR MEDICINE BASED ON THE EXAMPLE OF [89ZR]ZR-PSMA-DFO.

Feodor Braun[1], Sergio Muñoz Vázquez[1], Felix Dietlein[1,2], Thomas Fischer[1], Evgenia Isachenko[3], Vlarimir Isachenko[3], Melanie Freifrau von Brandenstein[4], Philipp Krapf[1,5], Alexander Drzezga[1,5], Markus Dietlein[1] and Klaus Schomäcker[1]

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OBJECTIVE

This study developed [^{89}Zr]Zr-PSMA-DFO, a novel radiopharmaceutical for PET imaging in prostate cancer patients, particularly those with low PSMA expression. Unlike Gallium-68 and Fluorine-18, it offers a longer imaging window, improving detection. Nuclear medicine embodies the „bench-tobedside" philosophy, quickly translating lab discoveries into clinical practice. Interdisciplinary collaboration—spanning chemistry, radiochemistry, and clinical studies—ensures innovations like [^{89}Zr] Zr-PSMA-DFO reach patients efficiently, meeting regulatory and clinical needs.

METHODS

Radiopharmaceutical development is a multistage, regulated process, beginning with identifying a medical need. Nuclear medicine addresses early disease detection and targeted therapy. Radionuclide Selection: Zr-89 offers an extended half-life, ideal for imaging and therapeutic applications.
Radiolabeling: PSMA-DFO binds the radionuclide with stability, ensuring purity and safety.
Preclinical Studies: These evaluate the compound's affinity, biodistribution, pharmacokinetics, and efficacy.
Clinical Studies: Our feasibility and preclinical studies assessed performance in real-world settings, confirming safety and efficacy.

RESULTS

Preclinical studies demonstrated that [^{89}Zr]Zr-PSMA-DFO had a significantly higher tumor-tobackground ratio compared to established tracers such as [^{68}Ga]Ga-PSMA-11 and [^{18}F]F-JK-PSMA-7. In LNCaP tumor-bearing mice, the tracer showed prolonged tumor retention and enhanced imaging quality at later time points (up to 48 hours), confirming its specificity and stability. In a feasibility

study with 14 prostate cancer patients, [^{89}Zr]Zr-PSMA-DFO detected PSMA-positive lesions in 8 patients previously missed by conventional tracers. This improved sensitivity positions [^{89}Zr]Zr-PSMA-DFO as a valuable tool for detecting low PSMA-expressing tumors and enabling targeted therapies.

CONCLUSION AND OUTLOOK

[^{89}Zr]Zr-PSMA-DFO represents a significant advance in prostate cancer diagnostics, especially in patients with low PSMA expression or biochemical recurrence. Its longer half-life supports better tumor detection and personalized treatments such as salvage radiotherapy and metastasis-directed therapies. The clinic is also investigating new radiopharmaceuticals, including radioestrogens for hormone-dependent tumors and tracers targeting cytoskeletal structures, to further enhance diagnostic precision and therapeutic outcomes.

INTEGRATIVE CUT&TAG-RNA-SEQ ANALYSIS: A UNIFIED WORKFLOW FOR ENHANCED INSIGHTS HUMAN INDUCED PLURIPOTENT STEM CELL REPROGRAMMING

Niccolò Liorni[1], Alessandro Napoli[1], Marta Adinolfi[1], Tommaso Mazza[1], Manlio Vinciguerra[2*]

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TEXT

Human induced pluripotent stem cells (iPSCs) are inefficiently derived from somatic cells by overexpression of defined transcription factors. Overexpression of H2A histone variant macroH2A1.1, but not macroH2A1.2, leads to increased iPSC reprogramming by unclear mechanisms. Cleavage Under Targets and Tagmentation (CUT&Tag) is a recent methodology used for robust epigenomic profiling that requires only a limited amount of cells as starting material. RNA sequencing (RNA-Seq) reveals the presence and quantity of RNA in a biological sample, describing the continuously changing cellular transcriptome. The integrated analysis of transcriptional activity, histone modifications, and chromatin accessibility via CUT&Tag is still in its infancy. This work describes a robust bioinformatics methodology and workflow to perform an integrative CUT&Tag/RNA-Seq analysis, to unravel macroH2A1-dependent orchestration of iPSCs reprogramming using human endothelial cells. We demonstrate wider genome occupancy, predicted transcription factors binding, and gene expression regulated by macroH2A1.1 during reprogramming, compared to macroH2A1.2. MacroH2A1.1, previously associated with neurodegenerative pathologies, specifically activated ectoderm/neural processes. CUT&Tag and RNA-Seq data integration is a powerful tool to investigate the epigenetic mechanisms occurring during cell reprogramming.

ACKNOWLEDGMENTS

This research was partly funded by the European Commission Horizon 2020 Framework Program (project 856871—TRANSTEM).

ADVANCEMENT AND DEVELOPMENT OF HPSCREG FOR HPSC-BASED CLINICAL STUDIES

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SUMMARY

Recent decades have witnessed significant advancements in the development of induced pluripotent stem cells (iPSCs), highlighting their promising potential in regenerative medicine. An increasing number of human pluripotent stem cells (hPSCs) have been established as clinical-grade and utilized in clinical studies. However, there is a lack of publicly accessible resources that systematically compile and update the status of hPSCs in clinical studies. Major public clinical trial registries, such as ClinicalTrials.gov, do not have dedicated collections for hPSC-based trials, despite the significant differences between hPSC-based trials and traditional drug development processes. Since 2019, hPSCreg (Human Pluripotent Stem Cell Registry: https://hpscreg.eu) has been collecting

and providing information on hPSC-based clinical studies, extending its original functionality beyond the registration of human pluripotent stem cell lines. This initiative relies on manual searching, data collection and curation, following our established protocol (Kobold et al., 2020). As of October 11, hPSCreg has documented 160 clinical studies and traced 32 cell lines utilized in these studies.

CONCLUSION

The hPSCreg clinical study database is a crucial resource for providing information on hPSC-based clinical studies, addressing the lack of publicly accessible data in this field.

PERSPECTIVES

With the increasing number of clinical studies in recent years, we face challenges in automating data collection and standardizing the data curation process using artificial intelligence to enhance efficiency and data accessibility. Additionally, we are focusing on establishing more connections between clinical studies and the source cell lines used, which will enrich the database and improve the relevance of the data by fostering relationships between different datasets.

THE IMPACT OF ADIPOSE TISSUE DERIVED STEM CELLS ON INFLAMMATION, METABOLIC, AND CANCER PATHWAYS

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ABSTRACT

Introduction: Adipose-derived stem cells (ADSCs) play crucial roles in various physiological and pathological processes by influencing inflammation, metabolism, and cancer initiation and progression.

OBJECTIVE: This study aimed to provide a comprehensive overview of the impact of ADSCs on inflammatory processes and glucose homeostasis, and their relationship with the tumor microenvironment. Additionally, we present experimental findings focusing on the role of resistin in promoting epithelial-to-mesenchymal transition (EMT) and acquisition of cancer stem cell (CSC) properties in breast cancer cells.

METHODS: We conducted an extensive literature review and performed experiments using gene and protein expression analyses as well as imaging techniques.

RESULTS: Our findings demonstrate that resistin promotes EMT in breast cancer cells in vitro and induces the expression of key CSC genes.

CONCLUSION: These results contribute to the growing body of knowledge regarding the importance of adipose tissue, particularly ADSCs, in immunological processes and related pathological conditions. Our findings enhance our understanding of the mechanisms underlying the adiposity-cancer relationship and suggest potential therapeutic targets for future research and clinical applications.

ACKNOWLEDGMENTS

This work was supported by a grant from the Gerald J. and Dorothy R. Friedman New York Foundation for Medical Research.

SOME HIGHLIGHTS IN MODELING OF ENDOMETRIAL MATURATION DURING PREGNANCY

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OBJECTIVE

The functional layer of human endometrium has inherent plasticity to progressively specialize into decidua starting from secretory phase of endometrial cycle and ending by the 12th gestational week of pregnancy. Prompted by the site and time of blastocyst implantation, decidua differentiation diverges into parietalis and basalis type, the latter becoming a part from the maternal part of the placenta supplying nutrients and oxygen to the fetus. The whole process of endometrial transformation is subordinated to the post-ovulatory increase of progesterone, to which endometrial stromal cells (ESC) are particularly

responsive. ESC progressively upregulate/ downregulate the expression of thousands of genes that unfold morphological, biochemical and functional changes and shape the decidual response. ESC gradually turn to decidual stromal cells (DSC) that represent "cellular matrix" of decidua and are in contact with epithelial, local immune, trophoblast cells and the vasculature, constituting the integrity of the placenta. The state and effectiveness of DSC differentiation update the intercellular communications, receptivity of decidua and selection of good quality embryos. Maturitydependent homeostasis of decidua is suggested to control pregnancy outcome – health, failure, and probably placental abnormalities, such as preeclampsia, placenta accreta, fetal growth restriction etc. It is suspected that latent, first-trimester events are underlaying factors for unfolding pathology in second and third trimester. To big extent these cues remain elusive, leaving science still unable to discern the key determinants of the conditions and their balance with the compensatory capacity of the living systems. To establish and postulate a prognostic power of candidate determinants, in vitro modelling should be combined with findings from retrospective/ prospective studies, and specific state markers in an attempt to elucidate the complexity of fundamental processes.

ACKNOWLEDGMENTS
The study is supported by project KP-06-N61/7 from the Scientific Research Fund, Bulgaria.

BIOPRINTED MESENCHYMAL STEM CELLS FOR CRITICAL BONE DEFECT REGENERATION

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OBJECTIVE
Bone transplantation is second to blood transfusion in the world. Globally, 4 million people require bone transplantation or bone replacement surgery each year. Critical size bone defect is one that would not heal spontaneously. It can be secondary to bone loss from trauma, infection, tumor resection, or developmental deformities. Traditional therapeutic approaches such as autografts, allografts, and xenografts have been restricted. Tissue engineering holds promise in the treatment of large bone defects due to advancement of stem cell biology, novel biomaterials, and 3D bioprinting. Three-dimensional printing is a suitable method for manufacturing grafts with precise patient-specific customisation of scaffold geometry.

Mesenchymal stem cells (MSC) promote tissue regeneration during bone repair in osteoporosis, bone fracture, osteoarthritis, and bone defects and are considered a good cell source in regenerative medicine. MSC have regenerative properties due to their ability to differentiate into bone cells, but they also suppress inflammation locally and secrete factors that improve tissue repair.
The aim of our study is to produce scaffolds loaded with MSCs for the treatment of critical-sized bone defects in a mouse model.

METHODS
The role of MSC in the process of regeneration of large bone defects will be studied in in vivo and in vitro models. The in vitro model offers a study of the condition of MSC bioprinted with bone tissuemimic material. In the prints we have created, we checked the viability of MSC, proliferation and their degree of differentiation in different bioprinting protocols and in different commercial bioinks. Selected prints with and without MSC were then implanted in the in vivo mouse model of critical size cranial defect. Degree of bone regeneration was estimated by microcomputer tomography, histological and immunohistochemical methods.

RESULTS
GelXA Bone and CellInk Bone (CELLINK) bioprints, containing 1x10□ MSCs/ml produced using BioX (CELLINK) were analysed by viability, MSC proliferation and osteogenic differentiation. We observed rapid proliferation of MSC in the first week after bioprinting and this led as to the selection of conditions of prints for implantation in critical size cranial defect in a mouse model. After 6 weeks the degree of bone regeneration shown by microcomputer tomography analysis revealed CellINK Bone prints containing MSC to generate more bone tissue compared to GelXA Bone-MSC prints. Defects treated with ink-only constructs

and empty defects showed the background signal. Mason histological staining confirmed the microCT results. Immunohistochemical detection of osteogenic markers pointed to newly osteogenic cells in prints implanted.

CONCLUSION

This study is a step further in MSC applications in personalized and regenerative medicine. Our results will contribute to the improvement of strategies for the treatment of large bone defects, and this will alleviate the condition of patients with orthopaedic, oncological and dental bone losses.

ACKNOWLEDGMENTS

This work was funded by KP-06-H53/7, Bulgarian National Science Fund. BioX bioprinter is part of the equipment of the InfraACT, grant agreement DO1-178/29.7.2022, National Roadmap for RI.

REGENERATION OF IMMUNE FUNCTION THROUGH THYMIC EPITHELIAL STEM CELLS

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INTRODUCTION

The thymus plays a vital role in the maturation and differentiation of T lymphocytes, essential components of the immune system. However, it degenerates early, already during puberty and is particularly vulnerable to cytotoxic treatments. As a result, the thymus is no longer able to support the proper functioning of the immune system and can no longer produce new T lymphocytes.

This can lead to a variety of diseases, including immunodeficiency and cancer. Aside of lymphocytes, thymus is composed of several stromal cells of which thymic epithelial cells (TEC) are particularly interesting being the ones responsible for shaping the T cell repertoire.

OBJECTIVE

The objective of the current research is to understand the fine interplay between thymic cells in order to gain knowledge and procedures that will allow repairing and restoration of a disrupted immune system.

METHODS

This research focuses on developing methods to repair and restore a compromised immune system through human thymus regeneration using epithelial stem cells from postnatal human tissues. A novel approach involves the characterization of thymic epithelial stem/progenitor cells (TESCs) ex vivo, utilizing their capacity to form thymospheres in low-attachment culture conditions.

RESULTS

A novel epithelial cell precursor within the human postnatal thymus was identified and the techniques explored for in vivo regeneration in xenogeneic environments or in vitro organoid formation.

CONCLUSIONS

The findings may pave the way for creating mini thymic organs, significantly benefiting patients with immune deficiencies.

ACKNOWLEDGMENTS: This work was supported by the Croatian Science Foundation, IP-2020-02-2431 and the European Union's Horizon Europe Research and innovation programme under grant agreement n°101092269.

DESIGN OF HBEST1-CONTAINING NANOSTRUCTURES WITH POTENTIAL FOR TREATMENT OF BESTROPHINOPATHIES

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OBJECTIVE

Human bestrophin-1 (hBest1) is a Ca2+-dependent transmembrane protein expressed in the RPE. Mutations in the BEST1 gene cause retinal degenerations collectively termed "bestrophinopathies". Our group established MDCK II – hBest1 eukaryotic cell line, stably expressing wild type hBest1 and developed original scheme for the isolation and purification of hBest1 from these cells. We have demonstrated the surface organization of hBest1, its interactions with essential membrane lipids such as POPC, Sphingomyelin (SM) and Cholesterol (Chol) in models of biological membranes, and its association with various microdomains which has a direct effect on hBest1 conformation, surface organization and its functions. These results are fundamental for the main aim in search for opportunities to integrate already purified hBest1 protein into polymeric and/or bicontinuous nanoparticles, which could be used to intercalate hBest1 into the cell membranes and restore transport functions impaired by mutant forms of hBest1. The main research objective of our investigations is the synthesis and characterization of such hBest1-containing nanostructures. Specific multidisciplinary steps have been already applied and will be used to achieve this main aim: purification of hBest1 from MDCK II-hBest1; design, synthesis and characterization of a variety of nanostructures (micelles, vesicles, bicontinuous nanoparticles) based on lipids, amphiphilic copolymers, surfactants and integration of hBest1 protein therein; determination of the surface behavior of the nanoparticles; determination the association/dissociation coefficients of nanoparticles with different membrane lipids; fusion of the nanoparticles with the cell membranes and tracking the delivery of the protein into eukaryotic cells; determination of nanoparticles' biotolerance, localization and half-life of the protein in the membrane, morphology and growth rate of the cells; pathways of endocytosis and degradation of the protein in cells. If this approach proves successful, the functionally active nanostructures could subsequently be used in preclinical tests.

ACKNOWLEDGMENTS: This work was supported by grant from the Ministry of Education and Science of Bulgaria under contract DO1-178/2022 and the bilateral project IC-PL/11/2024-2025 within the scientific cooperation agreement between the Bulgarian Academy of Sciences and the Polish Academy of Sciences.

DUAL ACTIVATION OF SGC BINDING SITES: NITRIC OXIDE - DEPENDENT AND INDEPENDENT REGULATION OF STRETCH-ACTIVATED CHANNELS IN RAT VENTRICULAR CARDIOMYOCYTES

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OBJECTIVE: The mechanoelectrical feedback (MEF) in the heart, significantly contributes to the occurrence of arrhythmias and involves the flux of cations through nonselective stretch-activated channels (SACs). It is well-documented that nitric oxide (NO) can act as a regulatory mediator of MEF. The objective of this study was to investigate the regulatory mechanisms of SACs in rat ventricular cardiomyocytes, specifically examining the roles of NO-dependent and NOindependent pathways, as well as the impact of S-nitrosylation on SAC functionality. Methods: In freshly isolated rat ventricular cardiomyocytes, we applied the patch-clamp method in whole-cell configuration to measure nonselective stretch-activated cation currents (ISAC) during controlled cell stretching.

The NO donor SNAP, alphaα-1 subunit of sGC activator BAY41-2272, sGC blocker ODQ, PKG blocker KT5823, PKG activator 8Br-cGMP, and S-nitrosylation blocker ascorbic acid were employed to elucidate how NO and S-nitrosylation contribute to the modulation of MEF in the heart and to explore their potential implications in preventing arrhythmogenic SAC activity.

RESULTS: Our findings indicate that physiological NO concentration in the cell is necessary for SAC functionality. An increase in NO due to SNAP in an unstretched cell induced a Gd3+-sensitive nonselective cation current, an analog of ISAC, while cell stretching in the presence of NO abolished ISAC. The activation of SACs by BAY41-2272 suggests a NO-independent regulatory pathway, which can be interrupted by ascorbic acid. Since S-nitrosylation inhibition fully blocked ISAC, we conclude that nitrosylation is integral to this mechanism. Notably, ODQ prevented ISAC upregulation by SNAP, and in the presence of the background stretch, it enhanced ISAC in response to ODQ. This suggests that the lack of NO as a result of ODQ inhibition may reduce PKG activity, increase SAC phosphorylation, and ultimately amplify ISAC via S-nitrosylation. These results underscore the significant role of S-nitrosylation in SAC regulation.

CONCLUSION: Our study highlights the critical role of NO and S-nitrosylation in the regulation of SACs in rat ventricular cardiomyocytes, demonstrating that SAC functionality relies on physiological NO levels. We identified both NO-dependent and NO-independent mechanisms that modulate ISAC with S-nitrosylation acting as a key regulatory pathway. These findings advance our understanding of SAC regulation in MEF, emphasizing the importance of nitrosylation in the prevention of arrhythmogenic SAC activity and offering potential therapeutic insights for managing cardiac arrhythmias.

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TARGETED/CONTROLLED RELEASE OF BIOACTIVES - AN EXPERIMENTAL APPROACH

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OBJECTIVE
In the presented study, a system for registration of smooth muscle spontaneous contractile activity was used to investigate the biopharmaceutical behaviour of iron oxide magnetic nanoparticles and to demonstrate their potential for laser-controlled and targeted delivery of biactive molecules.

METHODS
Magnetic nanoparticles consisting of an iron oxide core covered by a coating of peat extract were prepared by green synthesis. Smooth muscle strips were isolated from the corpus region of guinea pig stomach and isometrically fixed in individual organ baths filled with modified Krebs solution. The mechanical muscle activity was recorded by tensometric system.

RESULTS
Nanosized iron oxide structures with potential therapeutic application were obtained using peat extract as reducing agent in green synthesis. The nanoparticles consist of an iron oxide core surrounded by a corona of bioactive molecules extracted from peat. Spontaneous contractile activity of the smooth muscle strips was not affected after treatment with peat-coated magnetic nanoparticles. In contrast, peat extract induced significant α-adrenergic agonist effect. After application of infra-red laser irradiation to the corona-coated nanoparticles, the release of bioactive molecules from the samples was triggered, resulting in a sharp increase in contractile activity comparable to the control assay.

CONCLUSION
Infra-red laser irradiation activates the release of bioactive molecules from peat exract evidenced by modulation of the spontaneous smooth muscles contractile activity. Therefore, peat-coated magnetic nanoparticles may serve as potential carriers for targeted and controlled release of bioactive molecules.

ACKNOWLEDGMENTS
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LONG-READ NANOPORE SEQUENCING: UNLOCKING NOVEL THERAPEUTIC TARGETS IN ADVANCED CASTRATION-RESISTANT PROSTATE CANCER

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Castration-resistant prostate cancer (CRPC) remains a significant challenge in oncology due to its ability to progress despite androgen deprivation therapy. The identification of novel therapeutic targets is critical for advancing treatment options and improving patient outcomes in advanced stages of this disease. Immune surveillance escape is a critical mechanism that enables metastatic progression in CRPC. Tumors evade immune detection by altering the expression of immune checkpoint molecules and secreting immunosuppressive factors. Long non-coding RNAs (lncRNAs) play a pivotal role in this process by modulating gene expression and immune responses, thereby enhancing the tumor's ability to escape immune surveillance. Additionally, mRNA isotype switching and splicing alterations in tumor cells contribute to the evasion of immune detection, promoting the metastatic capabilities of prostate cancer.

Long-Read Nanopore Sequencing offers several advantages for epi-transcriptomics studies, notably its ability to generate extensive continuous reads that span entire transcripts, thus providing a more comprehensive view of transcriptomic complexity. This technology facilitates the accurate identification of isoform variations and splicing events, which are crucial for understanding gene expression regulation and disease mechanisms. Additionally, it enables the detection of epigenetic modifications directly on RNA molecules, offering insights into the epigenetic landscape that were previously inaccessible with short-read sequencing. The real-time sequencing capability of nanopore technology accelerates the discovery of novel biomarkers, aiding in the identification of therapeutic targets. Furthermore, its flexibility makes it an invaluable tool for epi-transcriptome studies, significantly enhancing translational applications by enabling precise biomarker discovery and the identification of novel therapeutic targets in oncology and other medical fields.

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GLOBAL MICRORNA EXPRESSION PROFILE IN LARYNGEAL CARCINOMA UNVEILS NEW PROGNOSTIC BIOMARKERS AND NOVEL INSIGHTS INTO FIELD CANCERIZATION

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OBJECTIVE

Laryngeal carcinoma is still a worldwide burden that has shown no significant improvement during the last few decades regarding definitive treatment strategies. The lack of suitable biomarkers for personalized treatment protocols and delineating field cancerization prevents further progress in clinical outcomes. In the light of this perspective, MicroRNAs could be promising biomarkers both in terms of diagnostic and prognostic value. The aim of this prospective study is to find strong prognostic microRNA biomarkers for advanced laryngeal carcinoma and molecular signatures of field cancerization.

METHODS

Sixty patients were enrolled and four samples were collected from each patient: tumor surface and depth, peritumor normal mucosa, and control distant laryngeal mucosa. Initially, a global microRNA profile was conducted in twelve patients from the whole cohort and subsequently, we validated a selected group of 12 microRNAs with RT-qPCR. The follow-up period was 24 months (SD ± 13 months).

RESULTS

Microarray expression profile revealed 59 dysregulated microRNAs. The validated expression levels of miR-93-5p ($\chi2(2) = 4.68$, log-rank $p = 0.03$), miR-144-3p ($\chi2(2) = 4.53$, logrank $p = 0.03$) and miR-210-3p ($\chi2(2) = 4.53$, log-rank $p = 0.03$) in tumor samples exhibited strong association with recurrence-free survival as higher expression levels of these genes predict worse outcome. Tumor

suppressor genes miR-144-3p (mean rank 1.58 vs 2.14 vs 2.29, p = 0.000) and miR-145-5p (mean rank 1.57 vs 2.15 vs 2.28, p = 0.000) were significantly dysregulated in peritumor mucosa with a pattern of expression consistent with paired tumor samples thus revealing a signature of field cancerization in laryngeal carcinoma. Additionally, miR-1260b, miR-21-3p, miR-31-3p and miR-31-5p were strongly associated with tumor grade.

CONCLUSION

Our study reports the first global microRNA profile specifically in advanced laryngeal carcinoma that includes survival analysis and investigates the molecular signature of field cancerization. We report two strong biomarkers of field cancerization and three predictors for recurrence in advance stage laryngeal cancer.

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COMPREHENSIVE GENETIC STUDIES IN HEREDITARY CANCER SYNDROME

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OBJECTIVE

Hereditary cancer syndromes (HCSs) account for nearly 10% of cancers even though they are often underdiagnosed. Germline genetic testing for HCSs came to the forefront in the precision medicine era since it is with rising matter for risk assessment, screening and also treatment decisions. Main challenge for germline genetic testing is finding the most appropriate indication
guideline choosing which patients to refer to genetic testing and what to be tested. Using guidelines 48% of patients with pathogenic variant (PV) would not have been detected. For these reasons we have chosen to perform whole exome sequencing (WES) or broad gene panel testing for patients with HCSs.

METHODS

After genetic counselling and signing informed consent, DNA was extracted from peripheral blood from patients with HCSs. WES on NovaSeq6000 or panel testing on MiSeq was performed and the sequencing data was analyzed by DRAGEN/ BaseSpace(Illumina) and for variant interpretion we used VarSeq(Golden Helix). Carrier genetic testing for the patients' relatives was performed with Sanger sequencing. In this study we included patients with colorectal cancer (CRC), multiple endocrine neoplasia, retinoblastoma and breast/ ovarian
cancer (HBOC) who were negative after preliminary screening for common BRCA1/2 mutations.

RESULTS

Among the 18 patients with CRC we found PV in 15 patients (83,33%). The PV were in common mutated genes in CRC like APC, MLH1, MUTYH but also in FANCD2, RECQL4, CHEK2 as well as prostate cancer risk susceptibility gene EHBP1. In 14/29 (29%) patients with HBOC and negative for common BRCA1/2 mutations we found the genetic cause for the disease and in some of them the PV was not in gene recommended by guidelines. In addition, in a patient with early onset breast cancer and family history for immune deficiency (ID) we found
the causative variant for cancer in NBN (not in the gene panel recommended from NCCN). The patient was also carrier for PV in CD40LG which explained the X-linked recessive ID in her family. In 5/6 patients with retinoblastoma we detected PV with different penetrance in RB1. Only 2/27 patients with MEN1 were with family history of the disease and we found the causative variant. Among 5/25 patients with no family history we found PV in MEN1.

CONCLUSION

Our data shows that using guideline recommendations lead to skipping the causative variants in some of the patients and therefore broader gene panels or even WES are more suitable for HCSs.

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MICRO-RNAS AND FUSION GENES AS BIOMARKERS IN PROSTATE CANCER

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Prostate cancer is the second most common malignancy in men, with complex genetic alterations that contribute to tumor progression and metastasis. One of the most significant genomic events in prostate cancer is the TMPRSS2 gene fusion, which is present in approximately 50% of cases. This fusion leads to the overexpression of the ERG transcription factor, driving oncogenic processes such as epithelialmesenchymal transition (EMT) and promoting metastatic spread. Moreover, micro-RNAs (miRNAs) play a crucial role in the regulation of these oncogenic pathways.

Our research has identified miR-204 as a key regulator of the TMPRSS2 fusion, with dualistic behavior in prostate cancer. While it typically functions as a tumor suppressor, miR-204 can act as an oncomiR in TMPRSS2 fusion-positive metastatic prostate cancer, promoting the expression of oncogenic transcription factors RUNX2 and ETS1. These transcription factors further drive the aggressive metastatic phenotype of prostate cancer cells. We also demonstrated that miR-204 expression correlates with altered methylation of the TMPRSS2 promoter, thereby modulating fusion gene expression at both the mRNA and protein levels. Utilizing a novel proximity ligation assay (PLA)-based method, we developed a highly sensitive assay to detect the protein products of fusion genes, enabling better stratification of prostate cancer patients. Combining qPCR with our fusion-detection approach overcomes limitations in current diagnostic methods by providing insights into both mRNA and protein expression levels of TMPRSS2, facilitating the selection of appropriate therapeutic strategies, such as chemotherapy or hormone deprivation. This study highlights the potential of miRNAs and fusion genes as biomarkers for improved prostate cancer diagnosis and treatment.

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IN VIVO AND EX VIVO GENE THERAPY STRATEGIES FOR TISSUE REGENERATION: APPLICATIONS IN PERIPHERAL ARTERIAL DISEASE AND MUSCLE CONTUSION

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Gene and cell therapies offer promising strategies for enhancing tissue regeneration in conditions such as ischemic limb disease and traumatic muscle injury. Granulocyte macrophage-colony stimulating factor (GM-CSF) and mesenchymal stem cells (MSCs) are key components influencing vascularization and tissue repair. Our studies show that MSCs, derived from bone marrow, exhibit proangiogenic and myogenic properties that aid in recovery from ischemia. When combined with GM-CSF, MSCs promote the formation of larger vessels, improving reperfusion, though potentially increasing fibrosis without compromising muscle function. Additionally, MSCs from different genetic backgrounds demonstrate variable myogenic but consistent angiogenic responses, with a higher muscle regeneration rate observed in BALB/c-derived MSCs compared to C57/BL6-derived cells. Furthermore, using a plasmid vector for transient GM-CSF expression post-muscle contusion enhances M1 macrophage polarization, reduces fibrosis, and promotes angiogenesis and myogenesis, ultimately leading to improved muscle mass and function. These findings underscore the potential of both in vivo plasmid-based and ex vivo cell-based gene therapies in promoting tissue regeneration, highlighting their applications in peripheral arterial disease and muscle healing.

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CAR T-CELL THERAPY IN AUTOIMMUNE RHEUMATIC DISEASES

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ABSTRACT
Chimeric antigen receptor (CAR) T-cell therapy is a form of immunotherapy where the lymphocytes, mostly T-cells, are redirected to specifically recognize and eliminate a target antigen by coupling them with CARs. The binding of CAR and target cell surface antigens leads to vigorous T cell activation and robust anti-tumor immune responses. Areas of implication of CAR T-cell therapies include mainly hematological malignancies (i.e., advanced B-cell cancers); however, recent studies have proven the unprecedented success of the new immunotherapy also in autoimmune rheumatic diseases. We aim to review the recent advances in CAR T-cell therapies in rheumatology but also to address the limitations of their use in the real clinical practice based on the data on their efficacy and safety.

HUMAN IPSC-DERIVED RPE CELLS TRANSPLANTATION AS A POTENTIAL ADVANCED MEDICINAL THERAPY PRODUCT FOR TREATMENT OF AGE-RELATED MACULAR DEGENERATION

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OBJECTIVE
Age-related macular degeneration (AMD) is a leading cause of blindness in developed countries, severely impairing central vision due to the loss of retinal pigment epithelium (RPE) and photoreceptors in the macula. Current treatments can only slow disease progression, but transplanting functional RPE cells may halt or partially reverse the damage. This study evaluated the viability, survival, and functional activity of human iPSC-derived RPE cells seeded on a poly(L-lactide-co-DL-lactide) nanofibrous scaffold.

These scaffolds were implanted subretinally in immunosuppressed minipigs for six weeks. Animals were monitored both non-invasively and invasively, and retinal samples were collected for histological analysis.

Our results indicate that transplanted hiPSC-RPE cells maintain typical RPE characteristics, such as a uniform hexagonal layer and functional phagocytosis, support photoreceptors, sustain vision, and show no rejection of the xenotransplant with continuous immunosuppression.
hiPSC-RPE on PDDLA nanofibrous membranes appears to be a promising therapy for RPE degenerative diseases

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ANALYSIS OF EXTRACELLULAR VESICLES IN A MODEL OF HUMAN ENDOMETRIAL CELL DIFFERENTIATION

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INTRODUCTION: Decidualization is a process of endometrial differentiation occurring in the secretory phase of the menstrual cycle up to the 12th week of gestation. Cell metabolism shifts to transcription factors (C/EBPB, HAND2, FOXO-1 etc.) that induce tissue remodelling and angiogenesis. Decidualization of stromal cells can be monitored by proportional prolactin
release. A key component of the cellular cross-talk are extracellular vesicles (EVs) – comprised of lipid membranes and conjoint protein receptors (CD9, CD63, CD81), integrins etc. EVs function as delivery system of mRNAs, siRNAs, DNA and versatile proteins, which can activate signalling and affect the fate of recipient cells. Aim of the study: Investigation of adhesion receptors and FOXO-1 and HAND2 transcription factors in EVs from decidual stromal cells (DSC) in different functional states.

MATERIALS AND METHODS: DSC from healthy (h) and miscarrying (mi) donors were decidualized for 10 days and their state checked via prolactin quantification. EVs were collected by ultra-filtration. EVs were lysed for RNA extraction or precipitated for FACS analysis. Tetraspanins CD9, CD63 and CD81 and adhesion molecules β1, α3, α5, α6, αVβ3 were assayed. FOXO-1 and HAND2 transcript levels were analysed using qPCR.

RESULTS AND CONCLUSION: Analysis showed increase of CD63, decrease of CD9 and stable CD81 expression in hDSC at decidualization. The corresponding MVs expressed CD63 and CD81 but lacked of CD9 across the EV from healthy donors. EVs from miDSC showed 30% reduction of CD81, suggesting miscarriage-related profile. Adhesion receptors composed of beta1 integrin (β1) and some partner alpha chains – α3 and α6 were detected in decreasing intensity in hDSC at differentiation, while in corresponding MVs the reduction affected α3 and α5 integrins. In contrast, miscarriage EVs showed no change in integrin receptors at differentiation. Transcription factor FOXO-1 was upregulated in MVs after day 4 of decidualization of hDSC but not in MVs from miDSC, whereas HAND2 was expressed in all samples and conditions.

CONCLUSION: miDSC have impaired decidualization. As a consequence, EVs' receptor pattern and FOXO-1 mRNA content are disrupted, which probably causes loss of selectivity and aberrant signalling capacity to target cells. Parallel DSC models of status differentiation may help reveal the mechanisms of cell communication in the decidual niche.

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TRANSCRIPTOME ALTERATIONS CONTRIBUTE TO IMMUNE EVASION IN THE PROSTATE CANCER STEM-LIKE PHENOTYPE

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OBJECTIVE
The heterogeneous nature of prostate cancer (PCa) is associated with the acquisition of cancer stem-like cells (CSCs) cells with self-renewal properties and differentiation capacity, which is a prerequisite for the development of increased therapeutic resistance in castration-resistant metastasis. CSCs are characterized by the expression of CD44, a receptor of hialoronic acid, that is related to the stemness, tumorigenicity and increased metastatic potential of cancer cells. Another CSCs-associated molecule,CD24, play role in cell proliferation and cell survival. Both CD44 and CD24 has been intesivlely studied as immunotheraupheptic targets. The aim of the present study was to analyze the epi-transcriptome of bone metastatic prostate cancer cell line -PC3, to elucidate the mechanisms involved in the evasion

of immune surveillance.
PC3 cell line (ATCC) was cultivated in RPMI-1640 with or without fetal bovine serum under low-binding conditions to promote formation of stem-like enriched spheroids. Both wild-type adherent cells and spheroids were analyzed flow cytometrically and then sorted (SONY MA900) based on the cell surface expression of anti-CD44, CD24 monoclonal bs. Non-poly-A long non-coding RNAs (lncRNA) and mRNA were simultaiosuly detected in non-sorted and sorted spheroids using a modified library preparation protocol for long-read nanopore direct RNA sequencing (GridION MK1, SQK-RNA002).

The obtained mRNA and lncRNA expression profiles may provide insigh into new potential targets for immune evasion checkpoints that might enhance immune-based treatment strategies in oncology.

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DEVELOPING AN EXTENSIVE MITOCHONDRIAL DAMAGE RESEARCH PROTOCOL INCORPORATING ADAPTIVET SAMPLING AND FLUORESCENCE IMAGING TECHNIQUES

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OBJECTIVE
Mitochondria, as membrane-bound organelles, are pivotal in cellular energy production, regulating various biological processes. mtDNA is highly susceptible to damage due to its unprotected structure, redox environment, and lack of robust repair mechanisms. Heteroplasmic mtDNA variants reaching high thresholds can trigger mitochondrial dysfunction, leading to a variety of clinical manifestations.
While traditional methods for studying mtDNA defects are limited by cost, throughput, and sensitivity, third-generation sequencing technology offers a powerful alternative.

METHODS
Oxford Nanopore's sequencing platform has been implemented for mtDNA defects investigation. Using nanopores, we are able to sequence long DNA fragments, overcoming limitations of shortread technologies, and can identify base modifications alongside structural variations. Adaptive sampling has been employed to enrich mtDNA without PCR amplification, thus minimizing bias.
We used choriocarcinoma cell line Jar to test the approach. After optimizing the protocol, it was applied to adipose-derived mesenchymal stem cells (adMSCs), because these cells are intended for a future biomimetic chip project that aims to replicate an in vivo-like metabolic inflammatory state, facilitating multiomics analysis and drug screening.
Additionally, mitochondrial fluorescent dyes were employed to assess mitochondrial status.
Preliminary results with MitoBright ROS Deep Red for mitochondrial superoxide detection were captured using Andor's BC43 confocal microscope and Nanolive's 3D cell explorer.

RESULTS
The sequencing analysis allowed us to assess the levels of heteroplasmy across the mitochondrial population. It can be distinguished what percentage of the mitochondria possess mtDNA defects that can potentially lead to clinical manifestation. Exposing the cells of interest to different environment stimulus and chemicals prior applying this protocol, may help for more comprehensive understanding in mitochondrial biology. Using the fluorescence dye – MitoBright ROS Deep Red and stimulating some of the cells with an appropriate reagent, we were able to observe and compare the levels of ROS generation.

CONCLUSION
This study presents a novel approach for mtDNA analysis using adaptive sampling and demonstrates the potential for comprehensive mitochondrial profiling in various cell lines, with broad applications in disease modeling and therapeutic development.

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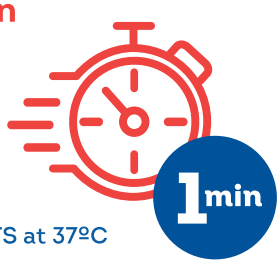

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MSC TREATMENT IN GERMAN SHEPARD HOMOZYGOUS FOR THE HIGH-RISK FACTOR FOR CDM IN EXON 2 OF THE SOD1-GENE – A PROMISING PROSPECTIVE CASE REPORT

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OBJECTIVE
To present a case report of treatment of CDM with alogenous MSC transplantation. Canine degenerative myelopathy (CDM) is a progressive neurodegenerative disease of the spinal cord, originally described in the German Shepherd dog (GSD). CDM is associated with a mutation in the superoxide dismutase 1 gene (SOD1:c.118G > A) which leads to specific ethiology. Initial clinical signs include asymmetric progressive upper motor neuron paraparesis and ataxia of the pelvic limbs which progresses to lower motor neuron paraplegia within 9 to 18 months, usually necessitating euthanasia.

METHODS
Here, we present a case study of 7 years old German Shepard dog, diagnosted via neurological exam, MRI and PCR of the exon 2 of SOD1 gene. Toghether with the paliative care, the patient was treated with intravenous infusion of alogenous adipose tissue derived MSC from a healthy donor.

RESULTS
The treatment was performed twice and resulted in slowing the progression of the disease, based on subsequent neurological examinations. Furthermore, some of the reflexes of the pelvic limbs were partialy restored.

CONCLUSION
There are at lest three mechanisms by which MSCs may exert beneficial effects,(growth factor secretion,

neuroinflammation attenuation and exosomes/ miRNA activity), though the precise contribution of each of them needs further evaluation.

While definitve treatment is still not avaliable, MSC based therapies may play a significant role in the complex therapeutical approach to CDM and other neurodegenerative diseases, both in veterinary and in human medicine.

CRYOPRESERVATION AND BIOBANKING OF HUMAN HEMATOPOIETIC STEM CELLS (HSC) IN BULGARIA – AN OVERVIEW

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SUMMARY
The first successful transplant using cord blood-derived hematopoietic stem cells was conducted in October 1988. To date, over 40,000 umbilical cord blood transplants (UCBTs) have been performed globally. At the present moment, around 10 milion frozen samples, most of them in North America (35%), are stored in public or private cryobanks worldwide. Global cord blood banking services market is USD 33.8 billion in 2023, expected to rise to USD 51.3 billion in 2030. Ten major cryobanks control 80% of that market.

In Bulgaria, 12 tissue banks are collecting and cryopreserving umbilical cord blood. Six of them freeze and store the samples on our country and the rest are transproting them abroad. Their activities are regulated by the Executive Agency Medical Supervision.

In the current report, information about the operation of the tissue banks in our country and the regulations imposed in the legislation will be presented.

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FOETAL STEM CELLS - CURRENT ADVANCES, ETHICAL ISSUES AND FUTURE PERSPECTIVES

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First reports of foetal tissue transplantation have been published in the 1920-ies. Since then, there have been a number of investigations for the assessment of foetal cells based treatments for wide variety of conditions, such as neurological diseases, heart failure, limb injuries,
diabetes, persistent wounds, etc. The sources for their derivation are fetuses from abortions, either spontaneous, or due to medical reasons (ectopic pregnancy, spina bifida, etc.). Foetal stem cells and tissues are promising for transplantation, because they are less immunogenic, as they lack the expression of MHC-1 and 2 and produce HLA-G to induce immune tolerance during pregnancy. They are polypotent and less differentiated than adult stem cells, with higher potential for migration and repopulation. In addition, foetal stem cells secrete a unique complex of cytokines and growth factors, which promote tissue regeneration.
Rising ethical concerns, however, have set challenges for the use of foetal stem cells and tissues in clinical practice. There are several issues related to this matter: the successful application of foetal tissue may make abortion procedures more socially acceptable, women could be persuaded to undergo abortion for donation purposes and the procedure may be altered based on medical needs. The possibility to obtain a rightful informed consent in such cases is also debatable. For that reason, in the legislation of many countiries the "principle of separation" was adopted, which states that the decision to undergo abortion should be completely independent from the consent for foetal tissue donation.
A few of years ago, the International Society for Stem Cell Research issued a Coalition Statement, signed by a hundred other societies in support of studies using fetal tissue. The statement was later backed up by the International Society for Cell and Gene Therapy, publishing an open letter to rescind the research restrictions in USA. As the unique properties of foetal stem cells hold significant promise for promoting tissue regeneration, still a number of issues exist, regarding the optimal conditions for their cell culture and processing prior to transplantation, maintaining the stem cell niche, the potential generation of iPS cells from foetal stem cells and others.

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BULGARIAN HEMATOPOIETIC STEM CELL DONOR POOL AS A POTENTIAL SOURCE OF HLA-HOMOZYGOUS INDUCED PLURIPOTENT STEM CELLS FOR HAPLOBANKING

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Induced pluripotent stem cells (iPSCs) are a promising tool in regenerative medicine. Notably, the HLA-compatible homozygous iPSC lines have the potential to be a source of cells for allogeneic therapy and the Public Cord Blood Banks are an extensive source of HLA-typed cells suitable for reprogramming into iPSCs. Moreover, cord blood units (CBUs) are collected and processed in an approved manner for clinical use. Therefore, it is justified that the establishment of HLA homozygous iPSC banks (haplobanks) using HLA-typed CBUs has been initiated in several countries. The goal is to create iPSC lines that will provide cells for personalized treatment in a large part of the population.
Thus, we aimed to investigate the CBUs stored in the National Public Stem Cell Bank for the presence of HLA-A-B-DRB1 homozygous units and to assess the representativeness of these units for the Bulgarian population.
The study included 591 CBUs stored in the National Public Stem Cell Bank and 635 healthy adults (≥18 years) from the Bulgarian population. The samples were characterized for HLA class I (HLA-A, -B) and class II (-DRB1) polymorphism by using low-resolution (PCR-SSOP) and high-resolution (SBT and NGS) genotyping techniques.
Among the investigated CBUs, we found four homozygous for the HLA-A, -B, and -DRB1 loci (0.7% of the units) with the following genotypes:

HLA-A*01-B*08-DRB1*03, A*02-B*18-DRB1*11, and A*02-B*27-DRB1*16. Furthermore, HLA-A*01-B*08-DRB1*03 (3.1%) and A*02-B*18-DRB1*11 (2.9%) were found as the two most frequent haplotypes when evaluating the frequency of the HLA-A-B-DRB1 three-loci haplotypes in the entire studied group of 1226 individuals (CBUs units and healthy adults) from the Bulgarian population. On the other hand, HLA-A*02-B*27-DRB1*16 was found among the ten most common three-loci HLA-A -B-DRB1 haplotypes (frequency greater or equal to 1%) in the studied group. Additionally, a comparison of HLA-A, -B, -DRB1 allele and haplotype distribution in the CBUs and the ethnically matched healthy adults revealed that the units serve as a highly representative HLA pool of the Bulgarian population.

The present study confirms that the Bulgarian Public Stem Cell Bank is a relevant source of well-characterized grafts suitable for allogeneic stem cell therapy. Our analysis found the availability of HLA homozygous CBUs stored in the Bank inventory. Moreover, the HLA genotypes of these homozygote units correspond to the most common three-loci haplotypes observed in the Bulgarian population. We conclude that the Bulgarian hematopoietic stem cell donor pool may be the best candidate for generating homozygous iPSC stocks, as an efficient option for allogeneic iPSC therapy in our country.

SUGAR MOLECULE –AN ALTERNATIVE APPROACH FOR STEM CELL BASED REGENERATIVE MEDICINE

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As an important part of regenerative medicine, stem cell biology has become more developed over time, and exciting studies in this field suggest new approaches related to their use. Researchers have studied many approaches to date, but each approach has certain limitations. Recent literature advocates using growth factors, especially it's VEGF, as the best option. But, researchers should consider its dose-dependent adverse effects, such as how a reasonable dosage may lead to tumor progression, severe vascular leakage, and hypotension. Other factors to consider include the shortened circulation life of growth factors, purification issues, and the high cost of the product. Additionally, an epigenetic approach poses challenges such as unstable miRNAs and the need to design an appropriate miRNA delivery system for a specific tissue and molecular target. Lastly, preparing the right cocktail involves selecting a medium with the correct supplements to prevent non-specific cell lineage during cell co-culture.

To avoid side effects of growth factors such as VEGF, which can occur upon overstimulation, or unstable miRNA, or insufficient loading of EVs, a focus on using small chemical molecules could be a basis for new approaches.

In the present study, we've focused on the use of small chemicals, not only due to their low cost and easy control of their delivery, but also because they are molecules that help the target cells to internalize receptors, so leading to activation of signalling cascades. By using these molecules, cells can induce a multitude of signaling pathways at their initial stages, which will play a role in all subsequent steps of the cascades necessary for producing the required growth factors. This can similarly change the endogenous stem cell fate. It can be beneficial for reprogramming cells without genetic manipulation and can contribute to rejuvenating strategies. We test how a new derivative of M6P affects the osteo-differentiation of stem cells and in angiogenesis, since the M6P-ligand plays a role in various cellular processes, including the TGF-β pathway. Our findings are encouraging, providing us a clue for furthering experiments on derivatives of M6P as a potential bone-forming drug.

CELL LINE-BASED MODELS FOR STUDYING OPTINEURIN AND COLLAGEN TYPE IV-RELATED INHERITED EYE DISEASES

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OBJECTIVE

Eye diseases and visual impairments are extremely common and affect approximately 2.2 billion people worldwide. Since most of the information from the outside world is perceived through the eye, the quality of life of people with visual impairments is significantly
decreased. There are many different causes of eye disease – infections, other chronic conditions, previous injuries and genetics. Some of the genes associated with eye problems encode the proteins optineurin and collagen type IV.

Human optineurin is a cytosolic non-secreted protein with roles in various processes such as membrane trafficking, exocytosis, and maintenance of the Golgi complex. It is found in some ocular tissues such as the retina and trabecular meshwork, as well as in non-ocular tissues. Mutations in the OPTN gene have been identified as causes of the diseases normal tension glaucoma and amyotrophic lateral sclerosis. Recently, a patient with signs of retinitis pigmentosa and myopia was found to be a carrier of a nonsense mutation in the OPTN gene (p.Glu135Ter) leading to a premature stop codon.

Human collagen type IV, on the other hand, is a secreted protein and a major constituent of basement membranes, including the lens capsule, Bruch's membrane, and the inner limiting membrane of the retina. Mutations in the COL4A1 gene, encoding the α1-chain of collagen type IV, are associated with various abnormalities. A heterozygous missense mutation in the COL4A1 gene (p.Arg1656Ser) was recently identified in a patient with signs of retinitis pigmentosa and macular dystrophy.

Cell lines are widely used experimental models to study gene function, signaling pathways, and other mechanisms. Therefore, the aim of the present study was to generate four stably transfected cell lines expressing wild-type or mutant form of optineurin and collagen type IV, respectively. For this purpose, MDCK II cells were chosen and vectors containing the human optineurin and collagen type IV genes or their mutant forms were introduced into them. All genes were conjugated with GFP at the C-terminus to facilitate confirmation of successful transformation. The established cell lines can be used as a model to study protein structure and function, as well as the molecular mechanism of pathogenicity associated with optineurin
and collagen type IV.

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INVESTIGATING CELL-AUTONOMOUS COMPLEMENT BEHAVIOR IN BREAST CANCER

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OBJECTIVE

Breast cancer is the most common cancer in women and a leading cause of cancer-related deaths. The immune system significantly impacts its prognosis and treatment. Recent studies revealed the key but not fully understood role of the innate immune complement system in breast cancer progression.

Moreover, over the last 10 years, it appeared that complement proteins act not only in the plasma complement cascade but also in a cell-autonomous, even intracellular manner. As we found cellautonomous expression of complement genes in breast cancer patients, our study aims to investigate the cell-autonomous roles of C1r, C3 and Factor B complement proteins in this malignancy.

METHODS
Tumor tissues from 40 breast cancer patients were studied, categorized into three groups: Her2+ nonmetastatic, Her2- nonmetastatic, and Her2+ metastatic. The study methods included immunohistochemistry, gene silencing in 2D and 3D tumor cell cultures, and analysis of TISCH and TCGA transcriptomic databases.

RESULTS
RNAseq analyses from the TCGA database showed that overexpression of complement genes C1R, C3, and CFB are linked to a better prognosis for breast cancer patients. Single-cell RNAseq analyses via TISCH revealed that genes for C1r, C3, and Factor B are expressed by cancer-associated fibroblasts, as well as by some tumor cells across various histological and molecular subtypes of breast cancer, confirmed at the protein level by our immunohistochemistry analysis of patient tumor sections. Two patterns were detected - cell-autonomous production and complement cascade activation. C1r and Factor B showed presence in all tested tumor tissues, whereas C3 displayed intraand inter-tumor heterogeneity. Knocking down these genes in vitro in the MDA-MB-468 breast cancer cell line reduced tumor cell proliferation and viability in 2D cultures, and tumorosphere formation in 3D cultures.

CONCLUSION
Our findings indicate that the impact of complement in breast cancer represents a balance of opposing mechanisms: complement cascade activation through intratumoral secretion, which appears to be anti-tumoral, versus tumor cell proliferation driven by certain complement proteins, such as C1r and Factor B, with cell-autonomous functions. Overall, the balance is tipped to anti-tumor immunity, as its overexpression correlates with a favorable prognosis.

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PHENOTYPE (CD90) RESPONSE OF HUMAN ENDOMETRIAL STROMAL/STEM CELLS TO CHRONIC INFLAMMATION

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OBJECTIVE: Endometrial stromal cells (ESCs) make up the majority of human endometrium and express CD90, a well-established stem cell marker. Chronic inflammation creates an atypical environment for these cells, potentially impacting their normal function and phenotype. Our aim is to investigate whether chronic inflammation influences CD90 expression in normal ESCs, providing basic findings for comparison to ESCs derived from endometriosis patients. Notably, CD90 has shown promise as a biomarker, with elevated soluble levels detected in the serum of women with endometriosis.

METHODS: ESCs from seven women have been used in a model of chronic inflammation. ESCs were treated with IL-1β (10 ng/ml), TNF-α (20 ng/ml), and IFN-γ (10 ng/ml) every 48 hours for eight days. Membrane CD90 density was analyzed using flow cytometry and cd90 RNA levels were assessed through quantitative PCR. Results were statistically analyzed.

RESULTS: All cytokines reduced membrane CD90 expression on ESCs, with IFN-γ causing 30% response compared to the untreated control. Statistical analysis using the t-test revealed significant changes with treatments of IL-1β (*p = 0.0123), TNFα (*p = 0.0297), and IFN-γ (**p = 0.0025). Combinations of cytokines did not exhibit synergistic effect on the level of CD90 expression and often resulted in diminished response compared to individual cytokines. Quantitative PCR confirmed a decrease in cd90 RNA transcripts (n = 6) showing a statistically significant reduction in cd90 gene expression (*p = 0.0231). Although, IL-1β also demonstrated decreased cd90 expression, it did not reach statistical significance, while TNF-α treatment showed the weakest effect.

CONCLUSION: These findings indicate that pro-inflammatory cytokines modulate CD90 expression in ESCs and highlight potential avenues for further research.

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LIVER DECELLULARIZED EXTRACELLULAR MATRIX AS A NATURAL 3D SCAFFOLD FOR CELL CULTURE

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Patients with end-stage liver diseases have a very high mortality rate due to associated complications, with liver transplantation being the only effective treatment at present. However, organ transplantation creates complications due to the shortage of donors, necessitating the development of new alternative approaches that have been studied in recent years.
Tissuemimicking cell culture methods that promote liver-specific functions are promising tools for engineering liver tissue. They are based on the use of biomaterials that attempt to recapitulate cell-cell and cell-matrix interactions. Decellularized extracellular matrix is preferred over synthetic matrix as it preserves the natural microscopic structures and microenvironment, and various bioactive molecules. In addition to transplantation, these models are valuable for drug testing and toxicity studies, offering a more accurate model than traditional cell culture. Processed into hydrogels, dECM materials have applications in tissue engineering, wound healing, and drug delivery.

OBJECTİVE
The study is aimed at investigating the possibility of using decellularized extracellular liver matrix to create a functional 3D in vitro liver model with application in tissue engineering and drug testing.

METHODS
A xenogeneic dECM was obtained from porcine liver and evaluated through macroscopic, histological, and DNA analysis to confirm successful decellularization. Recellularization was performed using the HepG2 cell line, and biocompatibility was assessed by fluorescence imaging and examining the expression of key liver markers (GSTA1, HNF4a, ALB) and conducting functional tests, including urea production and albumin synthesis. The results were compared with a conventional 2D monolayer culture of HepG2 cells. Additionally, co-culture with hepatic stellate cells (LX-2) was performed to explore further model development.

RESULTS
The 3D culture in the dECM matrix demonstrated preserved cellular phenotype after 7 days of culture and the expression of key liver markers, showing its biocompatibility. Functionality tests confirmed improved urea production and a small reduction of albumin synthesis in the 3D model. The decellularized matrix also supported co-culture with hepatic stellate cells, suggesting potential for more complex liver models.

CONCLUSİON
The dECM-based 3D liver model provides a promising platform for liver tissue engineering and drug testing. Future work will focus on further optimizing the co-culture conditions and exploring the model's applications in drug toxicity studies.

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PHAGE – BACTERIA INTERACTIONS AS A MODEL SYSTEM FOR ANALYSING CRISPR FUNCTIONALITY IN BACTERIA.

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CRISPR systems (Clustered Regularly Interspaced Short Palindromic Repeats) constitute an adaptive immune system in bacteria leading to the development of phage resistance. In recent years many studies report those systems as inactive in different bacterial populations due to several different reasons (the presence of antibiotic resistance genes, self-targeting spacers etc.). The aim of this investigation is to study phage - bacteria interactions, in order to establish the functionality of CRISPR systems. Enterococcus faecalis and its phages were used as a model system. This system consists of two E. faecalis strains (one with potentially active CRISPR system, BM15 and one with potentially inactive CRISPR system, NBIMCC 3915) and one E. faecalis specific bacteriophage. CRISPR loci and cas genes were initially identified by PCR. E. faecalis BM15 contains cas gene in its genome and E. faecalis NBIMCC 3915 do not have any cas genes. Each bacterial strain was subjected

to two consecutive phage treatments. Strain E. faecalis BM15 (initially susceptible to the phage) became phage resistant (bacteriophageinsensitive mutant, E. faecalisBIM BM15), while strain E. faecalis NBIMCC 3915 stayed phage sensitive. The total genomic DNA of E. faecalis BM15 and E. faecalisBIM BM15 were sequenced and analyzed by CRISPRCasFinder 4.2.20, CRISPRTarget software and
PHASTEST web server. The results showed inclusion of a whole new CRISPR locus, containing phage spacers in the genome of E. faecalisBIM BM15. Moreover, previous to the viral infection, the E. faecalis BM15 did not contain any intact bacteriophage DNA in its genome compared to the mutant strain. In conclusion, the treatment of bacterial strains with bacteriophages could be a potentially good model system for investigating the functionality of CRISPR systems in bacteria.

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GENOME EDITING IN SARCOMA

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New gene-editing technology, including CRISPR-Cas9 system, Cre-loxP, ZFNs, and TALENS have been used to engineer de novo translocations in sarcoma cells and establish models of cancer.
Sarcoma result of genetic alterations that are directly involved in the tumorigenic process and thus genome editing technologies have application potentials in sarcoma models and therapies.
CRISPR-Cas9 can be used to explore drug treatment and resistance. Modeling sarcoma processes is a multistep process that involves many genetic alterations and epigenetic changes. Thus were induced cancer-relevant t(11;22)(q24;q12) translocations found in Ewing sarcoma. EWSR1-FLI1 fusion protein has a similar activity to that expressed in primary ES cells, providing a powerful tool
for cancer studies. Application of this system will help development of mouse models. The epigenome editing could be applied to clinical and translational applications in biomedical research.

INVESTIGATION OF INFLAMMASOME ACTIVITY DURING SARS-COV-2 INFECTION THROUGH CONFOCAL IMAGING OF ASC SPECK FORMATION

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OBJECTIVE: Replication of the SARS-CoV-2 virus initiates NLRP3 inflammasome assembly that recruits ASC, which then groups into a large protein complex, termed „speck". ASC (apoptosisassociated speck-like protein) contains a CARD that is bind to pro-caspase-1. Activation of caspase-1 leads to the pyroptotic cell death in infected cells. This can lead to inflammatory reactions that are closely related to the development of severe disease.

METHODS: Microvascular lung endothelial cell line HULEC-5a was transfected with 0.2 µg/µL plasmid expressing the ASC protein coding sequence, fused to the fluorescent protein LmCerulean. The ASC fusion emission was monitored using a blue light filter for 24 hours. Consequently, a stimulus for the ASC speck formation was added: 1 µg/µL lipopolysaccharide purified from the E. coli and 13 µM Nygericin, or were co-transfected with a 0.2 µg/µL plasmid encoding for SARS-CoV-2 ORF3a accessory protein (Wuhan wild type sequence), fused to a fluorescent protein mRFP emitting in red light spectra. ASC specks formation was observed in real time using a spinning disk Andor BC43 confocal microscope, and identical size ROI was captured in different wells, every 30 minutes, and analyzed using Imaris software.

RESULTS: The number of the ASC specks per cell was compared in two groups: induced with LPS, followed by nigericin, and co-transfected with the ORF3a SARS-CoV-2 plasmid. In both groups ASC was detected automatically by spot detection protocol with Imaris software. The most active formation of ASC per cell appears after 14 hours after co-transfection with ORF3a Sars-CoV-2 in the first group and after 9 hours of exposure to LPS together with 6 hours of stimulation with nigericin.

CONCLUSION: ASC speck formation can be used as a simple upstream readout for inflammasome activation. Here, we describe a method for analyzing inflammasome activation by ASC speck visualization. Other experiments for detecting the

caspase-1 correlated to ASC formation and analysis of the speck sizes after different stimulation are ongoing.

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INVESTIGATING THE EFFECT OF THE SECRETOME OBTAINED AFTER CO-CULTURING RPMI8226 CELLS AND HUMAN MESENCHYMAL STEM CELLS (HMSCS) ON RPMI8226 CELLS

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Multiple myeloma is the second most common hematologic malignancy characterized by clonal proliferation of malignant plasma cells in the bone marrow. The tumor microenvironment plays a key role in the progression of multiple myeloma. Within this microenvironment, mesenchymal stem cells (MSCs) are crucial components that interact closely with multiple myeloma cells (MMCs). This communication (both direct and indirect) supports survival, proliferation and treatment resistance of MMCs, thus contributing to disease aggressiveness. In the laboratory, reproducing the indirect interaction via treatment with the cells' secretome is essential. By keeping cell morphology and interactions more authentic, 3D models better capture the physiological significance of the bone marrow microenvironment. The development of such models is critical for advancing our understanding of multiple myeloma pathogenesis and for testing novel therapeutic strategies.

OBJECTIVE
To establish an optimized protocol for obtaining human mesenchymal stem cells' and multiple myeloma cells' secretome after co-culture to evaluate the effect of the obtained secretome in establishing a 3D in vitro model of multiple myeloma.

METHODS
Human bone marrow mesenchymal stem (hMSC) cell line was used for co-culture with the multiple myeloma cell line RPMI8226. To determine the presence of cell-cell contact and phenotype change in the cell populations after direct co-culture was done using enzyme linked immunosorbent assay (ELISA) as well as light microscopy imaging. After treatment of the multiple myeloma RPMI882 cells with secretome the cell viability was determined by trypan blue staining. Further evaluation on the secretion of inflammatory cytokines was done using Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit.

RESULTS
A protocol for secretome accumulation after direct interaction between hMSCs and RPMI8226 cells was successfully developed, and data on the cell phenotype changes was obtained. To our knowledge, this is the first time a secretion of IL-8 is detected in RPMI8226 cells. Furthermore, treatment with hMSC secretome (after mono- and co-culture) resulted in an increase in IL-8 secretion. The cell populations keep their viability upon treatment with all of the secretome types.

CONCLUSION
The protocol demonstrated significant potential in developing a functional multiple myeloma model, with data supporting the impact of MSCs on multiple myeloma cells. Further assessments of the model's adequacy are ongoing to optimize its application for future research and therapeutic purposes.

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CASPASE-1 AND CASPASE-3 INTERPLAY IN SERTOLI CELLS: BALANCING INFLAMMATION AND APOPTOSIS IN MALE INFERTILITY

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OBJECTIVE
Recent developments in assisted reproductive technologies have brought to light a growing incidence of „male factor" infertility among couples experiencing reproductive difficulties. Autoimmune reactions targeting germ cells may contribute to infertility, alongside mechanical injuries, infections, or unidentified factors. To address these issues, it is essential to investigate the types of cell death in Sertoli cells, associated with the activation of pro-apoptotic and propyroptotic caspases, as well as their connections and activation pathways. Using flow cytometry, we detected pyroptotic cells with caspase-1 activity, which aids in pathogen clearance and the restoration of homeostasis. However, the prolonged release of inflammatory cytokines can disrupt spermatogenesis through gasdermin-mediated pyroptosis. Our study demonstrated that the activation of the pyroptotic inflammasome signaling pathway can switch to the less harmful apoptotic pathway. We identified this as the preferred cell death mechanism through the detection of active caspase-3 and apoptosis occurrence. By nanopore sequencing, we established the presence of the CD300a receptor and its positive regulation by innate immune signaling pathways recognizing molecular signals for pathogens and its relationship with cell fate in the context of the type of cell death and caspase activity.
Our research revealed that the activation of the pyroptotic inflammasome signaling pathway can transition to the less destructive apoptotic pathway of programmed cell death. This switch occurs through the direct activation of caspase-3 by caspase-1, dependent on an Nlrp3/Asc mechanism. Furthermore, there is a regulatory feedback mechanism from the effector molecule GSDMD that shields Sertoli cells from the actions of caspase-1 and caspase-3.

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MITOPHAGY AND MACROAUTOPHAGY IN PROSTATE CANCER: THERAPEUTIC POTENTIAL OF MODULATING MIR-141 AND MAPK1/ERK2

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OBJECTIVE: Prostate cancer is the most common cancer in adult men worldwide. Timely diagnosis is key to its treatment. Castration-resistant metastatic phenotype formation is supported by reprogramming the cellular responses to oxidative stress. These processes are associated with alteration of autophagy and mitophagy. Determining the molecular reasons of dysregulated miR-141, autophagy-modulating MAPK1/ERK2, and mitochondria-related PCa will aid in the development of novel therapeutic strategies.

METHODS: We used cell culture, transfection, Oxford Nanopore sequencing, mRNA extraction and cDNA synthesis, flow cytometry, RT-qPCR, fragment analysis, fluorescence microscopy Results: Our research shows that MAPK1 upregulates LC3 in LNCaP cells, while it downregulates ATG16L and LC3 in PC3 cells. Under starvation, silencing MAPK1 led to the most pronounced increase in macroautophagy in LNCaP cells, whereas in PC3 cells, starvation remains the primary activation mechanism. MiR-141 rescue leads to enrichment of autophagyrelated and NLR innate immune signaling pathways. MiR-141 enhances macroautophagy and mitophagy in LNCaP cell line, but in PC3 cells, this effect occurs only when miR-141 is combined with starvation.

CONCLUSION: Our study underscores the pivotal role of miR-141 in the regulation of mitophagy in PCa cells, particularly in the context of p53 status. The differential responses of LNCaP and PC3 cell lines to miR-141 mimic and siMAPK1, especially under starvation, emphasise the nuanced roles of both in autophagy and mitophagy. Our findings suggest that the restoration of miR-141 expression offer a promising therapeutic approach for specific PCa genotypes, especially those mirroring the characteristics of the PC3 cell line.

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PROLIFERATION OF MESENCHYMAL STEM CELLS BIOPRINTED USING OSTEOINDUCTIVE BIOINKS

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OBJECTIVE: One of the most recent approaches to treating critical-size bone defects is the implantation of bioprinted scaffolds of biocompatible and biodegradable inks containing osteoprogenitors, for example, mesenchymal stem cells (MSCs). In the development of such alternative bone grafts, the challenge is to use a material with adequate composition and rheology, able to provide support in the regenerative processes both in the tissue architecture and at the cellular level. The aim of the current study is to investigate the proliferation and viability of MSCs bioprinted in two commercial bioinks with osteoregenerative applications in vitro.

METHODS: GelXA Bone and CellInk Bone (CELLINK) bioprints, containing 1x10 MSCs/ml were produced using BioX (CELLINK). Bioprinting was performed using a Temperaturecontrolled printhead in droplet-printing mode. Photocuring toolhe ad (405nm) was used for the crosslinking of GelXA and CaCl crosslinking agent was used to crosslink CellInk. The prints were cultured under standard conditions. Cell proliferation in the prints was measured using Alamar Blue (BioRad) and the results were analyzed using FluostarOptima. Cell viability in the prints was evaluated by confocal microscopy immediately after staining with the Live/Dead kit (Sigma-Aldrich).

RESULTS: Both primary MSCs cultured in prints showed a dependence of the proliferation on the UV intensity in the cross-linking. The prints, cross-linked at 75% intensity of the Photocuring toolhead, were the most stable in shape and size, and cells in them continued to grow until day 28 in contrast to the cells printed under the other tested conditions in both experiments. The addition of CaCl crosslinking agent did not affect proliferation in double cross-linked prints, and cross-linking of GelXA prints with CaCl alone was weakly effective and no cell proliferation was detected in these prints after day 7. Cells bioprinted in CellInk Bone showed a dependence of proliferation on the size of the prints. In small prints (2 to 4 mm droplet diameter), cells slightly increased proliferation until day 35, whereas cells in large prints (4 to 6 mm diameter) showed an increase in values on day 5/7, then steadily decreased in Alamar fluorescence. Confocal microscopy showed an increase in the number of live cells (calcein-positive) in the prints over the culture time corresponding to the proliferation curves, as well as a rapid decrease in the number of dead cells observed only immediately after printing and after that only single PI-positive cells were detected.

CONCLUSION
All data show high survival levels of MSCs in bioprinting with GelXA Bone and CellInk Bone as well as normal levels of proliferation and viability of the cells in the prints in long-term culturing in vitro. GelXA prints, cross-linked at 75% Photocuring toolhead intensity showed the best stability of shape, and size combined with high levels of cell proliferation and vitality.

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SPERM VITRIFICATION: FROM EARLY INNOVATIONS TO FUTURE BREAKTHROUGHS IN REPRODUCTIVE CRYOTECHNOLOGY

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OBJECTIVE
Vitrification (ultra-rapid freezing) is a modern biotechnology used routinely in assisted reproduction centers for cryopreservation of oocytes and embryos. In recent years, the possibility of vitrification of male gametes has been increasingly explored, and the first babies have already been born after using the method.
Sperm vitrification has a number of advantages over conventional (slow) freezing. The duration of the procedure is significantly shortened. The use of penetrating cryoprotectants, which are toxic to the gametes, is avoided. The method allows the conservation of seminal fluid with deteriorated indicators, as well as single spermatozoa obtained by puncture of the testicle. Comparative studies show that vitrification has the best results in terms of sperm motility, apoptosis rate and epigenetic damage.
This report will present the modern achievements in the field of vitrification of male gametes and the pros and cons of different cryopreservation techniques.

IN VITRO OSTEOGENIC DIFFERENTIATION OF BIOPRINTED MESENCHYMAL STEM CELLS

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OBJECTIVE

Critical-size bone defects and diseases leading to impaired bone repair are socially significant health problem. In the search for suitable three-dimensional (3D) models for tissue regeneration and engineering, bioprinting with primary mesenchymal stem cells (MSCs) is one of the newest and promising approaches in stem cells-based therapies. Human bone marrow-derived MSCs play a key role in bone reconstruction in vivo, by differentiating to osteoblasts and osteocytes, making MSCs preferred cells in bioprinting bone implants.

AIM

The focus of this work is to investigate in vitro osteogenic differentiation of bone marrow-derived MSCs bioprinted in 3D constructs.

METHODS

In the present study, bone marrow-derived MSCs, routinely isolated and characterized by the expression of specific stem cell markers and differentiation potential in two-dimensional conditions were used. MSCs containing 3D constructs were printed using BioX (CELLINK). To induce osteogenic differentiation in bioprinted MSCs, constructs were cultured in osteogenic media (DMEM, 10% fetal bovine serum, 100 nM dexamethasone, 10 mM betaglycerol phosphate, 0.2 mM ascorbic acid 2-phosphate) for 28 days. Cell morphology and viability of osteogenic and control prints were visualized by fluorescent live/dead kit. Evaluation of the osteogenic differentiation was estimated by measuring the alkaline phosphatase (ALP) activity and the expression of Runt-related transcription factor 2 (RUNX2) at the mRNA and protein levels.

RESULTS

Confocal microscopy of the bioprinted MSCs showed a typical for 2D cultures fibroblastic morphology and a larger cell size in the control group, while the cells in prints cultured in osteogenic media for 14, 21 or 28 days were smaller and compact, showing a shift in morphology caused by osteogenic differentiation. 13.89 fold higher ALP activity was detected in bioprints cultured in osteogenic media for 28 days in comparison to DMEM cultured controls. Furthermore, RT-PCR analysis showed osteoblast inducer RUNX2 mRNA levels to be elevated 1.9 fold on the 7th day of the osteogenic differentiation compared to the control MSCs and a decrease observed on day 14 and 21 (1.7 fold and 0.6 fold change to DMEM cultured prints). RUNX2 expression was also confirmed at a protein level using immunofluorescence staining.

In Conclusion, the increased expression of Runx2 at mRNA and protein levels, as well as the higher ALP activity confirmed a successful osteogenesis of MSCs in bioprints. Methods routinely used for osteogenic differentiation detection in 2D conditions can be easily modified for detecting in vitro osteogenic differentiation in bioprinted MSCs.

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Списание "Ембриология" е специализирано научно издание на Българска Асоциация по Репродуктивна Човешка Ембриология (БАРЧЕ). В него могат да бъдат публикувани оригинални научни статии и обзори в областта на експерименталната и клинична ембриология и асистираната репродукция. Кратките предварителни съобщения, публикувани в това списание, могат в последствие да бъдат отпечатвани в разгърнат вид и в други научни списания.

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GnRH - gonadotropin-releasing hormone;
QoL - качество на живот.

1. Ryeqo КХП EU/1/21/1565/001-003/23.02.2024
2. Giudice LC et al. Lancet. 2022;399:2267–2279.

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