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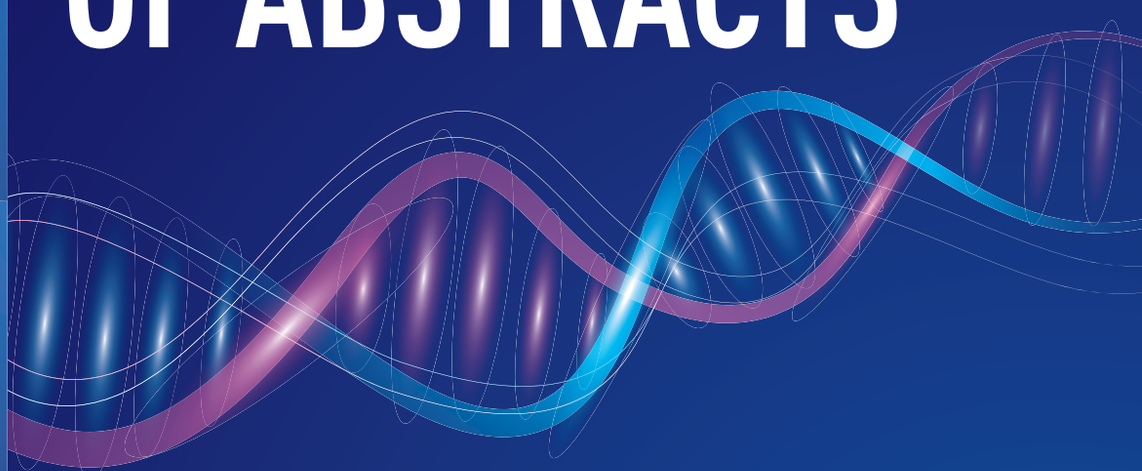
BOOK OF ABSTRACTS



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BIODESIGN

DESIGN OF TISSUE EQUIVALENTS: MSC SPHEROIDS AS BUILDING BLOCKS IN 3D BIOPRINTING

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Novel biomaterials for 3D bioprinting which are mechanically stable, biocompatible and provide proper cell density and viability are of high interest. In this work, we describe the 3D bioprinted system based on natural polymers containing spheroids formed from mesenchymal stromal cells (MSC).

To achieve printable and photocrosslinkable bioink, the mix of fibrin and gelatin was modified with PEG-acrylate and supplemented with riboflavin. As a cellular component of the bioink, spheroids formed from MSC isolated either from human gingiva or human adipose tissue were utilized. To print bioink, 3D extrusion bioprinter BioX CellInk was used. The printability and rheological properties were investigated after the printing. The physiological properties of spheroids (metabolic activity, cell proliferation, sprouting, migration, differentiation capacity) were analyzed at the different cultivation days.

The optimal bioink composition was found to be as follows: fibrinogen 25mg/ml and gelatin 7.5mg/ml (printing temperature 23°C). With the two-step crosslinking protocol (UV light and thrombin) the storage modulus of 188 Pa was achieved. All spheroids were metabolically active, and ones that were printed exhibited better proliferation than manually mixed. In the case of adipose tissue as an MSC source, spheroids shown more active migration and sprouting. Bioprinting promoted differentiation in osteogenic (gingival MSC) or chondrogenic (adipose tissue MSC) directions.

Overall, 3D bioprinting with gelatin-fibrin-based bioink provides the microenvironment for the maintenance of the major functional properties of MSC, which is crucial to the further formation of complex tissues and organs.

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ROLE OF GASOTRANSMITTERS IN VASCULAR SMOOTH MUSCLE CONTRACTIONS IN EXPERIMENTAL METABOLIC SYNDROME

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Hydrogen sulfide (H_2S), along with nitric oxide (NO) and carbon monoxide (CO), is a part of the family of gasotransmitters. H_2S plays a fundamental role in the regulation of cardiovascular homeostasis. It has been shown that H_2S can act as a protective factor for vascular endothelial and smooth muscle cells (SMC) protecting from the damaging effects of oxidative stress and dyslipidemia induced by metabolic syndrome (MetS).

The MS model was performed on male Wistar rats (n=33). Rats were separated into control and experimental groups. The rats from the control group were fed standard rat chow. The rats from the experimental group had a high-fat, high-carbohydrate diet for 12 weeks. The contractile activity of the smooth muscle segments of the rat aorta was studied by the mechanographic method.

It was found that the vasorelaxing effect of NaHS (5-100 μM) decreases in the setting of MetS. The endothelial NO synthase inhibitor L-NAME (100 μM) inhibits the effect of NaHS. The cystathionine-gamma-lyase inhibitor PAG (100 μM) decreases the vasodilating effects of acetylcholine (0.1-100 μM). Addition of the L-arginine (1 mM) as the endogenous NO synthesis precursor causes an increase in the effects of the H_2S donor. Thus, the contractile activity of vascular smooth muscles in MetS is not only due to the effect of H_2S, but also to the effect NO.

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METHODS OF OPTICAL SPECTROSCOPY FOR THE DIAGNOSIS OF EXTRA- AND INTRA-ARTICULAR INJURY

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Extra- and intra-articular injuries are common and prognostically unfavorable if diagnosed late or erroneously. The currently existing methods for diagnosis of osteoarthritis only allow the determination of cartilage damage at the macroscopic level associated with the final stage of the disease. Thus, the development of new techniques that allow minimally invasive or non-invasive rapid detection of early stages of cartilage degradation, occurring at the molecular level and not causing significant mechanical damage, is in high demand.

In this work, a device for ex vivo analysis of the cartilage was developed. The setup implements diffuse reflectance spectroscopy (DRS) and fluorescence spectroscopy in the near infrared spectral range. We examined explants obtained during the surgical operations carried out at the I.M. Sechenov First Moscow State Medical University. This study was approved by the LEC.

DRS measurements, allowing estimating the water content in the tissue, were carried out using the developed setup. Analysis of changes in the water properties in the cartilage tissue in normal and pathological conditions was supplemented by the study of intrinsic IR fluorescence. The cartilage mechanical parameters were determined as a result of indentation to understand their correlations with the features of optical spectra. Conducted experiments show that the water content in the cartilage tissue correlates with its thickness. It was also shown that the level of IR fluorescence depends on the cartilage condition, however, it is necessary to expand the number of samples measured to assess the reliability of the observed differences. The next step of the study will be the application of the developed methodology for assessing the state of the cartilage intraoperatively.

Acknowledgments: This work was carried out with the help of the Russian Science Foundation (grant 21-79-10325).

GENOMIC AND ANCESTRAL VARIATION UNDERLIES THE SEVERITY OF COVID-19 CLINICAL MANIFESTATION IN INDIVIDUALS OF EUROPEAN DESCENT

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Background: The coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is characterised by a wide spectrum of clinical phenotypes ranging in acuteness from asymptomatic, symptomatic with mild or moderate manifestation and severe involving pneumonia and respiratory distress. COVID-19 susceptibility, severity and recovery has demonstrated high variability worldwide. Advanced

age or presence of co-occurring attributes, such as type 2 diabetes, cardiovascular disease or extrinsic factors, such as smoking are known to exacerbate COVID-19 prognosis. Variances in the host genetic architecture may potentially control the inter-individual and population scale differences in COVID-19 presentation.

Methods: We performed a genome-wide association study (GWAS) employing the genotyping data from AncestryDNA COVID-19 host genetic study that included COVID-19 positive patients and healthy individuals who had tested negative for SARS-CoV-2 infection at the time of recruitment. We restricted our analysis only to the individuals of European descents to avoid genetic structure in the dataset, arising due to the presence of people from different ancestries. Further, we employed the asymptomatic individuals as controls instead of healthy individuals. We argue that the absence of perceptible known disease symptoms in the asymptomatic COVID-19 subjects make them more valuable as controls as their genetic make-up might be playing a crucial role in protecting them from severe disease outcomes. GWAS was performed in PLINK v1.9 with and without age correction. The genomic ancestry of COVID-19 patients was determined using ADMIXTURE v1.3 and qpAdm algorithm implemented in AdmixTools v5.1.

Results and Discussion: Our data revealed striking genomic differences between COVID-19 asymptomatic and severely symptomatic individuals. We identified 621 genetic variants that were significantly distinct (Multiple-testing corrected $P<0.001$) between asymptomatic and acutely symptomatic COVID-19 patients. These variants were found to be associated with pathways governing host immunity, such as innate and adaptive immune system, interferon signaling, interleukin signaling, antigen processing by MHC, cytokine signaling and known COVID-19 comorbidities, such as obesity, cholesterol metabolism and smoking. Our ancestry analysis, employing *qpAdm* algorithm revealed that asymptomatic individuals possess discernibly higher proportions of Ancestral North Eurasian (ANE) and Eastern Hunter Gatherer (EHG) ancestry and lower fractions of Western Hunter Gatherer (WHG) ancestry, while severely symptomatic patients have higher fractions of WHG and lower ANE/EHG ancestral components, thereby delineating the likely ancestral differences between the two groups. Overall, our studies suggest that asymptomatic individuals derived significantly larger proportions of their ancestry from ANEs/EHGs, which was introduced to Europe through Bell Beaker culture (Yamnaya related), a smaller proportion of indigenous WHG ancestry fractions, while severely symptomatic COVID-19 patients possess significantly larger fractions of WHG related ancestry.

POSSIBLE INTERCONNECTION BETWEEN DIFFERENT MORPHOLOGICAL PATTERNS OF CORNEAL ENDOTHELIUM/DESCEMET' MEMBRANE COMPLEX AND AQUEOUS HUMOR TGF-B1 LEVEL IN EYES WITH PSEUDOPHAKIC BULLOUS KERATOPATHY OR FUCHS ENDOTHELIAL CORNEAL DYSTROPHY

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Background: Chronic corneal edema is associated with Fuchs endothelial corneal dystrophy (FECD) or endothelial cell damage — pseudophakic bullous keratopathy (PBK). FECD is a genetic disorder characterized by loss of endothelial cells and deposition of extracellular matrix (ECM). Well known that TGF-β1 induces ECM deposition.

Purpose: To explore the interconnection between morphological changes of corneal endothelium/Descemet' membrane (EDM) complex and aqueous humor (AqH) TGF-β1 level in patients with PBK and FECD.

Methods: The study included 8 patients (8 eyes) with PBK and 12 patients (12 eyes) with FECD. AqH samples were collected during endothelial keratoplasty. The AqH TGF-β1 level was measured by TGF-β1 ELISA assay kit (Cloud-Cone Corp.) and detected at MultiSkan FC (Thermo Fisher). Intraoperatively obtained recipients' EDMs were immunostained using anti-ZO-1 antibodies with Alexa Fluor 594 label (both — Thermo Fisher Scientific). Cell nuclei were contrasted by Hoechst 33342. The images were captured in phase-contrast and fluorescence mode on Zeiss Axiovert A1.

Results: TGF-β1 level was elevated in FECD group compared to PBK ($p<0.005$). Both PBK and FECD specimens presented regions of enlarged endothelial cells with lower levels of ZO-1 labeling. In PBK there was a significant endothelial cell nuclei enlargement. In FECD corneal endothelial cells showed fibroblastic morphology. Elongated corneal endothelial cell nuclei clustered around ECM excrescences (guttae).

Conclusions: FECD and PBK are the conditions caused by different structural changes of EDM. TGF-β1 may play a pivotal role in ECM accumulation and guttae formation in FECD.

CYTOKINE LEVELS AND MACROPHAGE RESPONSE INDICATORS IN PATIENTS WITH IMPLANT LOOSENING AFTER *PRIMARY KNEE ARTHROPLASTY*

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Objective. To study the serum cytokine levels, levels of MIF, MSP in patients with implant loosening after *primary knee arthroplasty*.

Material and methods. We examined 47 patients with implant-associated inflammation (main group); 43 patients with aseptic implant loosening (comparison group), and 20 healthy individuals (control group). Cytokine serum levels, MIF, MSP were determined with ELISA in patients before the surgeries as well as in 1 and 12 month of them; in the controls these indicators were determined unitary. The statistical processing was performed with Mann-Whitney U-test and Wilcoxon signed-rank tests.

Results. TNFα, IL-6 levels increased 1.33-fold in 1 month and 1.67-fold in 12 months in the main group as compared to the controls. IL-10 decreased 1.23-fold in 1 month and 1.39-fold in 12 months. MIF increased 2.39-fold in 1 month and 3.60-fold in 12 months of the surgeries as compared to the controls. MSP increased 1.11-fold in 1 month, 1.18-fold in 12 months of the surgeries as compared to the controls.

Conclusion. Oppositely directed changes in serum of cytokines as well as the increase of MIF, MSP may be of concern in the progress of implant loosening after *primary knee arthroplasty.*

BIOTIC TECHNIQUES IN ARTHROPLASTY OF LARGER JOINTS IN OSTEOARTHROSIS PATIENTS

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Background. The primary arthroplasty of larger joints enables the recovery of the function that was lost due to the inflammatory destruction in certain segments of the human body. However, the influence of the initial specifics of metabolic and cell responses on the outcome of this type of high-tech medical intervention is understudied.

Material and methods. We performed a retrospective analysis of the pre-surgery examination of 36 patients of both genders 45.4±6.3 who needed revision joint replacements as well as 15 healthy individuals. We studied the pattern of lymphocyte subpopulations (T and B-lymphocytes, and natural killers) in the whole blood samples using Facs Canto II cytometer. The bone turnover markers were determined in blood serum with ELISA test using bone alkaline phosphatase (BAP) MicroVue Bone Health (BAP), USA; N-Mid Osteocalcin Elisa (IDS), and collagen fragments using Serum CrossLaps (Nordic Bioscience Diagnostics, Denmark).

Results. In 19 examined patients we observed the increase in SerumCrossLaps concentrations to 0.54 (0.49; 0.57) ng/ml along with BAP decrease to 24.2 (21.3; 26.4) u/l as compared to the controls (0.39 (0.37; 0.42) ng/ml and 37.1 (34.6; 39.1) u/l respectively. In 11 patients of this group we found the decrease in the blood concentrations of CD3+1009.2 (964.3; 1022.8) x 109/l due to CD3+CD8+ to 421.3 (408.2; 431.8) x 109/l and CD3+C4+:617.6 (611.5; 625.8) 109/l as compared to the controls (1740 (1701.2; 1779.1) x 109/l; 689.2 (677.4; 694.4) x 109/l and 998.2 (994.1; 1009.3) x 109/l.

Conclusion. The success of the joint implant integration in bone-implants interaction depends on the number of autogenous and exogenetic factors, it summons the simulation of biotic prediction models with regard to personalized indicants of bone turnover and cell immunity.

DIGITALIZATION OF NUTRIGENOMIC STUDIES AS A PROSPECT FOR NUTRITION PERSONALIZATION

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The current state of nutrigenomics in combination with digital technologies allows us to propose new approaches to assessing the effectiveness of functional foods (FF). The goal of the project is to use nutrigenomics to determine the dose-effect relationship, variability and duration of the effect when several FFs are exposed to the human body. The experiments used: Whey protein concentrate FitPROTEIN, SPORTAMIN® BCAA 6000, caffeine and glucan in the form of dietary supplements. Scrapings of epithelial cells of the oral mucosa served as biological material. The study involved 28 male volunteers of 20-25 years old. They were divided into 4 groups of 7 people. Members of each group consumed one of 4 studied FF for a period up to 20 days. The target genes for each FF were selected on the basis of the scientific literature data analysis. In particular, the CYP1A2 gene was for caffeine, the FTO gene was for FitPROTEIN and BCAA 6000 and the CADM1 gene was for glucan. The interaction between genes and FF (gene expression) was studied according to standard procedures.

Individual differences in gene expression were explained by the presence of different polymorphic variants in their genes. This means that a personalized approach to BCAA 6000 and caffeine intake is required, based on a preliminary determination of the consumer's genotype (genetic testing).

An algorithm for evaluating the effectiveness of FF based on nutrigenomic studies is proposed. The further development of nutrigenomics should rely on digital technologies. It seems promising to transfer experimentation to the virtual sphere through the use of digital twins technology, including in combination with the IOT.

A NEW METHOD BASED ON TERBIUM-INDUCED FLUORESCENCE FOR FAST ANALYSIS OF CARBOHYDRATE DEFICIENT TRANSFERRIN (CDT) THE MOST RELIABLE BIOMARKER OF ALCOHOL ABUSE IN CLINICAL AND FORENSIC CONTEXTS

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CDT, a group of transferrin glycoforms with a low degree of glycosylation, is well-known biomarker of chronic alcohol abuse, with application in traffic medicine, alcohol abuse treatment and follow-up of liver and heart transplants. Recent research has reported its better performance in comparison to traditional biomarkers such as GGT, liver enzymes, MCV, etc. However, accurate CDT determination, because of its complex nature, requires separation methods able to discriminate among a number of similar glycoproteins with high sensitivity and selectivity. Capillary electrophoresis and gradient HPLC with UV/Vis detection are currently the suitable analytical techniques for this purpose. In order to reduce instrumental complexity and costs as well as analytical runtimes, a new method has been developed which taking advantage of the high sensitivity and selectivity of terbium-induced fluorescence [based on Fluorescence Resonance Energy transfer (FRET)] shows higher analytical speed than the current methods with higher sensitivity and selectivity. In summary, the method uses a novel flow-modulated liquid chromatography technique with detection by recording the fluorescence signal at 550 nm wavelength (excitation at 298 nm). The chromatographic separation needs only 5 minutes.

The method was validated according to the current guidelines of analytical chemistry showing adequate accuracy and precision. The method was tested in parallel with HPLC-Vis on 131 sera showing an excellent correlation of results proved by a correlation coefficient of 0.995 (Pearson's r).

The proposed approach will make available the analysis of CDT outside specialized laboratories, such as in occupational medicine centers, doctor's offices, small laboratories and alcohol rehabilitation centers.

LEUKOCYTE TELOMERE LENGTH AND RESPONSE TO ANTIANGIOGENIC THERAPY IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a multifactorial disease that is becoming the main cause of vision loss in people over 60 years of age. The aim of the investigation was to study the relationship between leukocyte telomere length (LTL) and the parameters of the effectiveness of anti-VEGF therapy in the treatment of neovascular AMD. The dynamics of biomarkers of disease activity was analyzed according to optical coherence tomography and viso-functional results in 110 patients with AMD. LTL was assessed by qPCR. Positive dynamics of best corrected visual acuity (BCVA) was noted in 100% of eyes, while the median BCVA after 3 initial injections was 0.3 [0.1-0.6], after 5 injections — 0.5 [0.3-0.8]. The final visual acuity was 0.6 [0.4-0.9]. The central retinal thickness (CRT) decreased after the 3rd injection to 265 [234-306] μm, by the end of treatment — to 211 [190-262] μm. CNV type 2 (classical) determined lower BCVA values at baseline and in the course of treatment. The retention of activity of the subretinal neovascular membrane (SNM) at the end of the observation correlated with lower values of the initial BCVA and higher values of the initial CRT. In patients with shorter LTL, the signs of active membrane were more often detected after 3 injections. Patients with longer LTL are more likely to have an early response to the therapy, accompanied by a transition to an inactive SNM. Thus, LTL shortening is associated with greater SNM activity and the need for more intravitreal injections in AMD patients. Supported by the RSF grant No. 21-15-00047.

MATRIX-BOUND NANOVESICLES IN 2D- AND 3D CULTURE MODELS

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Matrix-bound nanovesicles (MBVs), a subgroup of extracellular vesicles (EVs) embedded within extracellular matrix, were reported to perform local regulation of the inflammation and healing and are therefore of great interest in tissue engineering and regenerative medicine. Cultivation conditions, namely 2D or 3D culturing, are known to affect the content of EVs, their properties, and functions. We established several culturing models allowing MBVs production under controlled conditions and assessed their yield and size.

MBVs were extracted from human umbilical cord mesenchymal stromal cells (UC-MSCs), and mouse NIH/3T3 fibroblasts cultured in 2D and 3D conditions via differential centrifugation. Their yield and size were assessed via dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) methods. Macromolecular crowding was used to maximize the MBVs' yield in 2D and 3D UC-MSCs cultures.

The size of the obtained nanoparticles corresponded to that reported in the literature (40-100 nm). NIH/3T3 fibroblasts showed significantly higher yield of MBVs than UC-MSCs, while the initial number of cells was equal. In the MSCs culture a greater yield of MBVs was observed when cultured in 2D conditions, while in the fibroblast culture it was observed in 3D conditions. The addition of ascorbic acid to UC-MSCs 2D cultures increased the MBVs yield ~1.5 fold, while the addition of both ascorbic acid and carrageenan increased their yield ~10 fold. In the samples from 3D cultures no positive effect of macromolecular crowding was observed.

Our findings might help to establish a culturing model with sufficient MBVs yield for their further thorough characterization.

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SNP GENOTYPING OF MYCOBACTERIUM TUBERCULOSIS STRAINS USING DNA HYBRIDIZATION PROBES

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Tuberculosis is an old and severe disease caused by *Mycobacterium tuberculosis* bacteria. Even though tuberculosis is curable and preventable nowadays, it is ranked 13th on the list of Leading Causes of Global Deaths in 2019, according to the WHO. At present, there is an increasing demand for new methods for diagnosing drug-resistant strains of *Mycobacterium tuberculosis* bacteria. The main criteria are analysis cost, simplicity, analysis speed, no need for expensive equipment and availability for countries with a low budget. Such criteria can be met by a modern genotypic method using the approach of DNA technologies. In this work, we propose a new diagnostic method based on DNA hybridization probes. They are represented by deoxyribozymes, synthetic single-stranded DNA molecules with catalytic activity. This technique can genotype a single nucleotide polymorphism (SNP) responsible for the resistance of this bacteria strains to antibiotics. The system utilizes a fluorescent signal for SNP detection: in the presence of SNP, the signal can be found. The main step included in this analysis is the amplification of the gene region of interest followed by the use of the diagnostic system and spectrophotometric analysis of the research results. The study is carried out using extracted DNA of *Mycobacterium tuberculosis* bacteria. The system is capable of diagnosing double-stranded DNA amplicons therefore the research can provide a foundation for a variety of applications in the express molecular diagnosis of human infections.

THE COOPERATION OF OSTEOGENESIS AND ANGIOGENESIS IN ADSC-DERIVED SPHEROIDS

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To provide better regeneration *in vivo* bone bioengineering techniques are aimed at pre-vascularization of bioequivalent. However, the cooperation of molecular pathways regulating osteogenesis and angiogenesis *in vitro* is still poorly understood, with insufficient vascularization in artificial bone tissue still being one of the challenges in tissue engineering. Thus, this study set out to investigate the influence of angiogenic and osteogenic induction factors on the 3D culture of human adipose-derived stromal cells (ADSCs). Four groups of spheroids (intact spheroids, spheroids with osteogenic induction, endothelial induction, and double induction) were analyzed by qPCR, immunocytochemical staining, Western Blot analysis, and angiogenesis assay in fibrin gel. Complex analysis of expression and synthesis of key factors indicated that ADSC spheroids were capable of spontaneous differentiation in both directions but with a predominance of osteogenic differentiation. The simultaneous addition of osteogenic and angiogenic inducing agents was able to modify this process through the angiogenesis-related genes promoting further maturation of osteoblasts. Spheroids from different groups formed CD34+ tubule-like structures in fibrin gel, and the addition of osteogenic factors led to the more branched and less structured net of tubules. The current study showed that ADSC spheroids were capable of spontaneous differentiation, while double induction stimulated osteogenesis, also providing angiogenesis. These findings can contribute to a better understanding of the cross-talks of differentiation processes and open new approaches to the generation of vascularized bone tissue bioequivalent.

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MECHANICAL ENHANCEMENT AND KINETICS REGULATION OF FMOC-DIPHENYLALANINE HYDROGELS BY THIOFLAVIN T

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The self-assembly of peptides is a key direction for fabrication of advanced materials. Novel approaches for fine tuning of macroscopic and microscopic properties of peptide self-assemblies are of a high demand for constructing biomaterials with desired properties. In this work, while studying the kinetics of the Fmoc-Diphenylalanine (Fmoc-FF) dipeptide self-assembly using the Thioflavin T (ThT) dye, we observed that the presence of ThT strongly modifies structural and mechanical properties of the Fmoc-FF hydrogel. Notably, the presence of ThT resulted in a tenfold increase of the gelation time and in the formation of short and dense fibers in the hydrogel. As a result of these morphological alteration higher thermal stability, and, most important, tenfold increase of the hydrogel rigidity was achieved. Hence, ThT not only slowed the kinetics of the Fmoc-FF hydrogel formation, but also strongly enhanced its mechanical properties. In this study, we provide a detailed description of the ThT effect on the hydrogel properties and suggest the mechanisms for this phenomenon, paving the way for the novel approach to the control of the peptide hydrogels' micro- and macroscale properties.

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THE USE OF AUTOLOGOUS CD34 + CELLS IN THE MEDICAL REHABILITATION OF NEUROLOGICAL DISORDERS CAUSED BY THE NOVEL CORONAVIRUS INFECTION COVID-19 SARS-COV-2: A REVIEW OF THE LITERATURE

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Justification. The long-term consequences of SARS-CoV-2 infection are of increasing concern. "Post-COVID-19 syndrome" characterized by impaired function not only of the lungs, but affects all levels of the nervous system.

Aim: to improve the efficiency of rehabilitation of patients with central nervous system damage caused by a previous coronavirus infection.

Materials and Methods: We searched for articles published in peer-reviewed journals indexed in pubmed, Wos, scopus, and RSCI. We analyzed 45 articles on cell technologies and immunotherapy in neurology, of which 39 are included in this review.

Results. The inclusion of stem cells (SC) in rehabilitation programs for patients with CNS injuries is a promising area. Possible mechanisms of therapy based on the use of pluripotent stem cells (SC), including CD34 +, include many aspects. On the background of SC transplantation, damaged nerve cells and surrounding tissues, including neurons and glial cells, can be restored, which helps to ensure the integrity of the nerve conduction pathway and restore nerve function. The introduction of autoCD34 + SC is performed intrathecally by spinal (lumbar) puncture. The dose of autoCD34 + SC is determined by the content of CD34 + cells and is not less than 1x106 CD34 + cells per 1 injection. Autologous hematopoietic stem cells (HSC) obtained from the patient himself do

not cause immunological conflicts, and, accordingly, do not require immunosuppressive therapy, unlike donor (allogeneic) and xenogenic cells. Thus, the patient does not experience disturbances in the natural mechanisms of anti-infectious and antitumor control.

Conclusion. A prospective, controlled, open, single-center study is planned, with the inclusion of patients who have undergone coronavirus infection caused by SARS-Cov-2, with the presence in the late period of the "postcoid" syndrome associated with lesions of the central and peripheral nervous systems. The study plans multiple injections of autoCD34 + SC with an interval of 28-30 days with rehabilitation courses in the intervals between injections.

ASSOCIATION OF POLYMORPHISMS OF CONNECTIVE TISSUE HOMEOSTASIS GENES WITH ADVANCED GLYCATION END PRODUCTS IN PATIENTS FOR INCISIONAL HERNIA

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Incisional hernia is one of the most frequent complications after abdominal surgery. Biomarkers for the prediction of hernias do not yet exist. The advanced glycation end products (AGEs) accumulate in tissues and organs, impairing their functions and causing age-dependent diseases. AGEs lead to intracellular damage and change the structure and function of proteins, damage DNA and form protein crosslinking during glycation.

Purpose. To study the association of connective tissue homeostasis genes (*EFEMP1, WT1*, *EBF2*) with advanced glycation end products in patients with incisional hernias.

Materials and methods. Our study involved 23 relatively healthy volunteers with no history of anterior abdominal wall hernias. The main group included 24 patients with incisional hernias. Genotyping was performed by TaqMan-based PCR. The AGEs study was measured using the a Skin Auto Fluorescence (SAF)-reader. All calculations were performed in the SNPStats program.

Results. When analyzing the association of connective tissue homeostasis genes, a reliable ($p = 0.018$) association of the skin auto fluorescence index with one (*EFEMP1*) of the three studied genes was revealed.

Conclusion. The revealed association may prove the effect of glycation end products on the risk of incisional hernias.

INFLUENCE OF BMP2, BMP4 AND TGFB3 ON THE DIFFERENTIATION OF CHONDROCYTES AND CHONDROPROGENITORS ON SCAFFOLDS IN VITRO

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Currently one of the most promising technologies in the field of cell therapy for damaged articular cartilage is autologous chondrocyte transplantation (ACI) and its scaffold-containing modification — MACI. Nevertheless, this technology is complicated by partial dedifferentiation of chondrocytes during in vitro expansion. We hypothesize that chondrogenic potential can be preserved during expansion period by morphogens of the BMP family.

We used a cellular 3D-model that combines chondroprogenitor cells isolated using the surface marker CD73 in rats and a commercial collagen scaffold Chondro-Gide®, pre-impregnated with various morphogens, including BMP2, BMP4, and $TGF\beta_3$. The cells were planted on the prepared membrane and thereafter cultured for 7 days.

Subsequent immunohistochemical analysis showed that all morphogens promote chondrogenic differentiation of cells, which was shown by an increase in the expression of SOX9, a specific marker of chondrocytes. However, BMP2 also directs cells (both chondrocytes and progenitors) to a hypertrophied state, which is fraught with further bone formation in place of cartilage. A similar effect, albeit less pronounced, was also observed in the

BMP4+chondroprogenitors and TGFβ$_3$+chondrocytes groups. Analysis of the expression of collagen II showed that TGFβ$_3$ promotes more collagen deposition in the culture of chondrocytes, while BMP4 promotes deposition in the progenitors group.

Based on the obtained results, we concluded that TGFβ$_3$ showed the most promising outcome because BMPs caused chondrocyte hypertrophy, the undesirable process during tissue engineering of articular cartilage. Generally, our data suggest that employment of morphogens during in vitro phase of MACI has a great potential to further improve this technology.

MONITORING OF DEGRADATION OF THE PERICARDIUM SCAFFOLDS BY LABEL-FREE MULTIPHOTON FLUORESCENCE LIFETIME IMAGING

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Artificial biomaterials can significantly increase the rate of tissue regeneration. However, implantation of scaffolds leads not only to the accelerated tissue healing, but also to an immune response of the organism, which results in the degradation of the biomaterial. The synergy of the immune response and scaffold degradation processes largely determine the efficiency of the tissue regeneration. Still, methods suitable for fast, accurate and non-invasive characterization of the degradation degree of biomaterial, which are applicable both for in vitro and in vivo, are highly demandable.

In this work we show the possibility of monitoring of the degradation of decellularized bovine pericardium scaffolds under conditions mimicking the immune response and oxidation processes using multiphoton tomography combined with fluorescence lifetime imaging (MPT-FLIM). We found that the average fluorescence lifetimes of genipin-induced crosslinks in collagen and oxidation products of collagen are prominent markers of oxidative degradation of scaffolds. This was verified in model experiments, where the oxidation was induced with hypochlorous acid or by exposureexposed to activated neutrophils. The fluorescence decay parameters also correlated with the changes of micromechanical properties of the scaffolds as assessed using atomic force microscopy (AFM). Our results suggest that FLIM can be used for quantitative assessments of the properties and degradation of the scaffolds essential for the wound healing processes in vivo.

This work was supported by Russian Foundation for Basic Research Project No. 20-015-00480 (scaffolds degradation experiments and FLIM) and by the Russian Science Foundation, Project No. 18-15-00401 (pericardium scaffolds preparation and analysis).

IMPROVEMENT OF THE ELECTRICALLY CONDUCTIVE PROPERTIES OF A MATERIAL BASED ON MULTI-WALLED CARBON NANOTUBES AND CALCIUM PHOSPHATE USING LASER PULSED RADIATION

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Abstract. One of the most important tasks of bone tissue engineering is the formation of new biomaterials and the search for methods to improve their properties. Calcium phosphate (CP) has properties similar to native bone. CP in the composition of a biomaterial is able to provide biocompatibility, increase the rate of proliferation and adhesion of osteoblasts. In bone tissue engineering, electrical stimulation (ES) is a new tool for accelerating cell growth. This method is able to influence intracellular processes and stimulate cell proliferation, migration and differentiation. To use ES, materials are required that combine biocompatibility and electrical conductivity. Multi-walled carbon nanotubes (MWCNTs) are characterized by high electrical conductivity, which allows them to be used as a component that improves electrically conductive properties.

The work presents a method for creating a biomaterial from CP and MWCNT with the addition of ethyl alcohol. The dispersions were airbrushed onto glass slides with varying numbers of layers. The resistance of the obtained samples was measured with a multimeter, and the electrical conductivity was calculated. Areas on the samples were exposed to different laser powers to improve electrical conductivity. The surface of the samples was studied under an optical microscope. The samples have properties that allow them to be used as a substrate for the growth of bone cells followed by ES.

BIOENGINEERING USING PHOTONIC AND CELLULAR TECHNOLOGIES

FLEXIBLE STRAIN SENSOR BASED ON CARBON NANOTUBES IN A POLYMER MATRIX FOR HUMAN MOTION DETECTION

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The development of smart and personalized medicine requires new devices for health monitoring. Flexible strain sensors are especially demand in rehabilitation after injuries and movement disorders. Due to their soft design, they can be easily attached to the patient's body and track the recovery process without causing discomfort. A fully biocompatible strain sensor based on Ecoflex polymer and multi-walled carbon nanotubes (MWCNTs) has been developed for human motion detection. The sensitivity mechanism is based on the change in the electrical resistance of the sensor in response to tensile/compression/bending deformations. Using laser radiation, electrically conductive MWCNT networks were formed and hermetically encapsulated in a non-conductive polymer matrix. The developed sensor demonstrates good sensitivity to deformations: the average gauge factor in tension is 4.8, the average gauge factor in bending is 0.9%/°, elongation is up to 725%, tensile strength is 1.4 MPa. The sensor showed a linear response with low hysteresis <5%. The electronic device for reading and processing the sensor signals was made based on the ATXMEGA8E5-AU microcontroller. The device was tuned to the sensor operating range in the electrical resistance range of 5-150 kOhm. The Bluetooth module made it possible to transfer the received data to a personal computer. The developed sensor is a fully wearable and can be useful for registering deformations of human body in health monitoring and rehabilitation. Effective reading of movements was demonstrated when the sensor was integrating with the joints of a human hand.

PANC-1 SPHEROIDS AS 3D MODEL FOR THE DETERMINATION OF ANTICANCER DRUGS ACTIVITY

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Three-dimensional (3D) cell culture has undeniable advantages in closely simulation the in vivo architecture and microenvironment of tissues and organs. Spheroids are the most attractive 3D model because of their uniformity and reproducibility. One of the key features of spheroids is that they can be composed of one or several types of cells. When testing anticancer drugs, the formation of heterospheroids from different types of cells opens up opportunities for much more accurate reconstruction of the tumor structure. However, is this complication of the model justified and does the more complex composition of the spheroids affect the activity of the drugs? To answer this question, we tested the activity of eight anticancer drugs on homospheroids consisting of pancreatic cancer cells (PANC-1) and heterospheroids consisting of a triple co-culture of PANC-1, primary human fibroblasts (HF) and endothelial cells (HUVEC). To assess the cytotoxic activity of the drugs, they were also tested on heterospheroids from HF and HUVEC. It was found that the use of heterospheroids from several types of cells, which more accurately reflect the heterogeneous tumor microenvironment, does not lead to a noticeable change in the activity of the drugs. The largest gap between the total (antiproliferative and cytotoxic) and net cytotoxic activity of drugs was observed for oxaliplatin and gemcitabine, which are classic drugs for the treatment of pancreatic cancer, indicating the relevance of spheroids as *in vitro* models for drug testing.

CARTILAGE TISSUE ENGINEERING ON ELECTROSPUN THERMORESPONSIVE MATRICES

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Traumas and degenerative processes of hyaline cartilage are among the most prevalent disorders in orthopaedics. Tissue engineering is a promising alternative to pharmacotherapy and endoprosthetic surgery, but available techniques possess certain limitations. Our study aimed to develop a new approach to obtaining scaffold-free cartilage-like tissue-engineered constructs.

The solution of thermoresponsive polymer pNIPAM-NtBa was electrospun to obtain fibrous matrices (average fiber diameter 2 μm) that were seeded with bone marrow-derived multipotent mesenchimal stromal cells (1.5×10^5 cells per cm^2). After cultivation in chondrocyte differentiation medium for 7, 14 or 21 days, the constructs were immersed into cold Hank's solution (4°C, 5 min) to dissolve the matrices. The collected cell sheets were washed to remove the residual polymer and analyzed with histological methods, immunocytochemical staining and atomic force microscopy (AFM).

Dense 70-100 μm-thick constructs with parallel-oriented collagen fibrils formed by day 21. The amount of collagen type I decreased and collagen type II (with parallel orientation) increased during the cultivation process. Cartilage-specific proteoglycan aggrecan and transcription factor Sox9 were widely represented in 21-day cell sheets.

The preliminary AFM results demonstrated that Young's modulus in areas with a prevalence of cells was 4.0±1.0 kPa, and in areas with presumably high extracellular matrix content reached dozens of kPa.

To sum up, the described approach allows to obtain the scaffold-free cell constructs with abundant oriented extracellular matrix, characteristic of cartilage tissue, which makes it a promising technology for the regeneration of articular cartilage injuries.

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REDOX-ACTIVATION OF NEUTROPHILS INDUCED BY PERICARDIUM SCAFFOLDS

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Implantation of scaffolds causes the local inflammatory response whereby the early recruitment of neutrophils is of great importance not only for fighting the infection, but also for facilitating the effective regeneration. Using the decellularized bovine pericardium (collagen type I) crosslinked with genipin (DBPG), we demonstrated the redox-activation of neutrophils in whole blood as well as in suspension of isolated neutrophils in plasma after the exposure to extracellular matrix scaffold. Oxidative burst of neutrophils, i.e. increase in the production of free radicals and oxidants, was demonstrated by luminol-dependent chemiluminescence and flow cytometry. ELISA revealed the secretion of a neutrophil-specific enzyme myeloperoxidase. Formation of neutrophil extracellular traps (NETs)-like structures in the blood was demonstrated by confocal microscopy. In addition, we studied platelets, another important player of immediate immune host response. We found that platelets amplified neutrophil redox-activation by the pericardium scaffold, and likely participate in the NETs formation. Free radicals generated by neutrophils and hypochlorous acid produced by myeloperoxidase are portent oxidizing agents which can oxidatively degrade biological structures. Hence, the scaffold-triggered activation of neutrophils causing modification of the extracellular matrix may affect the macrophage response and the wound healing process. Understanding the mechanisms and consequences of the pericardium scaffold oxidative modification by neutrophils is important for the development of new approaches to increase the effectiveness of tissue regeneration. This study was supported by the Russian Foundation for Basic Research (project no. 20-015-00480).

ANALYSIS OF PHOTOLUMINESCENT PROPERTIES OF UPCONVERSION NANOPHOSPHORS $NaYF_4:Yb^{3+}:Er^{3+}/NaYF_4$

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Upconversion nanophosphors (UCNPs) doped with erbium ions are actively used in biophotonics and regenerative medicine as highly effective photoluminescent labels and components: visualization of living cells *in vitro* and tissues *in vivo*, as well as the manufacture of biosensors in particular for local real-time temperature measurement.

Bare and coated with $NaYF_4$ inert shell UCNPs β-$NaYF_4:Yb^{3+}:Er^{3+}$ (20% Yb, 2% Er) in two size ranges (10÷25 and 200÷390 nm) were synthesized by thermolysis method. The intensity and lifetime of UCNPs photoluminescence enhance with increasing nanoparticle size and coating with the inert shell. This is due to a large amount of lanthanide ions inside the larger particles volume and relatively smaller contribution of surface quenching. It is shown that the integral radiation conversion coefficient can increase by a factor of 2÷10 due to the effective shielding of luminescent centers from surface defects after coating the UCNPs with an inert shell.

For the next studies, it is important to select UCNPs of a proper size for various tasks of non-invasive imaging of tissues and structures inside the body. On the one hand, nanoparticles can easily penetrate into living tissues, on the other hand, larger UCNPs with a high conversion coefficient can be applied for visualization of tissue-engineered structures.

The reported study was funded by RFBR according to the research project №20-32-90218.

BIOPHOTONICS AND MICROCIRCULATION (SPIE)

MODERNIZATION OF THE PARAMETERS OF THE DIGITAL DIAPHANOSCOPE LED APPLICATOR BASED ON THE SPECTRA OF PUS SINUSITIS

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Preliminary studies by digital diaphanoscope on healthy volunteers and patients with maxillary sinuses diseases, and conducted numerical simulation, have shown that the radiation sources with a wavelength of 980 nm are more informative for the diagnosis of patients with purulent contents. It was confirmed during the registration of absorption and scattering spectra of pus on a spectrophotometric complex. Thus, the purpose of this work was to determine the required parameters of the LED applicator and the selection of LEDs for the applicator's modernization.

Based on technical documentation analysis, the LA MI12WP4 LEDs (Light Avenue GmbH, Germany) (940 nm) were selected. These LEDs have similar characteristics to the LEDs F3453A (OSRAM Opto Semiconductors GmbH, Germany) (850 nm) that already used in the applicator. The maximum forward voltage for MI12WP4 is 1.85 V; for F3453A — 1.8 V, the maximum direct current for these LEDs is 100 mA. The manufacture of the modernized applicator was carried out using a specially made 3D printer mold for casting and medical silicone SILASTIC MDX4-4210. The upgraded LED applicator will allow studying patients with pus in the maxillary sinuses.

The reported study was funded by RFBR according to the research project No. 20-32-90147.

IN VIVO STAINING-FREE VISUALIZATION OF MAST CELLS AND MACROPHAGES IN HUMAN SKIN USING TWO-PHOTON FEMTOSECOND-PULSED TOMOGRAPHY WITH FLUORESCENCE LIFETIME IMAGING

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Mast cells (MCs) and macrophages (MΦs) are important multifunctional immune cells located in all tissues of the body. In the skin, resting and activated MCs, as well as M1- and M2-polarized MΦs are located in the dermis. Assessment of the quantity of MCs and MΦs and their activation and polarization states is currently limited to histomorphometric analysis of skin biopsies. *In vivo* non-invasive visualization in the skin is currently not possible.

We show for the first time that two-photon excited fluorescence lifetime imaging (TPE-FLIM), a label-free non-invasive method, can be used for the visualization of MCs and MΦs in human dermis *in vivo* with a high sensitivity and specificity. We demonstrate *in vitro* that human dermal MCs and MΦs exhibit specific TPE-FLIM parameters (lifetime and intensity) that distinguish them from the main components of the extracellular matrix and other dermal cells. We visualized the MC activation state, as well as phenotypes and phagocytosis of MΦs in the skin of healthy individuals *in vivo* using TPE-FLIM. First, we have recorded TPE-FLIM parameters of MCs and MΦs *in vitro*. Then, we confirmed the visualization MCs and MΦs in the skin biopsies *ex vivo* based on known TPE-FLIM parameters and cell-specific immune staining. Finally, we found cells with previously determined TPE-FLIM parameters for MCs and MΦs in the skin *in vivo* [1].

The developed non-invasive *in vivo* method can advance the understanding of the role of MCs and MΦs in health and disease diagnostics and therapy control in dermatology and immunology.

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IMPROVEMENT OF HEMODYNAMICS USING A MECHANICAL CIRCULATORY SUPPORT DEVICE IN PATIENTS WHO HAVE PASSED THE FONTAN PROCEDURE

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Objective: The Fontan procedure is a corrective hemodynamic operation. Blood flows from the vena cava to the pulmonary arteries. The Fontan procedure is used for many other congenital heart defects. We would like to study the possibility of using a rotary pump as a biotechnical system for mechanical support of a univentricular circulation.

Methods: Three various TCPC connections were selected for this study. We developed, a workbench consisting of (i) a hydraulic part, (ii) an optical part, and (iii) measuring equipment. Particle image velocimetry was used as a flow visualization technique. One of its most important advantages is the absence of disturbing influences on the flow.

Results: We obtained the flow velocity distribution vector field at the operating point for three different TCPCs. This allowed us to suggest the presence of stagnation zones in the flow. We have confirmed that the TCPC-3 configuration is the most optimal in the work of the cardiovascular system with a univentricular circulation. Also we have shown the effectiveness of using a rotary pump for mechanical support of a univentricular circulation. Most of the CVS parameters returned to the physiological norm.

Discussion: In further work, it is necessary to pay great attention to the hydrodynamic characteristics of the flow at the outlet of the MCS device and the design of the rotor of the MCS devices. Confirmation via experimental analysis of a real total pediatric cavopulmonary connection is also needed.

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SKIN MELANIN CONTENT AS AN AFFECTING FACTOR FOR LASER DOPPLER FLOWMETRY AND TISSUE REFLECTANCE OXIMETRY SIGNAL FORMATION

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Currently the microhemodynamic state assessment is quite a valuable instrument as the disorders of the blood microcirculation system are indicators of various diseases. Such techniques as laser Doppler flowmetry (LDF) and tissue reflectance oximetry (TRO) are widely used for non-invasive in vivo perfusion and saturation assessment. Both methods are based on tissue probing with laser light and registering the absorbing and scattering properties with their further analysis.

Nevertheless, there is a problem associated with differences in the optical properties of the skin for people of diverse ethnic groups. That's due to the varying concentration of chromophore melanin that can act as an optical filter and result in incorrect diagnostics.

The aim of the study was to carry out experiments and computational modeling that both consider the variety of melanin content in different ethnic skin types.

Experimental studies were carried out using a multi-functional laser non-invasive diagnostic system "LAKK-M" (SPE "LAZMA" Ltd., Moscow, Russia) that allows to register the LDF and TRO signals. Also, a Monte Carlo simulation was utilized in the task of describing and predicting the melanin effect on the diffusely reflected radiation component intensity.

The obtained experimental and simulation data shows that levels of registered signals for groups of people with different ethnic skin types is significantly different. The estimated diagnostic values turned out to be incorrect for people with an increased level of melanin. To prevent this from happening it is necessary to take into account the individual optical parameters of human skin.

THE STUDY OF OPTICAL PROPERTIES OF LIVER TISSUE AFTER PRELIMINARY PERFUSION

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The development of optical biopsy techniques for diagnosing liver tumors requires measurements of optical characteristics for modeling light propagation and correct interpretation of the results. However, this may be challenging. Hemoglobin in liver tissues is good at absorbing optical radiation in the visible range. It may be advisable to reduce the blood content in tissue samples.

In this work, liver perfusion before spectrophotometric measurements was studied as such a method.

The experiments were approved by the Ethics committee of Orel State University (minutes № 18 dated 21.02.2020). Male Wistar rats (n=2) were anesthetized with Zoletil 100 (Vibrac, France) in a standard dose. A laparotomy was performed to access the liver and catheterize the hepatic portal vein. It was connected to a system for infusion of isotonic solution with heparin at 37°C. The infusion rate was 1 L/h with the infusion volume of 2-2.5 L.

Measurements of transmittance and diffuse reflectance were performed on 1 mm slices placed between two glass slides using Shimadzu UV-2600 spectrophotometer with the ISR-2600Plus integrating sphere in the 220-1400 nm range. Absorption and reduced scattering coefficients were calculated using the adding-doubling algorithm by Scott Prahl.

The results showed a significant decrease in blood content, which enabled a more correct calculation of optical properties. This approach demonstrates its effectiveness. The scatter in the data suggests the need to refine the methodology in terms of changing the solution, rate and volume of infusion or preparation of slices.

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INVESTIGATION OF THE REACTION OF THE MICROCIRCULATION SYSTEM TO LOW-FREQUENCY BREATHING

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Breathing exercises contribute to the development of the ability to reduce the respiratory rate. A decrease in the minute volume of respiration presumably leads to a reaction of the microcirculatory bed to changed gas composition. The study aimed to evaluate the response of microcirculation parameters to breathing exercises.

Twenty five volunteers performed breathing exercises at a frequency of 3 (2, 1.5 or 1) times per minute for 5 minutes, free-breathing for 6 minutes before and after breathing exercises. Measurements were conducted on the forehead in right and left areas of supraorbital arteries, on the 3rd fingers and the 1st toes. It was carried out by a distributed system of 6 wearable laser Doppler flowmetry monitors (SPE "LAZMA" Ltd., Moscow): index of microcirculation (I_m), nutritive blood flow (M_{nutr}), amplitudes of endothelial, neurogenic, myogenic, respiratory and cardiac oscillations were analyzed.

After performing breathing exercises, an increase in microcirculation was observed at all breathing frequencies; breathing with a frequency of 1.5 and 1 per minute leads to a significant increase in blood pressure and M_{nutr}, which was accompanied by an increase in the myogenic mechanism. The most significant changes were achieved at the lowest respiratory rates, which may be associated with hypoxic-hypercapnic mechanisms.

No significant changes in microcirculation parameters after low-frequency respiration in the head in both groups of volunteers characterizes the work of homeostatic mechanisms for maintaining brain perfusion in stressful situations for the body (low-frequency breathing, hypercapnia and hypoxia). An increase in I_m and the M_{nutr} in limbs characterize the compensatory reaction of microcirculation to changes in respiration.

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SKIN-SAFE DISINFECTION WITH FAR-UVC-LIGHT

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Surgical site infections (SSIs) represent an important clinical problem resulting in increased levels of surgical morbidity and mortality. UVC irradiation during surgery has been considered to be a possible strategy to prevent the development of SSIs. Germicidal UV lamps, with a broad wavelength spectrum from 200 to 400 nm, are an effective bactericidal option against drug-resistant and drug-sensitive bacteria [1,2).So far, however, they are assessed as a health hazard to patients and staff. We investigated a newly developed far-UVC LED source with a peak emission wavelength of 233 nm for its suitability of killing microorganisms, especially Methicillin-sensitive and Methicillin-resistant Staphylococcus aureus (MRSA/MSSA), on germ carrier plates. In parallel, we investigated the effect of germicidal radiation doses on skin for human application. Skin cell viability, DNA damage potential and radical production were assessed in comparison to UVA/B (280–400 nm) irradiation. Far-UVC radiation at 222 nm served as a negative control. At a dose of 40 mJ/cm^2 the far-UVC LED light source could reduce the MSSA and MRSA by 5 log10 levels if no organic substances were included in the medium. Organic substances reduced the germicidal effect to 2 log10 levels independent of the doses. At 40 mJ/cm^2, the investigated skin models showed no reduction in immediate viability: The resulting superficial DNA damage was below 0.1 minimal erythema UVB dose which can be regarded as safe. The low damage vanished after 24h, while irradiation with this dose on four consecutive days showed no DNA damage, at all. The radical formation was far below 0.25 minimal erythema UVA dose. This low radical load can be scavenged by the antioxidant defense system [3]. This disinfection technique is also very promising to reduce viruses such as Sars Cov II.

APPLICATION OF METHODS OF OPTICAL SPECTROSCOPY FOR THE DIAGNOSIS OF EXTRA- AND INTRA-ARTICULAR INJURY

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Extra- and intra-articular injuries are the most common and prognostically unfavorable if diagnosed late or erroneously. The currently existing methods for diagnosing osteoarthritis only allow the determination of cartilage damage at the macroscopic level associated with the final stage of the disease. Thus, the development of new techniques that allow non-invasive rapid detection of early stages of cartilage degradation, occurring at the molecular level and not causing significant mechanical damage, is in high demand.

In this work, a device for ex vivo analysis of the cartilage state was developed. The setup implements diffuse reflectance spectroscopy (DLS) and fluorescence spectroscopy in the near infrared range. We examined explants obtained during the surgery carried out at the Sechenov First Moscow State Medical University. This study was approved by the LEC.

The cartilage mechanical parameters were determined as a result of identification. At the same time, its correlation with the water content in the tissue, determined using DLS, was carried out. Analysis of changes in the water properties

in the cartilage tissue in normal and pathological conditions was supplemented by the study of intrinsic IR fluorescence. In preliminary experiments, it was shown that the water content in the cartilage tissue correlates with an increase in its thickness. It was also shown that the level of IR fluorescence depends on the area and cartilage condition, however, it is necessary to expand the number of samples measured to assess the reliability of the observed differences. The next step of the study will be the application of the developed methodology for assessing the state of the cartilage intraoperatively.

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LASER SPECKLE CONTRAST IMAGING AND THEIR CLINICAL APPLICATIONS

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The development of noninvasive methods of imaging and quantification of blood microcirculation *in vivo* has been an important problem of optical diagnostics for many years Microcirculation is a set of processes occurring in the peripheral blood vessels and intercellular space. This process is critical to maintain the viability of tissues and ensure the normal functioning of the body. Disorders of this process play an important role in the pathogenesis of many diseases such as diabetes, atherosclerosis, anemia, coronary heart disease, etc. Monitoring of cerebral circulation is also an extremely important research and clinical task.

A number of optical diagnostic and imaging techniques have been previously developed for noninvasive assessment of blood microcirculation. One such inexpensive and simple method is laser speckle contrast imaging (LSCI). This method is based on the irradiation of tissues with coherent laser radiation, which is backscattered from the various components of biological tissue forms a random interference speckle pattern. The movement of red blood cells causes fluctuations in this speckle pattern, which can be mathematically processed to visualize the perfusion of tissues.

The aim of this work is to investigate the current clinical applications of LSCI for imaging and analysis of tissue perfusion. The paper presents a brief description of the theory of speckle contrast imaging, a review of current clinical studies, and describes the limitations and problems of this technology.

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CARDIOLOGY AND BIONIC TECHNOLOGIES

INVESTIGATION OF MECHANICAL PROPERTIES OF THE TISSUE-ENGINEERING CONSTRUCTIONS FOR THE CARDIAC TISSUE RESTORATION

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One of the approaches for the recovery of the damaged hearth tissues as the result of injury or disease is the tissue engineering for the creation of the multilayered nanocomposite constructions. During the creation of this kind of materials you should consider different structural and mechanical properties, such as hardness, porosity, strength and fatigue strength. In this work, the presented multilayered nanocomposite constructions made from the polymers, which often uses in tissue engineering: bovine serum albumin, collagen, chitosan and also single-walled carbon nanotubes. For the investigation of hardness were used nanohardness tester with Berkovich form tip. By using this tester we measured the average hardness and also the hardness in the different layers of our samples. It was found that the maximum hardness inherent in the near-surface layer of structures with large nanotubes at a depth of 200 nm was 1.5 GPa. Measurements of the fatigue strength of multilayer nanocomposite structures under low- and high-cycle loads made it possible to prove their applicability for the restoration of defects in cardiac tissue. After simulating heart contractions for 2 months in a physiological environment at a temperature of 37 ° C, the tensile strength was 1.6 ± 0.1 MPa.

CONTROLLED BIOMATERIALS

NANOPARTICLE-BASED DELIVERY SYSTEMS FOR THE VISUALIZATION OF LIVER PATHOLOGIES

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Liver diseases is an urgent problem of current healthcare. More than 844 million people all over the world have diseases of hepatobiliary system with mortality rate about 2 million people per year (Marcellin P., 2018). Reactive oxygen species (ROS) plays an essential role in liver cell damage and the progression of diseases accompanied with chronic inflammation, including hepatocellular carcinoma. We analyzed and validated 3 different animal models associated with high ROS production: hepatectomy, CCl_4-induced injury, hepatocellular carcinoma (PCR-rt for antioxidant markers, serum ALT/AST, histological staining). The maximum of ROS level was observed in CCl_4-induced liver injury (0.05 ml/kg) and hepatectomy models. We synthesized nanoparticles with plasmid DNA encoding HyPer protein (sensitive to hydrogen peroxide) to visualize excess of the ROS in liver. In 2 weeks after injection HyPer-positive cells were observed in liver tissue using western-blotting and confocal microscopy. Increase of ROS production was detected by the analysis of mean fluorescence intensity of HyPer-positive cells from 20 min to 2h after hepatectomy of liver lobes.

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COMPARISON OF THE INFLUENCE OF BIOPOLYMER COLLAGEN-CONTAINING AND TISSUE-SPECIFIC SCAFFOLDS ON INSULIN-PRODUCING CAPACITY OF ISOLATED RAT AND HUMAN ISLETS

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In the creation of a tissue-engineered pancreas, the use of scaffolds can contribute to the maintenance of viability and function of islets.

Objective: To compare the effect of commercially available biopolymer collagen-containing hydrogel (BMCH) and tissue-specific (DPS) scaffolds on the secretory ability isolated rat and human islets.

Materials and methods. The islets were isolated using collagenase technique. The DPS was obtained by decellularization of pancreas fragments. The islets were cultured in monoculture(C_{rat} and C_{human}), with the BMCH(I_{rat} and I_{human}) and the DPS(II_{rat} and II_{human}). By ELISA the basal insulin concentration was determined.

The results. It was revealed rat and human islets culture with scaffolds allows to increase insulin secretion *in vitro*. In the groups I_{rat} and II_{rat}, the basal insulin concentration increased relative to the C_{rat} by 26.2% and 48.7%(one day), — by 62.1% and 102.9%(third day), — by 249.6% and 373.6%(sixth day), respectively. The basal insulin concentration in the groups I_{human} and II_{human}, compared the C_{human}, increased by 17.1% and 39.5%(one day), — by 37.1% and 55.3%(fourth day), by 41.4% and 68.1%(sixth day), respectively.

Conclusion. The BMCH and the DPS contribute to prolongation of the secretory ability islets compared to the islet monoculture, but a more positive effect of the DPS.

MAGNETICALLY RESPONSIVE PIEZOELECTRIC POLYMER SCAFFOLDS FOR POLY(3-HYDROXYBUTIRATE) (PHB) IS A PIEZOELECTRIC AND TISSUE ENGINEERING

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Poly(3-hydroxybutirate) (PHB) is a piezoelectric and biodegradable polymer. Spun scaffolds made from PHB are designed to mimic extracellular matrices and provide supporting surfaces for cell growth. With magnetic particles these spun scaffolds could be a promising stimuli-responsive biomaterial for biomedical applications. We suggest that external magnetic field may not only affect cells by itself but also trigger the surface compressive stress through movement of magnetic particles. This surface stress combined with electrical signal of piezoelectric material would also play a significant role in tissue regeneration.

In this work we fabricated four types of spun scaffolds by varying fiber diameters and using two ways of thread positioning: chaotic and parallel. Additionally, we produced four types of composite scaffolds with different magnetic nanoparticles. These composite scaffolds were manufactured by low spinning speed and had chaotically oriented structure unlike the parallel fibers produced by high-speed technique.

We investigated all the obtained scaffolds for biocompatibility and visualized the cell growth pattern on different structures. Experiments had shown that whether oriented or not, these scaffolds could support cell growth. Parallel positioning of fibers created a specific environment for cells so they grew stretching along them. In contrast randomly oriented threads stimulated cells to grow by stretching between them. Composite scaffolds with magnetic particles had shown similar biocompatibility demonstrating that the magnetic nanoparticles had no cytotoxic effect. In further experiments we will explore the influence of external magnetic field on composite scaffolds and seeded cells.

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MAGNETIC NANOPARTICLES IN CANCER HYPERTHERMIA

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Localized and selective heat dissipation in biological environment via magnetic nanoparticles (MNP) is nowadays a challenging method of cancer treatment that has shifted from laboratory research to clinical trials.

We have investigated the MNP use in cancer hyperthermia. We have paid particular attention to the spherical superparamagnetic nanoparticles with iron oxide (SPION). For γ-Fe_2O_3 SPION (average diameter d~11 nm), we have obtained the saturation magnetization to be M~4 emu/g [1], and for Fe_3O_4 SPION (d~5 nm), it has been equal to M~20-25 emu/g [2].

In hyperthermia with large Fe_3O_4 MNP (d≥20, nm) and low M~10 emu/g we have measured the tissue temperature to be increased by 0.7°C. If M~70 emu/g (large), the tissue is heated 2°C higher [3]. In hyperthermia, Fe_3O_4 MNP may be more effective than γ−Fe_2O_3 MNP, though the biocompatibility degree is higher for γ-Fe_2O_3 MNP than for Fe_3O_4 ones.

It has been also shown that in large rod-like nanoparticles (d~100 nm) the value of M may be higher than in spherical MNPs. A long MNP can generate a stronger local magnetic field improving the hyperthermia process, as well as the contrast in MRI, so bacterial magnetosomes and carbon nanotubes with MNPs are supposed to be the most promising ones.

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TACTICAL SENSOR BASED ON BIOLOGICAL NANOMATERIALS

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Background. Biological nanomaterials, which include carbon nanotubes, acquire the properties of a tensoresistive effect upon deformation. In the present work, changes in the resistance R/R_0 from the bending angle θ of the film of a biological nanomaterial and the possibility of its use as a tactile sensor are considered. Here R_0 is resistance in the absence of deformation, R is resistance in the presence of deformation.

Methods. Films with sizes of 0.05-0.2 μm×2 mm×10 mm made of bovine serum albumin (BSA) or microcrystalline cellulose (MCC) containing multi-walled carbon nanotubes (MWCNT) served as a strain-sensitive element. An aqueous dispersion of a composite nanomaterial was deposited on a flexible PET substrate ~ 30 μm thick. The deformation process and measurements of physical parameters were carried out automatically and the data were saved in a computer.

Results. The angular sensitivity for bending is $(R/R_0 \_ 1)/\Delta\theta \sim 1.5\%$/deg, the minimum recorded pressure is ~1 Pa. This tactile sensor allows you to distinguish between the type of deformation: convexity and concavity, i.e. is bipolar like natural leather.

Conclusion. The dispersions used contained, as a matrix, biological materials (BSA or MCC) and as a filler <0.5wt.% MWCNT. They have an acceptable degree of biocompatibility, and tactile sensors created from them can be used in the new direction of "Skin electronics", as well as in medical practice, as a basis for artificial skin or monitoring the healing of a surgical suture.

INJECTABLE FORMS OF COLLAGEN-CONTAINING BIOMIMETICS OF THE EXTRACELLULAR MATRIX AND MESENCHYMAL STROMAL CELLS FOR CARTILAGE REGENERATION

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An alternative method for regeneration of damaged articular cartilage is the administration of cell-engineered constructs (CEC) into the damaged area. An actual issue is the development of biodegradable scaffolds for CEC.

Aim. Comparison of the functional efficiency of CEC with a microdispersed tissue-specific scaffold from decellularized porcine articular cartilage (DCp) and biopolymer microheterogeneous collagen-containing hydrogel (BMCH).

Materials and methods. The cartilage microparticles were obtained by micronization with CryoMill. For decellularization 3 freeze/thaw cycles, surfactants and DN.ase were used. For comparison we chose SpheroGEL® (BIOMIR Servis JSC). CEC, included human adipose-derives mesenchymal stromal cells (hADSCs) and DCp or BMCH, were cultured in chondrogenic medium for 42 days. The functional activity was investigated on the experimental model of osteoarthritis (OA) of the knee joints of rabbits. The morphology was assessed by histological methods.

Results. On the 14th day of cultivation all CEC contained glycosaminoglycans and collagen. The hADSCs on the DCp adhered and proliferated better then on the BMCH. In the OA model the regenerative activity of the CEC with BMCH and hADSCs was higher than with the DCp.

Conclusion. Stimulation of the processes of regeneration of cartilage *in vivo* effectively occurs when using CEC with BMCH. The high ability of CEC with DCp to maintain adhesion, proliferation and differentiation of hADSCs *in vitro* makes it promising for the creation of tissue equivalent of cartilage.

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CARBON NANOTUBE INTERFACES FOR NERVE TISSUE

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Biocompatible interfaces are widely used in many fields, including medicine and bionic technology. For example, they are used in stimulation and recording of chemical signals. In gastroenterology, our research may be useful for the development of sacral nerve stimulation (SNS) electrodes for fecal incontinence. However, disadvantages of the common neurointerfaces are high hardness damaging adjacent tissue and low stability in organism. An alternative to metal and silicon devices can be implants that include carbon nanotubes (CNT). In this work, we have created prototypes of the conductive part of the interface in the laboratory, to identify among various types of nanomaterials containing CNT those materials that will provide low resistance and contact electrical conductivity comparable to the electrical conductivity of a nerve.

The experimental sample consists of a PDMS substrate and a conductive nanocomposite containing CNT deposited on it. The conductive support was tested on a porcine spinal nerve fragment. In addition, an experiment was proposed with coating of nanocomposite area of contact between the nerve and the interface to increase the electrical conductivity. For making of dispersions, we used such types of CNT, like CNT containing graphene (AMG), hydrophilic MWCNT, carboxylated MWCNT, "MD-Taunit" MWCNT and SWCNT. The lowest resistance of conductive pattern is possessed by SWCNT-ethanol (0.48±0.02 kOhm) and MWCNT-ethanol (2.00±0.12 kOhm) nanocomposites. Conductive MWCNT pattern showed a higher electrical conductivity of the contact (0.324±0.054 S/m) compared to SWCNT. Hovewer, in experiment with coating, the best results were achieved with a substrate containing SWCNT (174±63 S/m). Indeed, the coating increases the electrical conductivity, but it was noticed that the readings in the experiment with coating changed more often than in the usual experiment, which may indicate low stability of the contact between the nerve and the interface. SWCNT and MWCNT have great potential for creating flexible conductive neurointerfaces, but further research is needed to determine which one has the most suitable properties for this purpose. The study was supported by the Ministry of Science and Higher Education of the Russian Federation (No. 075-03-2020-216 from 27.12.2019).

DIGITAL CARDIOLOGY: BIG DATA, DATA PROCESSING ALGORITHMS

FEATURES OF INFLAMMATION IN PATIENTS WITH CHRONIC HEART FAILURE ASSOCIATED WITH FATIGUE

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Introduction. Chronic heart failure (CHF) is a syndrome that occurs as a complication of cardiovascular diseases, including arterial hypertension (AH), and is characterized by fatigue. It was suggested that the decompensated forms of cardiovascular pathology with fatigue are associated with inflammatory reactions determined by the individual reactivity.

The aim was to determine the immunological profile of fatigue in CHF.

Material and methods. 50 patients with AH (55±9.7 years) (group 1) and 62 patients with CHF (64.4±9.7 years) (group 2) were examined. Group 2 included patients with NYHA II (14.5%) III (80.6%), IV (4.9%) classes. Group 1 consisted of patients with I (73.4%), II (20.9%) and III (5.7%) stages. The severity of fatigue was assessed using MFI-20. The activity of leukocyte elastase and a1-proteinase inhibitor, antibodies to S-100B were determined in plasma.

Results. Group 2 had the higher scores in MFI-20 subscales compared to group 1 ($p<0,01$). Both groups were characterized by the increase in the activity of a1-PI in comparison with controls ($p<0,001$). In group 1, there was an increase in the activity of LE compared to the reference values ($p<0,001$). In group 2, the decrease in the activity of LE and the high Abs-S100B compared to controls were revealed ($p<0,05$).

Conclusions. The results confirm the role of inflammation in the pathogenesis of chronic heart diseases. The spectrum of the inflammatory markers revealed in CHF with fatigue differs from that in AH. The decrease in LE activity and the high Abs-S100B may be factors confirming the severity of CHF with fatigue.

DIGITAL ONCOLOGY

MAIN RISK FACTORS OF HPV-ASSOCIATED HEAD AND NECK CANCER: RESULTS OF A SELECTED STUDY

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Introduction. Most head and neck cancers are derived from the mucosal epithelium in the oral cavity, pharynx and larynx and are known collectively as head and neck squamous cell carcinoma (HNSCC). Human papillomavirus (HPV) is now established as the principal cause of an increase in incidence of a subset of head and neck squamous cell cancers (HNCs) in numerous geographic regions around the world. Further study of the epidemiology of HPV-positive HNC will be critical to the development and implementation of public health interventions to reverse these global incidence trends.

Methods. A retrospective study of the data of 295 patients with head and neck cancer who applied for medical care in the period from 2018 to 2020 at the University Clinical Hospital No.1 of the Sechenov University was carried out. The information was obtained from the medical records of an inpatient and by the method of questioning and telephone interview.

Results. The role of smoking OR = 2.07 (CI: 1.07-4.02), hookah smoking OR = 3.06 (CI: 1.06-8.80), drinking strongly hot drinks OR = 3.65 (CI: 1.44-9.25), the presence of a dental prosthesis OR = 7.32 (CI: 2.77-19.31), heredity OR = 7.38 (CI: 3.07-17.76), “poor »Dental status OR = 33.54 (CI: 15.01-74.95), positive HPV status in history OR = 7.31 (CI: 2.77-19.31), 5 or more sexual partners during lifetime OR = 4.95 (CI: 2.47 — 9.93) as risk factors for HPV-associated head and neck cancer.

Conclusions. Preventive measures against HPV infection plays an important role in reducing the morbidity associated with malignant neoplasms of the head and neck. The results of the study convinced of the need for preventive measures in relation to the identified risk factors for the development of HPV-associated head and neck cancer.

DETECTION OF SOMATIC MUTATIONS IN THE *BRAF* GENE BY PYROSEQUENCING

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The detection of *BRAF* somatic mutations and their identification can be used for differential diagnosis, patient prognostication and as a marker for targeted therapy. Pyrosequencing-based method is suitable for quantitative mutation analysis.

The aim of this study was to develop a set of pyrosequencing-based methods for detecting of *BRAF* somatic mutations using «PyroMark Q24» equipment. Sensitivity and specificity were examined using plasmid DNA samples encoding *BRAF* gene mutant variants: V600E, V600R, V600K, V600M and K601E. The clinical testing was performed on 132 biological samples from thyroid nodules.

A set of three methods was developed with an analysis algorithm: for determining mutations in the *BRAF* 592-602 codons, for differentiating mutations in the 600 codon and for detecting the K601E mutation. The proposed approach makes it possible to identify clinically significant mutations in the sequenced region and to detect 2% of the mutant allele for V600E and V600M, 1% — for V600K and V600R, and 3% — for K601E mutations in samples with high DNA concentration. For samples with low DNA concentration (< 500 copies per PCR) the limit of detection was 5%.

These methods enable to unambiguously differentiate all of the tested mutations even in samples with a mutant allele load of less than 10%. Clinical sample testing revealed 53 V600E mutations with 4.9-50% mutant allele load. This approach can also be applied for rare mutation search in *BRAF* gene as well as identification of somatic mutations in other oncogenes.

PROSTATE-SPECIFIC ANTIGEN DENSITY AS A PROGNOSTIC MARKER OF PROGRESSION-FREE SURVIVAL AMONG PATIENTS WITH LOCALIZED PROSTATE CANCER TREATED WITH COMBINATION OF EXTERNAL BEAM RADIATION THERAPY AND ANDROGEN DEPRIVATION THERAPY

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Abstract. Prostate cancer (PCa) is one of the most common malignancies in men all over the world. External beam radiation therapy (EBRT) is considered as one of primary treatment options for localized PCa. Almost 35% of patients will face PCa progression within 10 years following EBRT.

Purpose. To assess the ability of PSA density (PSAD) to predict biochemical failure (BF) among patients suffering from localized PCa treated with combination of EBRT and androgen deprivation therapy (ADT).

Material and methods. We evaluated 272 patients with localized PCa who were treated with combination of EBRT and ADT between January 2000 to July 2015. The assessment of prognostic and clinical significance of PSAD took place.

Results. PCa progression was observed in 52 (19.11%) of 272 patients. PSA (p=0.0005), PSAD (p<0.0001), PSA doubling time (p<0.01) and Gleason score (p=0.00005) were found to significantly correlate with PCa progression. Using ROC-analysis we established PSAD threshold of 0.376 ng/mL/cc, when exceeded, was associated with significant decrease in progression free survival (PFS). PSAD exhibited an AUC of 0.703 (95%CI: 0.236-0.434; *P*<0,001). We applied threshold value to categorize patients into high PSAD (H-PSAD) and low PSAD (L-PSAD) groups. PFS was calculated according to PSAD value. H-PSAD group demonstrated a significantly lower PFS compared to L-PSAD (P<0.0001).

Conclusion. We have clearly demonstrated a PSAD as an important prognostic tool of high clinical relevance, which may aid in BF risk estimation among patients with localized PCa treated with combination of EBRT and ADT.

PROGNOSTIC ROLE OF PSA DENSITY IN PATIENTS WITH LOCALIZED PROSTATE CANCER TREATED WITH RADICAL PROSTATECTOMY

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Prostate cancer (PCa) is among the most prevalent cancers in Russia. Radical prostatectomy (RP) has proven to be a safe and effective modality of localized PCa treatment. Despite its effectiveness, within 10 years following RP, almost 35% of patients will face PCa progression.

Given the high demand for new biomarkers of PCa progression, we assessed the ability of PSA density (PSAD) to predict biochemical failure (BF) and detect unfavorable pathological features (UPF) among men suffering from localized PCa treated with RP.

The study cohort consisted of 147 men with localized PCa who underwent an open or minimally invasive RP between February 2001 and August 2015. The assessment of PSAD clinical and prognostic value took place.

The overall BF rate was 36.01%. A statistically significant correlation was found between BF and PSAD (p=0.006), Gleason score (p=0.0006), pathological stage T (p=0.002), extraprostatic extension (p=0.019) and seminal vesicle invasion (p=0.001). We established a relationship between PSAD and UPF detection following RP. By utilizing

ROC — curve analysis (AUC=0,635, p=0,005) we have determined PSAD threshold of (>0,309 ng/mL/cc) — when exceeded, was associated with statistically significant decrease in disease — free survival. In multivariate analysis — PSAD, abnormal Gleason score and seminal vesicle invasion were found to independently influence disease — free survival ($p<0.05$).

We have clearly demonstrated a PSA density as an important prognostic tool of high clinical relevance, which may aid in BF risk estimation. A PSAD parameter incorporation into preoperative nomograms may increase the predictive potential of latter.

OPTIMIZATION OF THE WHOLE GENOME SEQUENCING DATA PROCESSING VIA PARALLEL COMPUTING

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Objectives. Estimate the performance gains of bioinformatic tools used for processing and analysing whole genome sequencing data via parallel computing.

Methods. For the comparative analysis, the whole genome sequencing datasets of 7 Moscow Clinical Scientific Center patients were selected. Processing was performed on a cluster with 32 vCPUs (Intel Cascade Lake platform) and 64 gigabytes of RAM. Comparative analysis results are reported as minutes and percentage reduction in analysis time with and without parallel computing.

Results. In this study, we were able to optimize resource utilization at the level of individual tools, and at the level of the entire processing pipeline with the help of GNU Parallel and Apache Spark. According to the benchmarking results, MarkDuplicates showed no significant reduction in processing time — 167.3 minutes with parallelization of calculations and 160.6 without. Time required for BQSR decreased from 230.4 minutes to 137.8 (40.2%). The biggest difference was observed with HaplotypeCaller: the processing time decreased from 2127.4 minutes to 520.5 (75.5%). According to benchmarking results, the average analysis time reduction for 7 samples amounted to 1416.9 minutes or 44.67%.

Discussion. The exponential growth of sequencing data amounts is a major challenge for its storage, structuring and analysis. Existing bioinformatics algorithms on their own do not allow efficient processing of such large datasets, which leads to an urgent need of developing scalable and powerful tools, as well as optimizing existing ones, in order to overcome this problem. The Genome Analysis Toolkit (GATK) is one of the most widely used set of bioinformatics tools when it comes to the analysis of next-generation sequencing (NGS) data. Our results show a clear increase in performance and analysis speed achieved via implementation of parallel computing.

Implementation of parallel computing simplifies the NGS data processing due to increase of computational efficiency, high performance and fast execution time, which leads to a higher performance while leveraging the specifications of the equipment used.

GLIOMA GENE NETWORK ANALYSIS APPROACHES USING ONLINE BIOINFORMATICS TOOLS

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Glioblastoma is the most aggressive type of brain tumors resistant to a number of antitumor drugs. The problem of therapy and drug treatment course is complicated by extremely high heterogeneity in the benign cell populations, the random arrangement of tumor cells, and polymorphism of their nuclei. The pathogenesis of glioma needs to

be studied using modern cellular technologies, genome- and transcriptome-wide technologies of high-throughput sequencing, analysis of gene expression on microarrays, and methods of modern bioinformatics to find new therapy targets. Functional annotation of genes related to the disease could be retrieved based on genetic databases and cross-validated by integrating complementary experimental data. Gene network reconstruction for a set of genes (proteins) proved to be effective approach to study mechanisms underlying disease progression. We used online bioinformatics tools for annotation of gene list for glioma, reconstruction of gene network and comparative analysis of gene ontology categories. We show high degree of connection of the network structure for glioma, referring to hub genes as potential drug targets. The available bioinformatics tools and the databases for cancer genes' analysis will be discussed together with the recent progress in this field.

FEASIBILITY OF USING CARBONIC ANHYDRASES FOR PREDICTING DISEASE PROGRESSION IN PATIENTS WITH EWING'S SARCOMA

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Background: Ewing's sarcoma (ES) is a common tumor disease that often affects the bones of the lower extremities, characterized by massive invasion and early metastasis to the lungs, bone marrow and nearby tissues. Enzymes of carbonic anhydrase (CA) family, more often CA9 and CA12, represent targets for anti-metastatic therapy.

Specific Aim: Our study is aimed to assess the role of CA in the cancerogenesis of Ewing's sarcoma as a prognostic marker.

Methods: Cox regression, gene expression analysis (GSE63155), PPI, gene ontology (GO), correlation, RNA seq, MTT, Migration.

Results: Differential gene expression analysis of GSE63155 showed 966 genes, including CA2 (-log (p) = 1.744). Next, the scRNA-seq was performed with human fibroblasts and primary-established cell culture ES33. The results indicate a high content of the stemness marker (CD99) in both samples, and the average level of integrated expression for MMP2, MMP14, MMP9 and CA12 was higher in cancer cells compared to fibroblasts. We assessed therapeutic efficacy of multiple chemical inhibitors with various affinity to CA1, CA2, CA9 and CA12 inhibition using patient-established primary cell cultures (ES33 and ES36) and human fibroblasts. Our MTT test showed no specific inhibition of cell proliferation for tumor cells, whereas migration was impacted by TAS19 (most affinity to human CA12). Specifically, we observed a statistically significant difference ($p<0.05$) between TAS19-treated vs. control groups.

Conclusions: CA12 may contribute to the pathogenesis of ES tumor cells which justifies considering CA12 as a potential therapeutic target.

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EMERGING VIRAL INFECTIONS

METABOLOMICS OF COVID-19 PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Metabolomics studies have been conducted in patients suffering from the coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus. Identification of metabolomic signatures of COVID-19 measured in human biological specimens are relevant to improve the understanding of this disease.

Objective: To systematically review available evidence from metabolomic analysis performed in patients with COVID-19.

Methods: Systematic search under execution in 2021 by using PubMed and Web of Science databases. Metabolomics studies conducted in human biological specimens were included, with the exception of cell line studies.

Results: 369 publications were initially identified. The final number of eligible studies for data extraction was 46. Forty-one studies reported analyses in blood based matrices (serum, plasma, red blood cells) and 2 in urine. Individual studies conducted in sputum, exhaled breath, feces, exosomes, tongue-coating, sebum and breastmilk were also evaluated. The studies included patients at different stages of symptoms (i.e. mild vs. moderate vs. severe symptoms vs. ICU patients). Most of the studies had confirmation of COVID-19 positive by reverse transcription polymerase chain reaction (RT-PCR). Significantly altered metabolites or metabolic pathways associated with COVID-19 included circulating levels of glucose, fatty acids, phosphatidylcholines and sphingomyelins from lipidomic analysis, alterations on tryptophan−nicotinamide pathway and cytosine metabolism, increased levels of ketone bodies, accumulation of mannose and reductions of select free amino acids relative concentration, among others.

Conclusions: Metabolomics has been used in COVID-19 patients mainly in serum/plasma at different disease stages. Specific metabolites that can potentially serve as complementary non-invasive biomarkers as well as metabolic pathways integrating the data from the available studies will be proposed.

STRUCTURE OF TEXT REPEATS AND ENTROPY PROFILING FOR PROKARYOTIC GENOMES: CASE OF CORONAVIRUS GENOME

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Viral disease epidemics such as the COVID-2019 coronavirus pose a serious threat to the world's population challenging bioinformatics problems of virus genome analysis related to the virulence estimates. The analysis of genomic rearrangements and mutations of the virus for the purpose of risk assessment and control presents a global public health challenge. Constantly updated data on genomic sequencing of the coronavirus provide detailed material for analyzing the structure of the genome and the possibility of its diagnosis using sequencing. Using our original computer tools we analyzed nucleotide repeats in the coronavirus genome using estimates of text complexity and

entropy. We used publicly available data to consider reference virus genome and reconstruct the phylogenetic tree of the strains. The study of the structure of repetitions of prokaryotic genomes allows one to find evolutionary relationships between different species, including for coronaviruses. We used own computer program to estimate the intergenomic distance by the number of sequence rearrangements. Estimates of the complexity of the text as a whole are important both for analyzing the structure of a genetic text, identifying evolutionary origins, and comparing complete genomes. A modified Lempel-Ziv text compression algorithm was used to assess the structure of repeats in the coronavirus genome. The application of estimates of the complexity of the text (linguistic complexity, entropy) for the study of the structure of prokaryotic genome sequences is discussed.

MASS SPECTROMETRY IN ONCOLOGY

METABOLOMICS OF PROSTATE CANCER IN BLOOD AND URINE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: There are metabolic signatures in blood and urine that may help to identify individuals at higher risk of developing or actively harboring prostate cancer (PCa).

Objective: To systematically review available evidence from metabolomic analysis performed in blood and urine in PCa.

Methods: Systematic search under execution in 2021 using PubMed and Web of Science databases. Metabolomic studies published since year 2000 were examined.

Results: In total, 749 publications were initially identified. After screening titles, abstracts, full texts and relevant references and reviews, 133 studies were eligible for data extraction: 84 studies on plasma or serum (7 trials, 11 prospective cohorts, 2 retrospective cohorts, 5 pre-post, 14 nested case-control, 35 case-control, 4 group comparisons, 4 cross-sectional and 2 individual characterization studies) and 52 studies on urine (2 prospective cohorts, 2 pre-post, 1 nested case-control, 33 case-control, 8 group comparisons, 1 cross-sectional and 5 individual characterization studies). The largest part of the studies included associations or comparisons with the Prostate Specific Antigen and with the Gleason score. Most of the papers reported significantly altered metabolites or metabolic pathways associated with the tricarboxylic acid cycle, amino acids, organic acids, fatty acid metabolism, alterations of steroidogenesis, porphyrin and purine metabolism, among others.

Conclusions: Metabolomics has been extensively used in PCa research. Most of the studies have been conducted in serum/plasma comparing disease versus control groups. Specific metabolites that can potentially serve as non-invasive biomarkers will be proposed through meta-analysis.

ENABLING TECHNOLOGIES IN ANALYTICAL SAMPLES PREPARATION

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In the past decade relevant results have been achieved both in improving solid/liquid extraction methods and in the greening of analytical chemistry. In particular, pharmaceutical industry has driven a wave of important innovations in analytical chemistry, with improved samples preparation procedures and much faster and efficient chromatographic separations. New extraction reactors based on ultrasound, microwaves, subcritical water, supercritical CO_2 and hybrid combinations (i.e. ultrasound/supercritical CO_2) could dramatically improve extraction efficiency, saving time and often getting rid to organic solvents. The rise of collaborations among big pharma companies and universities on new enabling technologies consortium in precompetitive R&D could lead to important innovations in the ongoing highly efficient green analytical processes. Besides surveying the current state of the art, the emerging trends will be highlighted.

MASS SPECTROMETRIC ANALYSIS OF PROTEOMIC SIGNATURES OF EXTRACELLULAR VESICLES FOR LUNG CANCER RECOGNITION

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The proteins of extracellular vesicles (EVs) that originate from tumors reflect the producer cells' proteomes and can be detected in biological fluids, e.g., blood plasma. Thus, EVs provide proteomic signatures that are of great interest for cancer diagnostics. By applying targeted mass spectrometry with stable isotope-labeled peptide standards, we assessed the levels of 28 EV-associated proteins, including the conventional exosome markers CD9, CD63, CD81, CD82, and HSPA8, in vesicles derived from the lung cancer cell lines NCI-H23 and A549. Furthermore, we measured their abundance in plasma samples from 34 lung cancer patients and 23 healthy volunteers. Overall, we detected and quantified the levels of seven proteins in undepleted blood plasma: TLN1, TUBA4A, HSPA8, ITGB3, TSG101, and PACSIN2. The most diagnostically potent markers were TLN1 (AUC, 0.95), TUBA4A (AUC, 0.91), and HSPA8 (AUC, 0.88). The obtained EV proteomic signature allowed us to distinguish between the lung adenocarcinoma and squamous cell carcinoma histological types. The proteomic cargo of the extracellular vesicles represents a valuable source of potential biomarkers. This research was funded by the Russian Science Foundation, grant number 21-74-20122. Mass spectrometry analysis and data storage were performed using the equipment of the "Human Proteome" Core Facility (Institute of Biomedical Chemistry).

ULTRAFAST MASS SPECTROMETRY BASED PROTEIN PROFILING FOR CANCER RESEARCH AND DIAGNOSTICS

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Mass spectrometry (MS) based proteome profiling is widely used to monitor the physiology state of cells and organisms. Living system responds to environmental change, biotic and abiotic stresses; any phenotype change can be captured at the level of protein quantities. Proteomic alteration scanning provides a unique assessment of the activity of biochemical processes by simultaneously and quantitatively measuring many protein products in

ongoing reactions. Liquid chromatography (LC) and tandem mass spectrometry (MS/MS) lay in the grounds of bottom up proteomics, a state-of-art method for relative quantitation of proteome changes. Protein profiling is accompanied by LC to resolve sensitivity issues of tandem mass spectrometry, i.e. low coverage of protein sequences. Chromatographic separation prior to mass detection may include single or multiple fractionation contributing to elongation of experimental times, up to few hours per probe. Multiplexing with chemical labeling (i.e. Tandem Mass Tag (TMT) systems, Thermo Scientific) allow analysis of up to 18 samples in one tube that significantly saves instrument time. Yet, labeling requires additional sample preparation with cost-consuming supplies, thus, leaving space for further improvements.

Ultrafast MS/MS-free proteome profiling has recently been revisited with high resolution MS instruments. We suggested DirectMS1, a novel method using ultrashort LC separation with MS1-only spectra acquisition and bioinformatic pipeline for interpretation of detected peptide features and FDR-controlled protein identification. We demonstrated that 5-min gradients with field asymmetric ion mobility spectrometry and high resolution peptide mass detection yield ~2000 proteins identified in HeLa proteome at 1% FDR. On-going study shows that DirectMS1 profiles the changes in cellular proteomes with efficiency comparable with label free and TMT-based quantitation. Obtaining a quantified proteome in a 5-min experiment attracts attention, since experimental time always matters as “labor/money cost” for the method. In comparison with TMT-based quantitation, our method still provides ten fold gain in the instrument time.

Currently, DirectMS1 is ready for challenges with new samples and applications. We have started its development for proteomic analysis of plant tissues, bacteria and microbiomes, cancer cells, and physiological liquids such as blood plasma. We suggest that DirectMS1 is of interest for clinicians and can be further developed for medical applications, if supported by productive collaboration, actual diagnostic needs and clinical samples.

APPLICATIONS OF DE NOVO SEQUENCING IN CANCER CELL LINE ANALYSIS

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Mass spectrometry-based methods are widely used in medicine including cancer research. The techniques commonly applied to this end include both quantitative and qualitative analysis; however, the latter usually amounts to identification of proteins and peptides via database search. At the same time, *de novo* sequencing can offer important advantages for better understanding of oncology diseases, allowing to detect unexpected post-translational modifications and sequence variants. The limitations arise because the *de novo* sequencing methods should be very reliable to be capable of processing complex samples, while this is often not the case.

In this talk, we will discuss the possibilities of applying the Twister algorithm for *de novo* sequencing of proteins and peptides from high-resolution mass spectrometry data to the analysis of cancer cell lines. In contrast to the other *de novo* sequencing approaches, Twister does not aim at elucidating the entire peptide amino acid sequence from a single spectrum but rather derives its accurate fragments, and further combines the obtained results across several spectra. In this way, it can capture the features essential for tumor characterization, appropriately complementing the existing onco-proteogenomic techniques.

MEDICAL ELEMENTOLOGY IN CARDIOLOGY AND ONCOLOGY

TWO-DRUG CHEMOTHERAPY DOXORUBICIN WITH INHIBITORS OF PI3K/MTOR KINASES MORE EFFECTIVE THAN MONOTHERAPY WITH DOXORUBICINOF CYTOMEGALOVIRUS-INFECTED THP-1 LEUKEMIC CELLS

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Resistance to chemotherapy decreases efficacy of the treatment and promotes cancer recurrence and metastases. Recently, data on the presence of HCMV in tumor cells of different origins have been accumulating.The role of human cytomegalovirus (HCMV) infection in tumor resistance is insufficiently studied. Previously, we have shown that the monocytic leukemia THP-1 cells after HCMV infection acquired resistance to the doxorubicin (DOX), both in transcriptionally active and latent cells. Gancyclovir are antiviral drug for treatmentcytomegalovirus infectiondid not restore the sensitivity of the infected THP-1 cells to DOX chemotherapy. The aim of the work was to assess the contribution of viral factors and cellular pathways (apoptosis, autophagy and mTOR) to the resistance of HCMV-infected leukemia cells to DOX and in overcoming it.Analysis of the mRNA content of Bcl-2 and caspase 3 genes and also Beclin 1 and LC3-II genes showed that prevention of cell death is associated with a decrease in the activity of apoptosis and autophagy. DOX treatment in the presence of PI3K (LY294002) and mTOR (rapamycin, Torin2) inhibitors significantly increased the death of both active and latent HCMV-infected THP-1 cells. The study of mRNA and protein content of the HCMV immediately early protein IE1 in the DOX treated cells suggest that the viral protein may be involved both in resistance and in the restoration of the sensitivity of THP-1 cells infected with HCMV to DOX.

MOLECULAR ONCOLOGY AND TARGETED DRUG DELIVERY

UCNP-BASED PHOTOLUMINESCENT NANOMEDICINES FOR TARGETED DELIVERY AND THERANOSTICS OF CANCER

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Theranostic approach is currently among the fastest growing trends in cancer treatment. It implies the creation of multifunctional agents for simultaneous precise diagnosis and targeted impact on tumor cells. A new type of theranostic complexes was created based on radioactive ^{90}Y-containing $NaYF_4$:Yb,Tm upconversion nanoparticles (R-UCNP) coated with polyethylene glycol and functionalized with the HER2-specific recombinant targeted toxin DARPin-LoPE. The aim of this study was to analyze the therapeutic potential and biodistribution of theranostic complexes in organs and tissues of animals with human xenograft tumors.

The results of assessing the biodistribution of complexes obtained by various methods are in good agreement with each other. It was shown that 6 h after administration, complexes predominantly accumulate in the tumor in comparison with other organs and tissues. A high concentration of complexes in the tumor remains up to 72 h from the moment of administration. A small number of complexes were noted in the spleen, liver, lungs, and peritoneum. In other organs and tissues, complexes were recorded in trace amounts. The results obtained showed that a single injection of theranostic complexes causes inhibition of tumor growth by 30-40%.b Theranostic complexes based on radioactive UCNP ($Na^{90}YF_4$:Yb:Tm) and low immunogenic targeted toxin DARPin-LoPE selectively accumulate in HER2-positive tumors and almost don't accumulate in healthy organs and tissues, and at the same time this complexes have a high therapeutic potential.

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KNOCKDOWN OF CD44 PREVENTS METASTASIS IN HT-29 XENOGRAFT COLORECTAL CANCER MODEL

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The transmembrane glycoprotein CD44 is involved in various functions of both normal and neoplastic cells including cell proliferation, differentiation, intercellular adhesion, invasion, migration, and angiogenesis. Despite its versatile physiological functions, CD44 is a commonly accepted marker of cancer stem cells (CSC). The current paradigm of CSC has stimulated numerous studies on CD44 aimed to use its expression as a prognostic biomarker in several tumor entities including colorectal cancer (CRC), one of the leading causes of cancer-related deaths throughout the world. As *CD44* mRNA undergoes alternative splicing giving rise to several protein isoforms, which obviously play different roles in cancer progression making the interpretation of experimental findings difficult. In the current study the distribution of *CD44* mRNA isoforms in primary CRC tumors was analyzed using RNA sequencing data from TCGA-COAD. CD44 isoform 3 (according to NCBI nomenclature) was revealed as the major isoform in CRC and was found to be upregulated in tumor tissue compared to normal mucosa. The human HT-29 colorectal adenocarcinoma cell line reflected the clinical isoform expression pattern well and was chosen for further shRNA-mediated CD44 knockdown. These cells were subcutaneously injected into SCID mice and despite only moderate, insignificant effects on tumor growth, the knockdown of CD44 led to a decrease in the number of spontaneous pulmonary, bone and hepatic metastases. The results of mRNA sequencing and proteome analysis of the xenograft primary tumors revealed a role of CD44 in EMT. The putative mechanism relies on the CD44-dependent regulation of STAT3, NF-κB, and/or HIF-2 transcription factor activity.

IDENTIFICATION OF MOLECULAR BIOMARKERS FOR HEAD AND NECK SQUAMOUS CELL CARCINOMA BY BIOINFORMATICS ANALYSIS

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer in the world. HNSCC includes several tumors that originate in the larynx, nasopharynx, hypopharynx, mouth, and neck (1). According to the global cancer statistics report, head and neck cancer accounts for ~ 900,00 cases and 400,000 deaths annually (2). The risk factors that are associated with the progression of head and neck cancer include tobacco smoking, alcohol consumption, Epstein-Barr virus (EBV) infection, and human papillomavirus (HPV) infection (1). Currently, HPV is a frequently used and well-studied biomarker in head and neck cancer (3). Here, we revealed a study to find new biomarkers and the most common genes which are associated with HNSCC oncogenesis using a bioinformatics approach.

Datasets containing whole-exome and -genome sequencing of head and neck cancer patients were downloaded from cBioportal (4). The MutaGene python package (5) was used to predict driver mutations and analyze the mutational signatures of predicted genes with driver mutation in HNSCC. Analysis of oncogenes and tumor suppressor genes was performed using the COSMIC (6) and the Tumor Suppressor Gene databases (http://bioinfo.uth.edu/). Gene ontology analysis was performed with the GOfuncR package in the Bioconductor tool to find the biological function of predicted driver mutation genes. The STRING database (http://string-db.org/) was applied to construct the protein–protein interaction (PPI) network of predicted driver genes. Afterward, to visualize the PPI network, the Cytoscape software (Cytoscape Consortium, San Diego, CA, USA) was used. The key module in the PPI networks was identified using the Maximal Clique Centrality algorithm of Cytoscape.

Analysis using the MutaGene package showed that 193 genes had at least one driver in HNSCC datasets. Gene ontology analysis is important in functional genomic research, which is applied to annotate different biological functions of genes in the genome. The biological processes associated with the predicted genes were mainly involved in the, regulation of the microtubules, and microtubule cytoskeleton organization. Analysis of protein-protein interaction network could provide insights into mechanisms underlying carcinogenesis and tumor progression. The protein-protein interaction network constructed by STRING between predicted genes with driver mutation revealed 129 nodes and 209 edges. The hub genes were extracted by the MCC algorithm, and ten upregulated genes (TP53, HRAS, PIK3CA, CDKN2A, ARID1A, RHOA, TGFBR2, SMARCA4, CASP8, GNAS). Consistent with the previous study (7), the TP53 gene is altered in 50 % of tumors in head and neck cancer patients. Mutations in PIK3CA (8) lead to inhibition of apoptosis and are most likely to carcinogenesis. Each mutational process generates a specific local DNA context in the genome which can be described in the form of mutational signatures. Such mutational signatures as Signature 11 that is associated with alkylating agents, Signature 3 (it is associated with failure of DNA double-strand break-repair by homologous recombination), and Signature 15 were found using the MutaGene tool. These findings revealed that mutational processes from different etiologies are involved in the development of head and neck cancer. Moving forward, the results from this study could help to explore the possibility of developing new biomarkers for HNSCC.

HYBRID TRICALCIUM PHOSPHATE/HYDROGEL CONSTRUCTS FOR BONE TISSUE REGENERATION FUNCTIONALIZED WITH AN ANTITUMOR DRUG

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Substitution of critical bone defects due to injuries, osteoporosis or oncological diseases is an important problem for biomedical research. The evolved promising “regenerative approach” emphasizes the creation of suitable conditions for gradual substitution of the biomaterial with a new-formed bone tissue. In our work, we studied novel 3D printed calcium phosphate-based implants capable for cell seeding or saturation with anticancer drugs.

Stereolithography 3D printing was used to create bioceramic implants based on non-stoichiometric β-TCP/HA biphase ceramic powder with granulometry 0.3-1 μm. Implants were printed according “Kelvin” 3D model modified by topological optimization. 3D printed constructs possessed an interconnected system of macropores (500-1000 μm), necessary for the penetration of blood vessels and nutrients inside and through the implants. The constructs demonstrated favourable cytocompatibility and provided suitable 3D conditions for adipose-derived MSCs seeding. To increase the therapeutic potential, the created model structures were saturated with the antitumor drug doxorubicin within the composition of the coating of UV-polymerizable hydrogel based on polyacrylamide/polyethylene glycol diacrylate (PAA/PEGDA). In addition, 3D constructs were saturated with doxorubicin or cisplatin admixed to coating gel compound. Drug release kinetic was studied using simulating body fluid (SBF) model and HPLC analysis. Saturation of the structures with the antitumor drug resulted in its prolonged (up to 7 days) release.

Thus, our data illustrate the possibility to create the osteoconductive calcium phosphate implants capable for bone tissue engineering and local drug delivery by means of additive manufacturing technologies.

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SYNTHETIC PEPTIDES-INHIBITORS FOR CANCER IMMUNOTHERAPY (IMMUNE CHECKPOINTS)

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Immune checkpoints blockade is one of the most promising strategies for cancer immunotherapy that can activate cancer patients antitumor immunity. Current approaches of inhibitory immune checkpoints blockade utilize monoclonal antibodies. We propose that peptides interacting with inhibitory immune checkpoints, instead of monoclonal antibodies, can be more efficient in activating antitumor immune response without side effects. Using microarrays containing 330,000 peptides with random amino acid sequences and recombinant human Fc chimera proteins (CTLA-4, PD-1, PD-L1 and B7-H3, R&D Systems) we identified peptides that interact with molecules that control the immune response. 3D modeling suggested that these peptides interact with parts of the molecules which are responsible for receptor-ligand binding. Incubation (ELISA) of CTLA-4Fc in the presence of p344, p346 decreased the binding of CTLA-4 to B7-1. Thus, these results indicate that the p344, p346 peptides, which specifically interacts with CTLA-4, can partially block CTLA-4 and B7-1 interaction. Peptides blocking interaction of recombinant receptors-ligands in ELISA were selected for functional tests for immunomodulating activity.

Peptides can provide an alternative approach for inhibitory immune checkpoints manipulation in cancer patients due to their small size, low immunogenicity and cost-saving chemical synthesis.

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LOW EXPRESSION OF CD24 IS ASSOCIATED WITH POOR SURVIVAL IN COLORECTAL CANCER

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CD24 is a small GPI-anchored glycoprotein whose aberrant expression is associated with progression and metastasis formation of various malignancies. In this study we analyzed expression of CD24 in a cohort of colorectal cancer patients using immunohistochemistry staining of CD24 in apical membrane and cytoplasm of primary tumors and liver metastases. Membranous CD24 immunoreactivity was significantly decreased in liver metastases compared to matched primary tumors, while cytoplasmic CD24 immunoreactivity was significantly increased. A significant association between absence / low expression of CD24 and shortened patient survival was revealed. Then, using TCGA-COAD RNA sequencing data, we showed that total CD24 mRNA level was two-fold decreased in primary colorectal cancers compared to adjacent normal mucosa. Like the protein staining data, ten percent of patients with the lowest mRNA expression levels of CD24 in primary tumors had significantly reduced survival compared to the ones with higher CD24 expression. To explain these findings mechanistically, shRNA-mediated CD24 knockdown was performed in human HT-29 colorectal cancer cells. It resulted in the strong increase of cell migration in vitro. As increased cell migration is a hallmark of metastasis formation, this experimental finding corroborates the association of a decreased CD24 expression with a poor prognosis. Differential gene expression analysis revealed strong upregulation of genes involved in cell migration in the group of TCGA patients with low CD24 expression, including integrin subunit α3 and α3, β3 subunits of laminin 332. Further co-expression analysis identified SPI1, STAT1 and IRF1 transcription factors as putative master-regulators in $CD24^{low}$ colorectal cancer.

MODELING OF THERMAL PROCESSES OF INTERACTION OF NANO-, PICO-, AND FEMTOSECOND LASER PULSES WITH BIOTISSUE PHANTOMS FOR INTRACELLULAR DRUG DELIVERY

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For optimal intracellular laser drug delivery using carbon black nanoparticles, it is necessary to investigate the energy transfer mechanisms involved in the irradiation of nanoparticles with near-infrared laser radiation. The biotissue phantoms that simplify the analysis of thermal processes are considered. A mathematical model that describes the thermal processes of interaction of pulsed laser radiation with dry biotissue phantom was developed. The modeling results are supported by experimental data on nano- and femtosecond irradiation of dry bone. The relationship between accumulative heating and the size of the carbonized area around the laser wound was established. To determine the regularities of fragmentation of carbon particles, cotton fabric and *ex vivo* porcine skin fragments colored with marker ink were irradiated with pico- and femtosecond pulses. The thresholds of particle fragmentation without damaging the surrounding tissue, depending on the presence of liquid, were established. To study the role of the liquid, experiments were carried out on irradiation of carbon particles with nano- and picosecond pulses in distilled water and an aqueous solution of glycerol. A mathematical model of bubble formation around a particle was developed. The mutual influence of cavitation, fragmentation, and accumulative effect is shown. An in-depth understanding of heat transfer mechanisms may allow developing systems for optimal drug delivery into cells.

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MULTIOMICA

INTERDISCIPLINARY PLATFORM OF MEDICAL GENETICS, GENOMICS AND BIOINFORMATICS TO INVESTIGATE MOLECULAR MECHANISMS OF THE DISEASES

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Molecular mechanisms of human diseases often have complex background demanding development of new bioinformatics methods and sequencing technologies. Reconstruction of gene networks, as well as protein structure modeling, presents modern computational approaches for search and analysis of drug targets. We have organized special journal issue at the International Journal of Molecular Sciences to continues paper collection “Medical Genetics, Genomics and Bioinformatics” in this journal based on the materials presented at the series of medical conferences in 2020. The methods and algorithms were discussed at the medical forum, organized by I.M. Sechenov First Moscow State Medical University, and medical symposia within the framework of the BGRS conference series in Novosibirsk presenting novel approaches for the drug targets discovery, e-Health and neurobiology studies. We discuss recent progress in the development of computational methods in the field of genomics, transcriptomics and proteomics in human diseases and in the modeling (https://www.mdpi.com/journal/ijms/special_issues/Medical_Genetics_2021). The topic on medical genomics application will be continued by the special journal issues on gene expression regulation analysis and applications of novel bioinformatics tools to human genomics.

FLIM AND SINGLE-CELL TRANSCRIPTOMICS REVEAL METABOLIC FEATURES IN THE CARTILAGE GROWTH PLATE DEVELOPMENT

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Background and Aims: The growth plate is a tiny cartilaginous structure, which facilitates bone longitudinal growth. We found that stem cell niche forms in postnatal growth plate yet it is unclear what triggers its formation. The aim of our study is to characterize the metabolic state of chondrocytes before and after the stem cells niche formation.

Methods: We used femur and tibia of wild type and PTHrP-CreERT2:tdTomato transgenic mice of the postnatal period 8-10 days (P8-10) and 28-30 days (P28-30). Tissues were analyzed within the first two hours postmortem. Metabolic state was studied using fluorescence lifetime imaging microscopy (FLIM) by estimating lifetimes of nicotinamide adenine dinucleotide phosphate (NAD(P)H). We analyzed single-cell RNAseq (10X sequencing) dataset from 1 month old mice using the Seurat package.

Results: We revealed the metabolic differences between the same populations at different stages with the tendency to oxidative phosphorylation switch: for progenitors (resting zone, where the stem cell niche is formed), proliferative (PC) and hypertrophied cells (HC). The analysis of P8-10 and P28-30 demonstrated the significant difference between progenitors and other populations (PC and HC) in a way that the progenitors' status was least glycolytic. Further validation using the single-cell dataset revealed the lowest glycolysis genes expression for the progenitors' population.

Conclusions: During the cartilage growth plate development the population of chondro-progenitors becomes less glycolytic. The progenitors show dramatically different metabolic state from PC and HC that can help in further analysis of their impact for the growth plate development and cartilage stem cell niche.

IDIOPATHIC THROMBOCYTOPENIC PURPURA GENE NETWORK ANALYSIS USING ONLINE BIOINFORMATICS TOOLS

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Werlhof's Disease (Idiopathic thrombocytopenic purpura, ITP) is an autoimmune disease that decreases blood coagulability due to isolated thrombocytopenia. It often follows various infections or vaccinations due to produced autoantibodies to glycoproteins of platelet membranes. In some cases, the etiology is unknown. Drugs of the first, second and third line of the ITP therapy are used in clinical practice today. Nevertheless, all of these drugs are ineffective in some cases. Research of etiology and pathogenesis of the Werlhof's disease using genomic, transcriptomic, proteomic analysis and methods of modern bioinformatics can reveal new therapeutic targets. The functional annotation of disease-associated genes can be collected using genetic databases and then verified by integrating additional experimental data. Gene network reconstruction for a list of genes and encoded proteins is an effective method of researching the molecular mechanisms of pathological processes. Online bioinformatics tools were used for functional annotation of the list of genes associated with the Werlhof's disease. After that, for the same genes and encoded proteins, categories of gene ontologies were comparatively analyzed and their network interactions were reconstructed. The reconstructed network structure is highly connected, genes and their expression products that located in its center are potential targets for new drug molecules.

PHARMACY

DIVERSITY OF *LEISHMANIA MAJOR* FROM YAZD REVEALED BY ITS1-PCR-RFLP AND MOLECULAR TYPING THE MITOCHONDRIAL CYTOCHROMES AND THE NUCLEAR *HEAT SHOCK PROTEIN* 70 GENES

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Cutaneous leishmaniasis is of public health concern worldwide. The diseases are caused by *Leishmania major*, *Leishmania tropica*, and *Leishmania infantum* in Old World. In Iran, the province of Yazd belongs to one of the most affected area by cutaneous leishmaniasis caused by endemic *L. major* and *L. tropica*. We previously documented the high prevalence of cutaneous leishmaniasis caused by *L. major* in the Yazd province and provided evidence on the circulation of isolates exhibiting "non-canonical" ITS1-PCR-RFLP (Internal Transcribed Spacers PCR — Restriction Fragment Length Polymorphism) pattern. Here, we test the suitability multigene sequencing methodology to point the molecular diversity of these isolates. DNA was extracted from the isolates stored in the BioBank of the Research Center for Food Hygiene and Safety of Yazd province. Specific primer pairs were used to amplify fragments of the mitochondrial cytochrome genes *CytC, CytB, cytochrome oxidase II* (*COII*), and nuclear *heat shock protein 70* (*hsp70*) genes. The amplified fragments have shown complex diversity patterns of *Leishmania* species studied. For first time, we showed that *CytB, CytC, COII, hsp70* were successful to identify the clinical isolates of *Leishmania* spp. when ITS1 approach failed to detect and identify it. These gene regions proved be able to serve as the reference for the phylogenetic analysis.

ADJUNCTIVE THERAPY WITH THE ANTIOXIDANT MEXIDOL FOR LATE-ONSET SCHIZOPHRENIA

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Antioxidants, including Mexidol, are successfully used in complex therapy in neurology and psychiatry, including gerontopsychiatry.

The purpose of this study is to test the hypothesis that among patients with late onset schizophrenia, a subgroup can be distinguished, for whom the use of Mexidol as an adjunctive therapy to antipsychotics would be the most favorable (effective) in relation to the symptoms most pronounced in severity in this subgroup of patients, as well as in relation to side effects of antipsychotic and antidepressant drugs.

The study included 3 groups of patients aged 45-78 years with a diagnosis of "schizophrenia" according to ICD-10, selected on the basis of patterns of their symptoms and treated by: (i) only antipsychotic drugs and antidepressants (n=22), (ii) Cerebrolysin or Actovegin (n=11), and (iii) Mexidol (n=10) as adjunctive therapy.

Measurements of the activity of platelet enzymes in all examined patients showed that in Mexidol group the activities of platelet glutamate dehydrogenase and glutathione-S-transferase were significantly lower than in other examined groups ($p<0.02$). After a 28-day course of therapy, levels of those clinical signs, the severity of which

was significantly higher in the Mexidol group, decreased and turned out to be indistinguishable from that in the comparison groups; the activity of platelet enzymes became also indistinguishable from the activity of enzymes in the comparison group. The severity of hypochondria decreased significantly already after a 2-week course of the treatment.

The result of the study demonstrates the validity of Mexidol usage as an adjunctive therapy in addition to antipsychotic and antidepressant therapy in the group of patients with late onset schizophrenia.

EFFECTS OF DEUTERIUM-DEPLETED WATER WITH VARIOUS DEUTERIUM CONTENT IN MOUSE MODELS OF DEPRESSIVE-LIKE BEHAVIOR

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The isotopic composition of drinking water has a significant impact on physiological processes due to the kinetic isotope effect. Previous studies revealed an epidemiologic relationship between natural variations of deuterium in drinking water and the incidence of depressive disorder that was further supported in the chronic stress mouse model of depression. Previous research showed positive effects of deuterium-depleted water on exploratory activity, emotional behavior, sleep, neurogenesis, and glucose tolerance. However, molecular mechanisms underlying these effects are poorly studied. To address the role of circadian and inflammatory responses to changes in deuterium content, gene expression of PER1 and PER2 that regulate day-night rhythmicity, cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2), pro-inflammatory cytokines interleukin-6 and interleukin-1β were studied in the brain of mice housed on the deuterium-depleted water (90 ppm). While no significant expression changes in the hippocampus and prefrontal cortex were shown for PER genes, decreases in inflammation-related genes had a trend to a significant level. Next, we studied the effects of water containing an ultralow amount of deuterium (5 ppm), on mouse despair behavior. Animals were exposed to repeated swim sessions on days 1, 2, and 5; on days 3 and 4, deuterium-depleted water was given. While we found an optical trend to decreased parameters of despair behavior in the experimental group, these changes between groups were not significant. Thus, increases in group sizes or longer supply of deuterium-depleted water are likely to exert an antidepressant effect of deuterium-depleted water. This question will be studied in our next experiments that are underway.

ANTIFUNGAL AGENT FOR THE PREVENTION AND COMPLEX TREATMENT OF ORAL CANDIDIASIS IN GERIATRIC PATIENTS WITH COVID-19

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Candida albicans (C. albicans) is a potentially dangerous pathogen for geriatric patients with systemic disorders and high risk of developing morbidity from COVID-19, especially for denture wearers with prosthetic stomatitis. As known, *C. albicans* infection, the main denture-associated candidiasis infection, can complicate COVID-19 and increase associated morbidity and mortality. Taking into account the age category of patients, it is important to develop more effective antifungal medicines and methods for the prevention of oral candidiasis. The main approach to treating various types of oral candidiasis involves the use of topical or systemic antifungals. It is advisable to use biologycally active complexes in the therapy of oral candidiasis to minimize side effects, reduce the risk of developing allergic reactions, and reduce the dose of topical or systemic antifungal agents. We have developed the original formulation of topical antifungal agent based on stigma and petal alcohol extracts

of Iran endemic Crocus species (*C. haussknechtii Boiss. & Reut. ex Boiss*., *C. speciosus M. Bieb.* (Bieberstein's crocus), and *C. sativus L.*). Developed antifungal agent also have a proven anesthetic, analgesic, antioxidant, anti-inflammatory effect due to the content of flavonoids, anthocyanins, alkaloids, saponins, and tannins. As well known, the main metabolites of biologically active complex of Crocus species, crocin (glycoside carotenoid) and safranal (cyclical terpenic aldehyde) suppresse inflammatory pain response, decrease the number of neutrophils, and have an antinociceptive effect. The developed formulation of antifungal agent for topical use based on stigma and petal alcohol extracts of Iran endemic Crocus species, after appropriate clinical trials, can be recommend for prevention and complex treatment of such types of oral candidiasis, as acute pseudomembranous candidiasis, acute and chronic atrophic candidiasis, and others.

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DIBENZOYL THIAMINE TO AMELIORATE NEUROINFLAMMATION ASSOCIATED WITH ALS-LIKE SYNDROME IN MICE

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease lacking efficient treatment. We used a novel transgenic mouse line based on the mutation of Fused in sarcoma protein (FUS), DNA/RNA-binding factor, a cause of 5-7% of clinical cases of ALS. Since oxidative stress and neuroinflammation are known as mechanisms of ALS development, current study was aimed at the investigation of possible protective effects of antioxidant thiamine compounds on key hallmarks of the ALS syndrome in FUS-tg mice. Mutant male mice bred on CD1 background and their wild type littermates at the age of 2 months were treated with thiamine at the dose of 50 or 200 mg/kg/day or its highly bioavailable derivative dibenzoyl thiamine (DBT) at the dose of 50 mg/kg/day administrated via drinking water for 6 weeks. All groups were monitored for motor functions in the wire test and pole test. ELISA assay and RT-PCR were used to measure neuroinflammation markers in brain and spinal cord. We demonstrated improved motor functions and decreased of mutant mice treated with DBT compared to non-treated FUS-tg mice. GSK-3β brain content, as well as expression of GSK-3β and IL-1β in the spinal cord were lower in FUS-tg mice treated with DBT in comparison with non-treated mutants, while administration of thiamine was not as effective even at the higher dose. As a result of the current study, we showed potential therapeutic effect of DBT on the development of ALS-like syndrome.

FULLERENE C60 AND ZIRCONIUM DIOXIDE AS NEW PROMISING COMPOUNDS TO AMELIORATE ALS-LIKE SYNDROME IN MOUSE MODEL

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Amyotrophic lateral sclerosis (ALS) is fatal disease that results in theneurodegeneration in the spinal cord and brain and muscle tissue atrophy. To date, riluzole and edaravone are the only drugs used in the clinic for the treatment of ALS, however, their use is largely ineffective.In our study we investigated the effects ofother potential therapeutic agents –zirconium dioxide (ZrO2) and fullerene on behavioral scores, motor functions and parameters of neuroinflammation in FUS (Fused in sarcoma) transgenic mice (FUS [1-351], FUS-tg) as novel ALS model. At the age of 2.5 months FUS-tg and wild type mice received ZrO2 at the dose of 200 mg/kg/day, fullerene at the dose of 0.1 mg/kg/day or standard ALS treatment riluzole at the dose of 8 mg/kg/day for 6 weeks. We demonstrated improved motor functions in the wire test and pole test in groups received both ZrO2 and fullerene compared to non-treated FUS-tg mice. Explorative behavior was also improved by both treatments as it was shown in the open field and novel cage tests. IL-1β expression in the spinal cord measured by RT-PCR assay was decreased in mutants treated with fullerene and ZrO2 in comparison with non-treated FUS-tg mice, while TNF expression was ameliorated only by fullerene administration. In most cases beneficial effects of new treatments were more pronounced than those of riluzole.Altogether, we may suggest fullerene and ZrO2 as new promising candidates for ALS treatment that require further investigation.

NANOBODIES TO PRIORITIZE THERAPY STRATEGIES: FOCUS ON BLOCKING OF SARS-COV-2 PROTEINS

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Single-domain VHH antibodies (also called nanobodies, or single-domain antibodies) are derived from camelid heavy-chain-only antibodies, whose antigen-binding sites are composed of just one peptide chain. This makes their coding regions straightforward to clone from cDNA (without combinatorial issues) into phage display vectors for subsequent selection of high-affinity binders. Nanobodies have been used for a wide range of applications, and their production in *E. coli* or yeast is potentially less expensive and more scalable than conventional antibody manufacturing. Previously, we discussed a method for the generation and binding optimization of VHHs that involves the grafting of the complementarity determining regions (CDRs) from already existing, non-camelid antibodies to VHH frameworks, followed by affinity maturation and target binding improvement using in silico site-directed mutagenesis. VHHs against SARS-CoV-2 (in various forms) have been described recently. Monoclonal anti-SARS-CoV-2 immunoglobulins now represent a treatment option for COVID-19. However, their production in mammalian cells is not scalable to meet the global demand. Using alpaca immune libraries against the receptor-binding domain of the SARS-CoV-2 Spike protein, recently 45 infection-blocking VHH antibodies were isolated. We will review recent advances in this field.

APPLICATION OF AN IN VIVO GEL BIOREACTOR IN ASSESSING THE REGENERATIVE POTENTIAL OF PERIOSTEAL CELL SPHEROIDS AND SCREENING POTENTIAL MOLECULES FOR IMPROVING IT

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Introduction. Modeling regenerative osteogenesis processes in animals is a current issue in a number of biomedical fields. It is especially important to properly conceptualize the mechanisms of osteoreparation when conducting preclinical studies of regenerative medicine products that maximize their potential in this area. There are in vivo bioreactors for growing regenerated skeletal tissues in artificial spaces in a living body, such as under a periosteum elevated with gel, under the titanium walls of regenerators, inside regeneratrons filled with solutions. In this study we would like to announce the development of an in vivo bioreactor in the form of a controlled space in a bone defect under a pseudosynovial membrane, filled with alginate gel, for quantitative and predictive analysis of bone regeneration after spheroid transplantation and for testing potential molecules and compositions that could improve the regenerative capabilities of skeletal tissues in bone surgery.

Materials and methods. Primary cell culture of rabbit periosteum was used in the study. Cell spheroids of standard size and shape were created by 3D cultivation in wells micro-molded in agarose for standard multi-well plates on precision micro-molds (MicroTissues Inc.®). Spheroid viability was evaluated using the LIVE/DEAD® method on days 3 and 6 after cultivation. The spheroids were transplanted onto a pseudosynovial membrane created using the Masquelet induced membrane technique. After removing the methylmethacrylate spacer the space was filled with a chemically defined alginate gel. The in vivo bioreactor (the space inside the pseudosynovial membrane) includes a tubular bioresorbable membrane and alginate gel in the centre. The percentage of the pseudosynovial membrane surface covered with attached and fused spheroids and the volume of newly formed skeletal tissues in the defect served as the quantitative criteria of the regenerative potential of cell spheroids. The kinetics of spheroid and regenerated skeletal tissue diffusion was evaluated by means of multiparameter histomorphometry with NIS Elements software; pictures were taken using light microscopy, scanning laser confocal and electron microscopy and X-ray microtomography with high resolution (SkyScan) on days 3, 7, 14, 21, 60 and 90.

Results. It was demonstrated that in agarose wells it is possible to form cell aggregates of round-shaped periosteal cells consisting of live cells and intercellular substance, capable of fusing with each other in vitro and in the recipient organism after autotransplantation. Periosteal cell spheroids were transplanted on a well-vascularized pseudosynovial membrane, fusing with each other and forming a continuous tissue structure integrated with the recipient surface in an orthotopic position corresponding to the periosteum. It was shown that spheroids densely packed in a single line can initiate osteogenesis in an in vivo gel bioreactor, filling the bone defect with regenerated skeletal tissues. Bone remodeling units passed from bone fragments into the regenerate, allowing for its regenerative remodeling. The dynamics of spheroid tissue fusion and the kinetics of their diffusion into the gel were analyzed. A quantitative evaluation of the regenerative capability of spheroids in vivo was performed and a favorable prediction was made for the realization of the regenerative potential of the biomedical cell product based on this homologue in the clinic.

Discussion and conclusions An in vivo bioreactor was developed for quantitative analysis of bone regeneration in an animal model by means of transplanting spheroids from periosteal cells or other cell products for bone regeneration. This makes it possible to predict the degree of realization of the regenerative potential of spheroids in clinical trials and can be applied for systematic screening of potential molecules capable of enhancing the regenerative potential of spheroids and allowing for effective reparative osteogenesis with their aid. The bioreactor opens up new horizons in finding the optimal physicochemical parameters of a local environment and biopharmacological means to improve the regenerative capabilities of bone and to boost the efficiency of the engraftment of tissue-engineered constructs and their involvement in cell regeneration.

EX VIVO ORGAN MODEL FOR TESTING THE REGENERATIVE POTENTIAL OF CHONDROGENESIS-INDUCING CELL SPHEROIDS AND CHONDROGENIC MOLECULES

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Introduction. Cell spheroids have a number of important advantages over cell-rich fluids in regenerative technologies, they are used as building blocks in bioprinting and tissue engineering. Furthermore, certain types of tissue spheroids composed of hyaline cartilage cells — chondrospheres — are already used in clinical practice to treat articular cartilage defects. Cartilage does not have its own blood vessels, it receives nutrients via diffusion and compression from the underlying bone and synovial fluid which allows for using organ cultures to model ex vivo cartilage regeneration. In this study we would like to announce the development of the first quantitative and predictive analysis of ex vivo cartilage regeneration with the aid of periosteal cell spheroids and articular cartilage chondrospheres for testing potential chondrogenic molecules and compositions that could improve the efficiency and regenerative potential of these homologues of biomedical cell products.

Materials and methods. Primary cultures of sheep chondrocytes and rib perichondrium cells were used. Chondrospheres of standard size and shape were produced using agarose wells (MicroTissues Inc.®). The viability of the produced spheroids was evaluated using the LIVE/DEAD® method. To evaluate the regenerative potential of the spheroids, they were placed in a small defect created in an isolated fragment of sheep articular cartilage with a part of the adjacent subchondral bone. The organ culture was in a bioreactor with aerosol delivery of nutrients. The kinetics of spheroid distribution on damaged cartilage surface and the kinetics of fusion of adjacent spheroids were evaluated with computer morphometry. Histology, immunohistochemistry and scanning electron microscopy were used to conduct a morphological analysis of ex vivo cartilage regeneration. Healing kinetics was evaluated on days 3, 5, 7 and 12. The percentage of the cartilage surface covered with adhered and spread spheroids and the kinetics of volume change of the tissue patch were used as the quantitative criteria of the regenerative potential of spheroids ex vivo.

Results. It was shown that periosteal cell and chondrospheres adhered and spread on the surface of the cartilage wound and then fused with each other, forming a continuous spheroid tissue structure integrated with the intact cartilage. It was demonstrated that densely packed spheroids completely fill the cartilage defect, with the perichondrium cell spheroids showing a more pronounced regenerative potential than the chondrospheres produced from mature cartilage cells. A multilevel analysis of spheroid distribution kinetics along with an evaluation of their tissue fusion kinetics by measuring the fusion surface and the morphometry of the tissue-engineering patch over the course of the interaction between the building blocks and the organ culture allow us to perform a quantitative evaluation of their regenerative potential ex vivo and to predict the regenerative capability of spheroids for cartilage restoration in vivo.

Discussion and conclusions. A new organ model and quantitative analysis of cartilage reconstruction ex vivo were developed for testing the regenerative capability of cell spheroids. This makes it possible to predict the regenerative potential of spheroids for articular cartilage repair in animal models and in the clinic. It can also be applied for systematic screening of potential molecules capable of enhancing the regenerative capabilities of tissue-engineering building blocks for hyaline articular cartilage repair.

IN VITRO EVALUATION OF THE CYTOTOXICITY OF NEW SYNTHETIC CATIONIC ANTIMICROBIAL PEPTIDES AGAINST HUMAN PERIPHERAL BLOOD CELLS

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Cationic antimicrobial peptides (AMPs) are considered as next-generation antibiotics that eliminate bacterial resistance. In view of potential clinical use, the possible effects of AMPs against the human body's cells should be assessed. This study evaluates the cytotoxicity of synthetic AMPs constructed by us earlier based on the analysis of the *Hirudo medicinalis* leech genome: (P1) Phe-Arg-Ile-Met-Arg-Ile-Leu-Arg-Val-Leu-Lys-Leu; (P2) Phe-Arg-Ile-Met-Arg-Ile-Leu-Arg-Val-Leu-Lys; (P3) Arg-Trp-Arg-Leu-Val-Cys-Phe-Leu-Cys-Arg-Arg-Lys-Lys-Val; (P4) Lys-Phe-Lys-Lys-Val-Ile-Trp-Lys-Ser-Phe-Leu;

(P5) Arg-Pro-Ile-Leu-Ile-Arg-Val-Arg-Arg-Ile-Arg-Val-Ile. The peptides can be ranked according to their bactericidal activity as follows: P1=P2>P3>P5>P4. The peptide's cytolytic activity was evaluated by the release of hemoglobin from erythrocytes or lactate dehydrogenase from mononuclear cells and neutrophils. The order of peptide's potency to disrupt the cell membrane was P1>P3≈P5>P2≈P4, with P3 causing cell lysis as well as aggregation. This order was the same with respect to the peptide-induced morphological changes in the cells. The erythrocyte membrane exhibited less resistance to peptides than that of other cells. Blood plasma and albumin inhibited the hemolytic effect of peptides, indicating peptide binding to plasma components, in particular albumin. Activation of respiratory burst in neutrophils in response to peptides was not detected (by chemiluminescence method). Nevertheless, morphological features characteristic of activated cells were observed and were especially pronounced for P1. The results suggest that P2 combines high bactericidal activity with low toxicity against human blood cells. It is noteworthy that P1 which only differs from P2 in the presence of additional Leu at C-terminus and has the same bactericidal activity was more toxic.

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CAN NEURODEGENERATIVE DISEASES BE DEFEATED NATURALLY?

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Rapid changes in life-style, environmental pollution and excessive use of fertilizers and hazardous toxic chemicals during the production of food materials, are seriously life threatening for human beings and causing health hazards.

These toxic chemicals produce neurotoxins that affect the transmission of chemical signals between neurons resulting into neurodegenarating disorders.Currently, there are no cures for neurodegeneration.

For each of the neurodegenerative diseases, there are specific drugs that can be used to minimize their symptoms (such as donepezil and memantine for Alzheimer's disease, L-dopa for Parkinson's, riluzole for ALS).

Plants, herbs, and ethnobotanicals have been used since the early days of humankind and are still used throughout the world for health promotion and treatment of disease. Plants and natural sources form the basis of today's modern medicine and contribute largely to the commercial drug preparations manufactured today.

Keywords: Neurodegenerative Diseases , Alzheimer's disease,Parkinson'sdisease,prevention, medicinal plants

HERBAL TREATMENT AND SYSTEMIC INFLAMMATION IN A MOUSE MODEL: A RELEVANCE FOR THE MANAGEMENT OF COVID-19-ASSOCIATED CYTOKINE STORM

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"Cytokine storm" is regarded as the core pathogenic mechanism of COVID-19 infection and, therefore, represents its key therapeutic target. However, no satisfactory treatment of such kind is currently available. Another major problem with COVID-19 management is the treatment availability in low-income countries, in which no vaccination programs is foreseen soon. In this context, we examined effects standardized herbal component (SHC) in mice. The main components of SHC are aethylalcohol, blackberry, chamomile, garlic, gloves and elderberry, whose application was previously shown to exert anti-inflammatory action. We modeled a "cytokine storm" in mice using R848, an agonist of toll-like receptor (TLR) TLR7/8, and lipopolysaccharide (LPS) which triggers TLR4, given that the activation of these TLRs triggers systemic inflammation. First, mice received SHC for two weeks and were challenged with R848. Gene expression of cytokines: IL-1β, IL-6, chemokines: CXCL1, CXCL10 as well as ACE-2, SAA-2, INF-γ was studied in the liver, brain, and spleen, using PCR. There was a trend to reduced mRNA levels of IL-6 and CXCL1 in the brain and CXCL1, CXCL10, and SAA-2 in the liver of R848-injected SHC-dosed mice, suggesting that the SHC may mitigate inflammation. Second, mice received SHC for 14 days, challenged with LPS, and investigated for behavioral tests of anxiety, exploration and despair. We found subtle effects of improved exploratory behavior but no changes in floating behavior that were suppressed by the injection of LPS. Together, SHC can be suggested as a cost-effective herbal agent with anti-inflammatory properties, potentially useful in a prevention of "cytokine storm".

COMPARISON OF THE ANTIOXIDANT POTENTIAL OF GROSSULARIA RECLINATA FRUITS AND LEAVES

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The Grossularia reclinata leaves can become a cheaper and more accessible plant source of flavonoid compounds than its fruits, which have traditionally been used in therapy due to their varied spectrum of antioxidant activity.

Aim. To analyze the antioxidant potential of the Grossularia reclinata leaves in comparison with its fruits to assess the usefulness of this plant raw material in the creation of new herbal remedies containing polyphenolic compounds.

Material and methods. The identification of flavonoid substances in the Grossularia reclinata leaves was carried out using qualitative reactions described in the State Pharmacopoeia. The quantitative flavonoids' content in terms of rutin was estimated spectrophotometrically using an Analitik Jena SPECORD 250 AG Germany device. The computer program Microsoft Excel 2010 was used for statistical analysis. The level of significant differences was determined at $p \leq 0.05$.

Results. It was proved that an alcoholic solution of Grossularia reclinata leaves contains polyphenolic substances. Their total content in terms of rutin ranged from 0.55 to 0.57%, averaging 0.56 ± 0.006%. The calculation of the index of the leaves' usefulness developed by us showed that the content of flavonoid substances in the Grossularia reclinata leaves exceeds their fruit's content by almost two times.

Conclusion. The obtained results of the study proved the usefulness of Grossularia reclinata leaves to expand the raw material base of phyto production and make it possible to consider this raw material as a promising source for obtaining new therapeutic and prophylactic drugs with antioxidant properties.

STUDY OF INTERACTION OF QUINAZOLINONE DERIVATIVES WITH TEICHOIC ACIDS OF S. AUREUS CELL MEMBRANE BY MATHEMATICAL MODELING METHODS

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It has been found that most gram-positive bacteria contain teichoic acid or related glycopolymers, which play a decisive role in various cellular processes, and also determine the ability of bacteria to survive in adverse conditions [3]. It is known that *S. aureus* produces wall teichoic acids and lipoteicic acids associated with the cytoplasmic membrane [3]. It is justified to consider them as an object for developing a strategy for the creation of new antimicrobial agents that do not cause resistance in the pathogen. One of the directions is preparation of inhibitors of their biosynthesis [4].

The antimicrobial effect of quinazolinones against a wide range of pathogenic strains has been substantiated [2]. Their ability to influence the teichoic acids of the bacterial cell wall is unexplored which is possible with the help of mathematical modeling methods (semi–empirical method PM3) which involve the use of GAMESS software complexes for calculations and the Mopac package for composing and editing structures. Visualization and processing of the results is carried out using the ChemCraft program and a software package for calculating the main characteristics of intermolecular interaction, in which the process of composing the Z-matrix using the output data from the MOPAC program is automated [1].

The calculation of energy characteristics and geometry of adsorption complexes made it possible to exclude from the many potentially possible active centers those that are involved in the formation of complexes with a low probability of formation. A table of the main energy and geometric characteristics was compiled for intermolecular modeling of interactions between molecules. The signature of the active centers of the molecules has been obtained. The energy of hydrogen bond formation is characterized on the basis of multidimensional, heterogeneous, output data of quantum-chemical programs.

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MOUSE MODEL OF GENE × ENVIRONMENT INTERACTION-ASSOCIATED PATHOLOGICAL AGGRESSION FOR DRUG RESEARCH AND DEVELOPMENT

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Pathological aggression often accompanies neurodevelopmental disorders, such as attention-deficit/hyperactivity disorder and autism spectrum disorders. However, up to date there is no effective treatment for aggression, and development of such treatments calls for animal models of pathological aggression. In particular, female aggression remains understudied. Excessive aggression in a clinic is associated with variants within the tryptophan hydroxylase-2 (Tph2) gene, a key enzyme in brain serotonin synthesis. This phenotype is recapitulated in naïve mice with complete, but not with partial Tph2 inactivation. It is known that alongside with genetic predisposition, environmental adversities, e.g., stress may contribute to pathogenesis of neurodevelopmental disorders and abnormal aggression. Recently, we showed excessive aggression and altered monoamine brain metabolism in heterozygous Tph2-deficient ($Tph2^{+/-}$) male mice subjected to rat exposure stress. Predation stress procedure increases measures of aggression, dominancy, and suppresses sociability in $Tph2^{+/-}$ mice, while opposite changes are observed in wild-type control mice. Stressed female mutants displayed excessive aggression along with altered expression of serotonin receptors Htr1a and Htr2a, markers of stress and inflammation glycogen synthase kinase 3-beta (GSK-3β) and AMPA-receptor subunit A2 (GluA2), plasticity marker synaptophysin (Syp), and myelination-related molecules, myelin basic protein (Mbp), proteolipid protein 1 (Plp1), myelin-associated glycoprotein (Mag), and myelin oligodendrocyte glycoprotein (Mog). Together these changes in gene expression may suggest altered myelination and neuroinflammation as putative mechanisms of excessive aggression in these mutant mice. In conclusion, $Tph2^{+/-}$ mutant mice may be useful for modeling G×E interaction in excessive aggression for drug research and development, particularly in females.

STUDY ON N-AXETYLTRANSFERASE (NAT2) GENE POLYMORPHISM USING BIOINFORMATICS TOOLS

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Reconstruction of gene network of the disease, and identify the interaction of genes, proteins and drug compounds are important for background studies of therapy. Using the online bioinformatics tools we analyzed the current data set related to the metabolism of xenobiotics, mediated by N-acetyltransferase 2 (NAT2) gene. The study of allelic polymorphism of the NAT2 gene has prognostic value, allowing to determine the risk of a number of oncological diseases, the degree of increased risk due to smoking and exposure to chemical carcinogens, including drugs. The aim of this study was to determine the frequencies of two important "slow" variants of the NAT2 gene (NAT2 * 5, rs1801280 and NAT2 * 7, rs1799931), which significantly affect the rate of xenobiotic acetylation among the indigenous Nenets population of Northern Siberia. The obtained frequencies of polymorphic variants among the Nenets occupy an intermediate value between those for Europeans and Asians, which might indicate specific features of adaptation. We present a model of the distribution of two polymorphic variants of the NAT2 gene involved in the biotransformation of xenobiotics to study the characteristics of their metabolism in the indigenous inhabitants of Yamal. The work was supported by Russian Science Foundation (grant 19-15-00219).

APPLICATION OF RTCA PROFILING OF CELL LINES FOR QUALITY CONTROL OF BIOMEDICAL CELL PRODUCTS

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Identity is one of the important quality attributes of biomedical cell products (BMCP) determined in the frame of quality control. Identity characterization includes the determination of the proliferative activity of the cell line that is part of the BMCP. To assess the proliferative activity of cells, the xCELLigence instrument can be used, which is a real-time cell analyzer (RTCA) that allows *in vitro* continuous label-free analysis.

The aim of the study was to obtain and compare the RTCA profiles of cell lines DF-2 (model, 12 passage), MRC-5 (normal, 28 passage) and A549 (tumor, 7 passage) at various concentrations (2500, 5000, 10000 and 20,000 cells/well) using xCelligence RTCA DP (Agilent, USA) according to the manufacturer's cell index (CI) analysis protocol during 92 h.

As a result of the experiment, it was shown that each cell line has its own characteristic kinetic profile. The CI_{max} values obtained for the model (DF-2) and normal (MRC-5) cell lines were similar and significantly different from those obtained for tumor cells (A549). The obtained data can be useful for quality control of cell lines as part of BMCP.

The study was carried out as part of a publicly funded research project No. 056-00005-21-00 and was supported by the Scientific Centre for Expert Evaluation of Medicinal Products (R&D public accounting No. 121021800098-4).

TECHNOLOGICAL PLATFORMS FOR THE CREATION OF INNOVATIVE GENE THERAPY DRUGS AND VACCINES

DNA-CONSTRUCTS BASED ON BINARY ANTISENSE TECHNOLOGY TOWARDS SELECTIVE GENE THERAPY

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In 2021, the WHO announced that cancer is the leading cause of death worldwide. Due to the complexity and heterogeneity of oncological diseases, some types of cancers cannot be treated with surgery, radio- and chemotherapy. As auxiliaries, GT agents still have limitations for clinical approval and the search for alternatives or optimization of current approaches remain an urgent global agenda.

Antisense oligonucleotide (ASO) is the mechanism of RNase H-mediated suppressing the gene of interest (e.g. vital housekeeping gene), which leads to decrease mRNA expression and will cause cell death. However, ASOs as drugs still have side effects to normal tissues due to insufficient selectivity.

Previously we have shown the developed Binary Antisense oligonucleotides (BiASO), which activates the cleavage of target GFP mRNA 6 times more effective in the presence of cancer marker KRAS RNA than in its absence *in vitro*.

To enhance the activity of chemically modified with thiophosphates BiASO *in vivo* (K562-GFP cell line), it was placed on a common DNA scaffold, which should increase the rate of activation due to the close arrangement of BiASO strands and decrease background signal in the absence of cancer marker.

It was found that the selective target mRNA cleavage using BiASO on DNA scaffold was 6 times more effective in comparison with original BiASO.

The study was supported by RFBR (No. 20-34-90071).

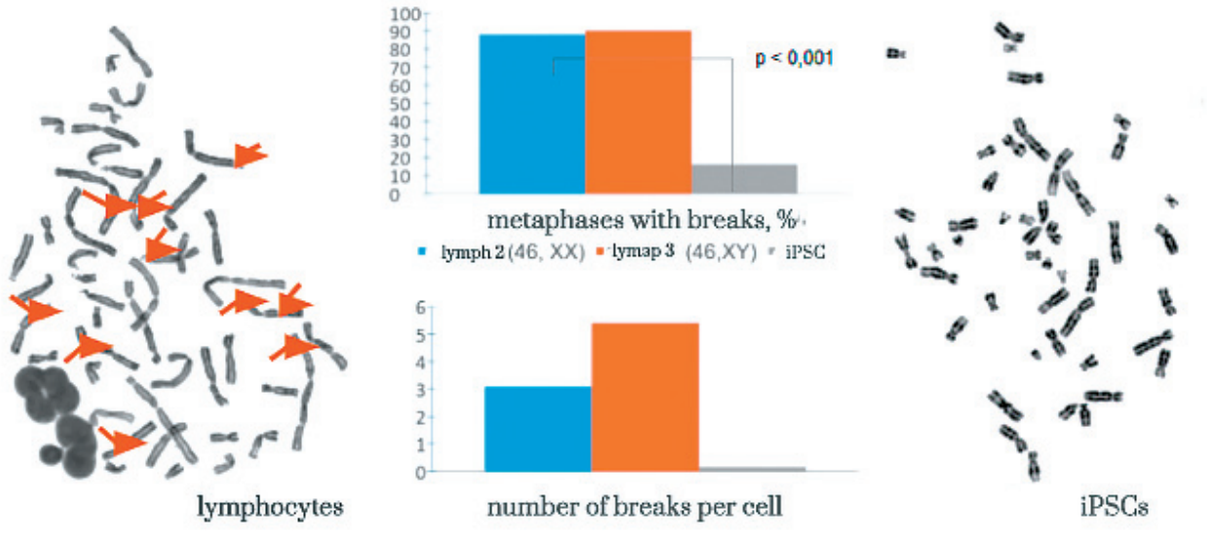

Figure 1. Comparison of the number of chromosomal breaks in cultures of lymphocytes and iPSCs at standard conditions of induction of CFSs.

Figure 2. Chromosome fragility in iPSCs when caffeine is used with aphidicolin.

STUDY OF CHROMOSOME FRAGILITY IN INDUCED HUMAN PLURIPOTENT CELLS

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Replication stress is considered to be one of the factors of mutagenesis in iPSCs. The most vulnerable to replication stress genomic loci are called common fragile sites (CFSs) and can be identified as repeated chromatid and chromosomal breaks and gaps on cytogenetic slides of metaphases[12]. Cytogenetic mapping of CFSs in iPSCs is complicated because of the peculiarities of these cells[3]. The study and mapping of CFSs are important for the use of iPSCs in regenerative medicine, for a better understanding of the mechanisms of genetic pathologies and oncogenesis[45].

The aim of the study is to identify the replication stress conditions of human iPSCs and cytogenetic mapping of

CFSs in iPSCs.To study the replication stress, mutagens are added to the cells, including aphidicolin, which inhibits DNA replication by inhibiting DNA polymerases α, ε, and δ[6], and caffeine, which inhibits ATR and ATM[7].According to the results, the standard conditions for the induction of CFSs (0.4 μM aphidicolin) do not cause chromosomal breaks in iPSCs.The addition of caffeine to aphidicolin made it possible to induce chromosome fragility in iPSCs and to select the optimal conditions for mutagenic action for further mapping of CFSs.

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THROMBUS FORMATION: EXPERIMENT, MATHEMATICAL MODELS, CLINICAL APPLICATION

THE DESCRIPTION OF CLINICAL OBSERVATION RECURRENCE OF PULMONARY EMBOLISM AFTER CESSATION OF 9-YEAR WARFARIN THERAPY

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Introduction. Among the causes of acute cardiovascular accidents, venous thromboembolic complications (VTEC) rank third in the world. Timely diagnosis of pulmonary embolism (PE) is often difficult. Risk factors (RFs) of PE are well known and categorized, however, secondary prevention is insufficient in situations with no identified RFs. The clinical case illustrates repeated PE in 9 years after the withdrawal of anticoagulant therapy (AT).

Description of the clinical case. Patient S. for many years suffered from arterial hypertension, type II diabetes mellitus and obesity, constantly taking antihypertensive therapy. In December 2010 (at the age of 76) she felt shortness of breath, chest pain and was urgently hospitalized at the Sechenov University Clinic. Chest computed tomography (CT) with contrast revealed massive PE of both lungs. Next 9 years the patient continued warfarin therapy, condition remained satisfactory. In September 2019, due to elective surgery, warfarin therapy was canceled and not resumed. After 3 months, shortness of breath, chest pain suddenly reappeared. Chest CT showed bilateral PE, vascular ultrasound revealed acute floating thrombosis of the left femoral vein. The therapy included: Clexane, Enalapril, Indapamide, Bisoprolol, Atorvastatin, Omeprazole. Later, Clexane was replaced by Apixaban. To date, the patient has been taking AT with Apixaban for almost 2 years, PE has not recurred.

Conclusion. The clinical case demonstrates difficulties in duration of AT, especially in patients with RFs of hemorrhagic complications. Thus, a longer follow-up of patients with previous PE, new approaches in risk stratification of recurrent VTEC, and determination the duration of AT are needed.

THE ANTIPLATELET EFFECT CHLORAMINE OXIDANTS CONTAINING AN ADENINE RESIDUE

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The work consisted of investigating the possibility of creating a new irreversible inhibitor of platelet function.

Objective: The work consisted of investigating the possibility of creating a new irreversible inhibitor of platelet functions based on chloroamine derivative of adenosine phosphate that can bind to receptors on the surface of cells.

Methods: Platelet aggregation was measured using the turbidimetric method according to Born on an aggregometer "Biola" (Russia). The addition of agonist (ADP) triggered platelet aggregation, leading to a gradual increase in light transmission of the sample. The quantitative index of platelet aggregation capacity was the maximum change in light transmission of their suspension.

R**esults**: We investigated an inhibitory action of N6-chloroadenosinephosphate on ADP-induced platelet aggregation in platelet rich plasma (PRP). The concentration of semi-maximal decrease in the degree of aggregation (IC50) was approximately 100 μM. AMP at this concentration decreased platelet aggregation only by 25–30%. Chloramine of AMP inhibits isolated platelets to a more significant extent than in PRP. In the case of isolated platelets, IC50 was approximately 10 μM. AMP in this concentration (10 μM) did not have an anti-aggregation effect on platelets. Chloramine of AMP inhibits (IC50=25 μM) thrombin-induced initial platelet aggregation in suspension of cells in a physiological saline. Chloramine of AMP reacts very rapidly with thiol compounds such as reduced glutathione, cysteine.

Conclusion: Since the chloramine of AMP is characterized by increased reactivity toward the sulfhydryl group, this may provide the tropism of the chloramine studied to purine receptors in platelets, which contain free cysteine residues.

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