ABSTRACTS BOOK

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Vasily A. Popkov[1,2], Nadezda V. Andrianova[3], Vasily N. Manskikh[1], Zorova L.D. [1,2], Pevzner I.B. [1,2], Egor Y. Plotnikov[1,2,4] & Dmitry B. Zorov[1,2]

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Pregnancy is a major factor, which affects all systems of mother organism, including kidney function. Kidney pathologies are quite common (up to 10% of all pregnancies) complication, during pregnancy and are very dangerous for both mother and child life (up to 40% mortality rate is some countries). However, there are studies, which report increased capacity for regeneration during pregnancy of some tissues, such as muscles, liver, and spinal cord. Other studies reported changes in oxidative status and altered vulnerability to oxidative stress, which is well known to play a crucial role in tissue damage during ischemia. All these facts together suggest that kidney susceptibility to acute kidney injury can be strongly affected by pregnancy. This study aimed to address acute kidney injury during pregnancy directly and to elucidate underlying mechanisms. We have shown, that pregnancy indeed dramatically affect kidney functions and damage during AKI: number of function and damage markers (such as serum creatinine and blood urea) has shown, that AKI during pregnancy is much less severe. From the other hand, we found decreased basal level of ROS generation and increased one after ischemia/reperfusion. Also, we have addressed fibrosis formation, mitochondria functions, oxidative stress, proliferation, and hypertrophy during the post-ischemic period, and suggested 2 hypothesis, explaining observed phenomena. In summary, our results indicated increased tolerance of kidney during pregnancy.

The study was partially supported by RSF grant 18-15-00058.

APPROACHES TO INCREASING THE DOCKING ACCURACY

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Molecular modeling, especially docking, is increasingly used in the development of medicines. With the help of docking, the molecule (ligand) of the test substance is positioned in the active center of the target protein and the protein-ligand binding energy is estimated: the more this energy, the more effective the drug. Reviews mention more than 60 docking programs in which a great variety of different simplifications are realized, because many decades docking programs are developed in the paradigm of «faster and faster» to dock many thousands of ligands on a laptop. But the calculation «on the lap» in Russian has a meaning: rough, slapdash, etc., and this reveals itself in the widespread use of docking programs. The positioning of ligands is performed satisfactorily by docking programs, but they tend to give large errors in binding energies.

The report discusses sources of docking errors, and approaches to their elimination, recently implemented in supercomputer programs FLM and SOL-P. The SOL-P program successfully docks flexible ligands to proteins with several dozen mobile atoms in the conformational space with an unprecedentedly large number of dimensions due to the application of a new method of global optimization - the tensor trains method. The conclusion is made that almost all existing simplifications that limit the accuracy of docking can be overcome, and a new generation of docking programs can be created, the use of which for the drug development should significantly increase its efficiency. The work was financially supported by the Russian Science Foundation, Agreement no. 15-11-00025.

ENRICHMENT OF VIRAL NUCLEIC ACIDS BY SOLUTION HYBRID SELECTION WITH GENUS SPECIFC OLIGONUCLEOTIDES

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Rapid and accurate identification of the infectious agent is a critical stage of epidemiologic surveillance. Most of the currently available molecular diagnostic tools, such as Polymerase Chain Reaction (PCR), Nucleic Acid Sequence-Based Amplification (NASBA), Loop Mediated Isothermal Amplification (LAMP), are based on specific amplification of nucleic acids. Meanwhile, most of the global virome remains undiscovered, resulting in a lack of reference sequences for novel pathogen identification. Using sequence-independent sequencing methods, such as high-throughput sequencing (HTS), can overcome this limitation; but, due to the extremely low fraction of pathogen genetic material in clinical samples, its application is only cost-effective in special, rather than routine, cases. Here we report a method for the enrichment of conservative pathogenic sequences by hybridization in solution with degenerate oligonucleotides. This approach has increased viral cDNA content for two distinct flaviviruses (yellow fever virus and Japanese encephalitis virus) by hybridization of HTS-ready libraries with pan-flavivirus oligonucleotides by three orders of magnitude. Moreover, it was possible to enrich the content of enterovirus (family: *Picornaviridae*, genus: *Enterovirus*) cDNA using an oligonucleotide designed to the FMDV virus (family: *Picornaviridae*, genus: *Aphthovirus*).

The proposed technique can enrich the share of pathogen genome fragments in a library, even if the nucleic acid sequence of a pathogen is currently unknown. It can also significantly enhance the utility of HTS for routine diagnostics.

INFLUENCE OF DIETARY RESTRICTION ON THE SEVERITY OF ACUTE KIDNEY INJURY IN YOUNG AND OLD ANIMALS

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Acute kidney injury (AKI) is a widespread disease affecting approximately one in five hospitalized patients. Revealing the mechanisms of AKI in aging organisms remains considerable problem while the mean age of patients with this diagnosis is 64 years. One of the approaches that prevents development of a wide range of age-related pathologies is dietary restriction (DR).

The aim of this research was to study the nephroprotective effect of DR on AKI in young and old animals.

We performed the model of ischemic AKI and revealed that in young rats after DR levels of AKI markers were 3.5 times lower than in young rats which were fed *ad libitum*. In old rats there was no effect of DR on AKI severity. To evaluate DR-mediated activation of autophagy we stained vital kidney slices with LysoTracker Green and also measured LC3II/LC3I ratio in kidney. A significant increase in LysoTracker Green fluorescence intensity and LC3II/LC3I ratio was observed in young rats after DR, but there were no such alterations in old rats underwent DR. We also evaluated the intensity of mitophagy by the level of PINK-1 in mitochondria, and it was decreased in young rats after DR.

Thus, DR has a nephroprotective effect on AKI reducing its severity, but only in young rats. This protective effect could be explained by more significant activation of autophagy in kidney tissue of young animals compared to old, as well as by more intensive elimination of the damaged mitochondria.

Supported by RSF grants 18-15-00058 (analysis of AKI) и 14-15-00147 (study of mitochondria).

RAPID DIAGNOSTICS OF HUMAN INFECTIONS AND AUTOIMMUNE DISEASES USING NOVEL OLIGONUCLEOTIDE HYBRIDIZATION PLATFORMS

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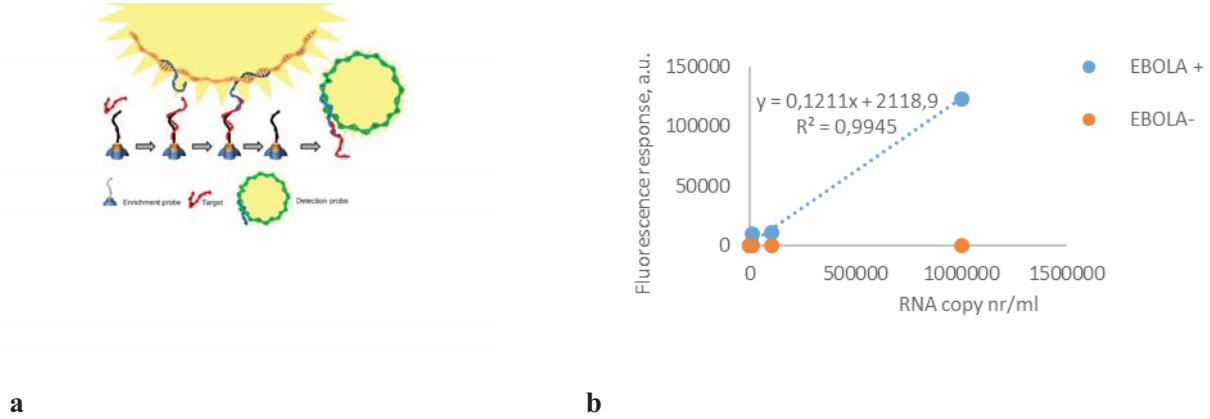
Minimal interference with a biological sample, or *bioorthogonality*, is crucial for reflection of genuine biology. Besides providing a way for the absolute quantification of a biomarker, bioorthogonal hybridization assays are free of amplification-related bias and do not require extensive work flow or computational data analyses.[1]

The hybridization platforms to be presented in this talk allow for enzyme-free, quantitative detection of diverse nucleic acid targets with advantages of robustness, high speed (< 2 h), previously unachievable sensitivity and specificity. Specifically, microfluidic platform for the detection of microRNA (miRNA) and viral RNA will be discussed (Fig. 1).[2] miRNA are a broad group of regulatory molecules playing an important role in biology and clinical diagnostics. In particular, several miRNAs are of considerable significance in the study of cancer and human autoimmune diseases due to post-transcriptional regulatory function of gene expression.[3] Similar approach has been applied to establish an assay for rapid detection of viral RNA in human blood (Fig. 1B).[4] As a final aspect, a new IT tool that enables the translation of the microfluidic platform to modern patient care, IBIO MED, will be presented.



a **b**

Figure 1. A) Key steps of the amplification-free diagnostics using microfluidic hybridization platform; B) Detection of EBOLA RNA from human blood using novel assay, vs. Influenza A negative controls (origin: BEI Resources, USA).

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SPECTROSCOPY AND IMAGING OF MALIGNANCIES IN TERAHERTZ FREQUENCY RANGE

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In this talk, we briefly discuss modern progress in terahertz (THz) diagnosis of malignancies. We pay special attention to the methods of early non-invasive diagnosis of skin neoplasms, as well as intraoperative diagnosis of brain tumors, being rapidly developed nowadays. We discuss our original contribution to this research area, which is associated with (i) studying *in vivo* the dielectric response of normal tissues, ordinary and dysplastic nevi of the skin at THz frequencies;

(i) studying *ex vivo* the THz dielectric permittivity of gelatin-embedded human brain tumors, including gliomas and meningiomas with different WHO grades, (iii) analyzing an ability for differentiation between normal and pathological tissues relying on the methods of THz spectroscopy and imaging, and, finally,**(iii)** developing novel THz instruments for the intraoperative detection of malignant tissues. The results of this study highlight potential of THz technology in non-invasive, least-invasive and intraoperative diagnosis of malignancies in various localizations.

THE ESTABLISHMENT OF HIV-1 PERSISTENCE: IMPLICATIONS FOR A CURE

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The latent reservoir for HIV-1 in resting memory CD4+ T cells is the major barrier to curing HIV-1 infection. Studies of HIV-1 latency have focused on regulation of viral gene expression in cells in which latent infection is established. However, it remains unclear how infection initially becomes latent. Here we demonstrate that a unique set of properties of CD4+ T cells undergoing effector-to-memory transition allow completion of steps in the viral life cycle through integration, but suppress HIV-1 gene transcription, thus allowing the establishment of latency. CD4+ T cells in this stage are significantly more permissive for HIV-1 latent infection than other CD4+ T cells. Establishment of latent HIV-1 infection can be inhibited by viral-specific CD8+ T cells, a result with implications for prevention and elimination of latent HIV-1 infection.

UNRAVELLING IMMUNOGENICITY OF NECROPTOSIS IN ANTI-TUMOR IMMUNITY

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The discovery of immunogenic apoptosis underlines the importance of tumor-host interaction, in which the activation of an immune response, specifically toward malignant cancer cells, results in a potent and long-lasting anti-cancer immunity (1). However, in order to overcome apoptosis resistance, which is often observed in tumors, it is of great importance to find other ways to kill tumor cells by triggering cell death modalities different from apoptosis. Necroptosis is one form of regulated necrosis and is mediated by RIPK1, RIPK3, and its substrate mixed lineage kinase domain-like and has been reported to contribute to inflammation under pathological conditions (2). Necroptosis is commonly induced by ligand- dependent activation of certain members of the TNFR and TLR families. To circumvent the use of cell death-inducing ligands or other immune active molecules that would not allow us to evaluate the immune response resulting uniquely from necroptosis induction, we opted for the use of inducible Tet-On systems for the expression of the downstream necroptotic effector proteins, allowing us to induce necroptosis independently of any receptor activation. In our genetic model a direct dimerization of FADD combined with inducible expression of RIPK3 promotes necroptosis. We report that necroptotic cancer cells release DAMPs and promote maturation of dendritic cells, the cross-priming of cytotoxic T cells, and the production of IFN-γ in response to tumor antigen stimulation. Using both FADD-dependent and FADD- independent RIPK3 induction systems, we demonstrate the efficient vaccination potential of immunogenic necroptotic cells. Our study broadens the current concept of immunogenic cell death and opens doors for the development of new strategies in cancer therapy.

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A NEW NON-TRAUMATIC ALCOHOLISM STAGE III DIAGNOSIS METHOD

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Chronic alcoholism and related diseases – one of the important medical and social problems. In recent years, more and more researchers' attention is attracted to the saliva investigation as clinical and laboratory object [1]. Atraumaticity, repeated sampling possibility, the absence of inconvenience to the patient make salivadiagnostics very promising. For many years, attempts have been made to develop reliable laboratory methods for diagnosing alcoholism based on blood tests. It should be assumed that the determination of biochemical parameters in saliva can serve as an additional criterion for the alcoholism diagnosis.

The aim of this study – development a non-traumatic diagnostic method for the alcoholism stage III estimation.

The material for the study was mixed saliva. Saliva was taken in the morning, on an empty stomach, after teeth brushing and rinsing the mouth with distilled water. Sampling of the material was taken without stimulation – by free stacking in a test tube for 10 minutes. The obtained mixed saliva was centrifuged for 15 minutes at 3000 r/min. For further work we used the supernatant. The activity of superoxide dismutase (SOD) was determined by H.P. Misra and J. Fridovich method [2]. Enzyme activity was expressed in units of activity (EA) per mg of protein in the sample.

Statistical processing was carried out with the definition of arithmetic mean, average error using MS Excel. The reliability of the differences between the control and clinical groups was judged by the magnitude of the Student's t-test after checking the dispersion of normality. Statistically significant differences were considered to correspond to the estimation of the probability $p<0.05$ error.

100 male patients aged 40 to 60 years with suspected chronic alcoholism were examined by SOD activity estimation in mixed saliva. 30 men with episodic alcohol drinking served as control group. A decrease of SOD activity by 50% or more In patients, suspected for the chronic alcoholism, of the age norm was revealed, which was accompanied by data of clinical and laboratory studies confirming the stage III of alcoholism.

The obtained data indicate the possibility salivadiagnostics using in patients with chronic alcoholism [3].

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AUTOANTIBODIES AGAINST VISUAL ARRESTIN AS BIOMARKER OF RENAL CELL CARCINOMA

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Background

Renal cell carcinoma (RCC) is the second most common cancer among uronephrological cancers that characterizes as highly aggressive and invasive with high frequency of metastasis. RCC is also lack in specific symptoms, therefore it is difficult to detect and it is usually diagnosed at late stage of disease in routine screening or during examinations for another pathology. Methods of early specific RCC diagnostic are poorly developed. Recent advances in diagnostic biomarkers of RCC are based on detection of tumor-associated proteins (TAAs) or nucleic acids in blood flow or in tumor itself. However, tumor heterogeneity and complexity of the biopsy procedure may be limiting factors. Contrariwise, blood is less invasive to obtain, but biomarkers undergo degradation by circulating nucleases and proteases, thereby their signals is damped in blood assays. Another type of biomarkers that could be applied for RCC diagnosis is autoantibodies against aberrantly expressed proteins. In contrast to TAAs and nucleic acids, antibodies are far more stable in blood and antibody response is early and enduring. The basis for antibody response against tumor is aberrant expression of TAAs by that tumor. Several TAAs were proposed to be aberrantly expressed in RCC and cancer-retinal proteins are one of that group of proteins. In this study, we analyzed whether aberrant expression of cancer-retinal protein visual arrestin can be detected in RCC and whether autoantibodies against visual arrestin will be produces.

Methods

Blood sera from 33 patients with diagnosed RCC and tumor tissue samples from 39 patients with diagnosed RCC or renal oncocytoma were collected. Western blot analysis was performed to detect autoantibodies against visual arrestin in patients' sera. To reveal the presence of vusual arrestin in renal tumor cells, immunohistochemical assay of tumor sections was performed.

Results

Western blot analysis revealed, that serum samples from 25 out of 33 (75,7%) patients with RCC produces immunostaining of band corresponding to visual arrestin. Immunohistochemical assay of 39 renal tumor tissue samples revealed arrestin-positive reaction in 3 out of 11 (27,3%) samples of clear cell RCC, in 4 out of 5 (80%) samples of chromophobe RCC, in 4 out of 6 (66,6%) samples of papillary RCC, and in 9 out of 10 (90%) samples of renal oncocytoma. Overall, 62,5% of tested by immunohistochemistry renal tumor tissue samples have shown arrestin-positive reaction, among which RCC sections were arrestin-positive in 50% of their cases.

Conclusion

Expression of visual arrestin can be detected frequently in different malignant renal tumor cells. Moreover, it was found that RCC patients often produce autoantibodies against aberrantly expressed visual arrestin. Taking in account such findings and advantages of antibodies over TAAs and nucleic acids as biomarkers, autoantibodies against visual arrestin could be promising biomarker for diagnostic and/or early RCC detection.

BEHAVIOURAL CHARACTERISTICS OF MICE WITH GENETIC DEFICIENCY OF ST3GAL5, THE KEY ENZYME IN BRAIN-SPECIFIC GANGLIOSIDE SYNTHESIS

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The lactosylceramide alpha-2,3-sialyltransferase (ST3GAL5) is a key enzyme in the biosynthesis of brain-specific gangliosides which are critical in many CNS functions. Genetic aberrations of ST3GAL5 in humans are associated with intellectual disability, epilepsy, blindness, anxiety, mental retardation, and altered neuroinflammation. Here, we studied the impact of genetic ST3GAL5 deficiency on motor, emotional and cognitive behaviour using a newly generated a mouse model lacking the ST3GAL5 gene (ST3ko). Young male and female ST3ko mice and wild type littermates were subjected to previously validated battery of behavioural tests for motor, emotional and cognitive behaviours. ST3ko mice demonstrated deficient motor functions in the wire and pole tests as well as increased anxiety-like behaviour in the open field test, which was also found specifically in females in other anxiety-related tests. Male ST3ko mice had increased locomotion. Moreover, ST3ko mice showed subtle changes in cognitive and social behaviour. We conclude, that genetic ST3GAL5 deficiency affects motor, emotional and cognitive behaviour suggesting validity of this mouse model in mimicking clinical aspects ST3GAL5 dysfunction.

BRAIN BIOPSY AS A NEW TECHNIQUE TO ADDRESS DYNAMICS OF GENE EXPRESSION DURING STRESS IN MICE: THE ROLE OF SEROTONERGIC SYSTEM

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Introduction: The aim of the study was to apply a newly developed method of biopsy of prefrontal cortex in wildy type male C57 BL6J mice and mice with genetically compromised expression of TPH2, tryptophan hydroxylase two, the neuronal-specific enzyme of serotonin synthesis, during rat exposure stress. As serotonin is known to play a crucial role in the regulation of stress response and associated with neuronal plasticity and emotional behaviour, including aggression, we studied the expression of several elements of serotonergic system in the prefrontal cortex of mice prior and following rat exposure rat in mice.

Material and methods: TPH2 heterozygous male mice bred on C57BL6J background and their wild type littermates we subjected to a 20-min stereotactic microsurgery, where a small amount of tissue was retrieved from a prefrontal cortex via customized needle. Thereafter they were subjected to a 5-day rat exposure predation stress, studied in the resident-intruder test, and killed for a routine dissection of the prefrontal cortex. RT PCR was run to study expression of genes of interest that are involved in stress response, neuroplasticity and serotonergic regulation.

Results and Discussion: TPH2 heterozygous mice displayed markedly increased scores of aggressive behaviour, while wild type showed their reduction. Stressed mutant mice had significantly upregulated AMPA receptor gene and diminished 5-HT6 receptor expression in comparison to wild type mice. We also found difference in expression of GSK3b and c-fos genes in between stressed and non-stressed groups of mice. Expression of 5-HT1a, 5-HT2a did not differ between two genotypes.

Conclusions: Our findings point to the involvement AMPA and 5-HT6 receptors in the mechanisms of excessive aggression associated with partial genetic reduction of brain serotonin synthesis that is triggered by stress. They also suggest the utility of brain biopsy as a new technique that enables to study dynamics of gene expression in the prefrontal cortex of small rodents.

DEVELOPMENT OF THE UNIVERSAL MIRNA PANEL FOR DIAGNOSTIC AND PROGNOSIS OF TUMOURS OF DIFFERENT LOCALIZATION

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Availability of DNA microarray techniques for micro RNA (miRNA) profiling demonstrated their tissue- and development specificity as well as differential expression in normal and tumor samples. The functional analyses suggest that miRNAs play role in cancer initiation, invasion and progression and, therefore, may be informative biomarkers of detection, diagnosis and prognosis. The expression patterns of miRNAs are even more informative and accurate than messenger RNAs, and due to their small size are less susceptible to degradation in formalin-fixed and paraffin-embedded tissue, allowing miRNA profiling for wider application. Moreover, miRNAs are detectable in plasma and other body fluids, which make possible to use them as non-invasive diagnostic tool.

Some miRNAs are deregulated in multiples cancers and with each miRNA able to target many genes involved in different oncogenic pathways. We proposed that a single panel of properly selected miRNAs could be used as biomarker in several cancers. Therefore, the aim of our study is to find such miRNAs.

To this end, we have analyzed the literature data and chosen five miRNAs known or suggested to play role in tumorigenesis for further investigation, namely miR-155, miR-10b, miR-34b, miR-204 and miR-18a-5p. To quantify their expression level in breast and gastric cancer patient samples, the real-time PCR was performed upon extraction of the total RNA from both tumour and normal tissue surrounded it. The differential expression was confirmed.

The further investigation requires estimation of practical applicability of the miRNA panel for non-invasive diagnostics.

DIFFERENTIAL OPTICAL VISUALIZATION OF TUMOR TISSUE AND INFLAMMATORY FOCUS WITH UPCONVERSION NANOPARTICLES

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Photoluminescent nanoparticles are a promising material for modern interventional diagnostics. Such nanoparticles are able to specifically label the tumor and provide tumor tissue visualization. Upconversion nanoparticles (UCNPs) are a class of luminescent nanoparticles with the ability to convert low-energy photons into photons with higher energy. UCNPs are inorganic nanocrystals (NaYF4) doped with lanthanide ions. Modification of the surface of UCNPs with biocompatible polymer coatings allows to reduce nonspecific cell uptake and provides a long-term visualization of marked structures in the depth of the tissue. The excitation wavelength and the photoluminescence of UCNPs located into the near-infrared window, providing tissue visualization up to several centimeters. The particle size (75±5 nm) determines the possibility of preferential accumulation in tissues with increased permeability of the vascular system, due to the EPR-effect.

We have established that UCNPs, coated with PEG, can circulate up to 10 hours in the bloodstream of C57BL/6 mice. C57BL/6 are efficiently accumulated in mice both bearing Lewis lung cancer and an inflammatory focus induced by a subcutaneously implanted collagen sponge. 24 hours after intravenous administration of nanoparticles, a stable uniform luminescence signal from the tumor and inflammation focus is recorded. When the walls of the vessels are normalized in the inflammatory focus (3-4 days), the signal from UCNPs significantly decreases, in relation to the tumor. On the 5th day after the administration of UCNPs, the inflammatory focus is not visualized, unlike the tumor. Widespread fensters in the vascular wall and lack of lymphatic drainage delay nanoparticles in tumor tissue for a long time, which makes it possible to differentiate it from the focus of inflammation and normal tissues.

EFFECTS OF ICV ADMINISTRATION OF NEURO-CELLS AND RILUZOLE ON MOTOR FUNCTIONS, BEHAVIOR AND INFLAMMATORY RESPONSE OF FUS-TRANSGENIC MICE, NEW MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, motor neuron disease or Charcot's disease, is a devastating fatal disease that is characterized by a progressive muscle paralysis due to the degeneration of motor neurons in the spinal cord and leading to death within about 2-5 years after the onset. Up to know, no effective disease-modifying therapy of ALS is known and the only generally approved drug Riluzole was reported to prolong life span expectancy of the ALS patients only by 10%. Given that Hematopoietic (HSCs) and Mesenchymal (MSCs) stem cells were shown to modify neuro-inflammatory processes that one of the causes motor neuron degeneration, we used Neuroplast's Neuro-Cells composition, an autologous bone marrow derived stem cell preparation containing both types of stem cells, to assess therapeutical of this pathology, on new mouse model of ALS.

We used a novel transgenic mouse line, which is based on the mutation of Fused in sarcoma protein (FUS), DNA/RNA-binding factor, a cause of familial form of ALS. We investigated motor, behavioral and basic physiologic parameters of FUS-transgenic (FUS-tg) male mice and their wildtype littermates during six weeks. Subgroups of mice received chronic dosing with riluzole via drinking water (8mg/kg/day), or single intracerebroventricular (i.c.v.) administration of human stem cells (Neuro-cell, 500 000 in 10 mkl), or i.c.v. treatment with vehicle.

Starting from Day 7 from the treatment onset, weekly monitoring of body weight, motor scores in Pole and Wire tests, novel cage and rotarod was performed. In a subgroup of mice, blood levels of cytokines IL1beta and IL6 were studied. On day 42, water- and food-intake, weight of muscles was assessed.

We found motor aberrations and impaired physiological parameters, alone with signs of systemic inflammation in FUS-tg mice. Positive effects of applied treatments on motor and basic physiological functions were shown in FUS-tg mice, where more pronounced effects of Neuro-cells were found and the effects of riluzole appeared to be limited.

EXPERIENCE IN DEPLETING COMPLEX GROWTH MEDIA FOR CHALCOPHYLIC ELEMENTS TOWARDS STUDYING KERATOCONUS PATHOGENESIS AS A VARIANT OF METAL-DEPENDENT ENZYMOPATHY

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Relevance

Confirmation of keratoconus development hypothesis predicting it to be caused by inactivation of copper-dependent lysyl oxidase in corneal stromal cell culture model required method of depleting growth medium for chalcophylic elements. After adapting basic method of metal-depleted media preparation with use of ion-exchange resin for different cationite medium was tested for its ability to sustain cell viability.

Material and methods

DMEM with 10% FBS was passed through a column with ion-exchange resin «Lewatit MonoPlus C249». After correcting pH and osmolarity, and regaining necessary ions, the medium was added into the cell culture. Elemental content was determined using ICP-MS and EDXS.

Results

Method allows depleting for metal cations by 50–300 times. According to MTS-test, cell survivability amounted to 50–60% during one week when the depleted medium was added to cell monolayer and 13% when passaged. Staining with Calcein-AM showed 90% and 20% of functionality. Cell viability remained high after 10-day incubation in the depleted medium. However, mitosis did not occur during the period.

Conclusion

Method of depleting growth mediums for chalcophylic elements may be carried out using «Lewatit MonoPlus C249». Further research is required for appropriate selection of the conditions necessary for sustained cell proliferation; it will allow future application of the method for modelling pathologic conditions of corneal stromal cells in keratoconus.

LANTHANIDE-BASED NANOPARTICLES WITH ANTI-STOKES LUMINESCENCE AS THERANOSTIC AGENTS

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Theranoctics is a new interdisciplinary research area, which is integration of therapy and diagnostics. Lanthanide-based upconversion nanoparticles (UCNPs) are promising nanoplatform for theranostics, which provide means for imaging biomolecular processes in broad physiological context. UCNPs are composed of inorganic crystalline ceramic host matrix, co-doped with pairs of different trivalent lanthanide ions (Ln^{3+}). Its distinctive feature is the ability to convert near-infrared (NIR) light into visible or ultraviolet light, known as Anti-Stokes luminescence. UCNPs are characterized by excellent photoluminescent properties and low cytotoxicity. Moreover, UCNPs being excited by NIR light enable detection of signal in conditions devoid of autofluorescence and provide deeper penetration of the excitation radiation into biosamples with no tissue damage.

The most common mechanism for passive nanoparticle delivery into solid tumors is the enhanced permeability and retention effect (EPR) which enables UCNP diffusion and accumulation in tumor tissues. The efficiency of the EPR-effect at UCNP delivery into the tumor is associated with circulation lifetime in blood. Thus, the surface modification leading to long UCNP presence in circulatory system remains the challenge.

Here, we report a new surface modification approach of UCNPs by using colominic acid (CA). CA is endogenous non-immunogenic, biodegradable, non-toxic compound with low serum protein adsorption. These peculiarities govern a long circulation time in living organism providing *in vivo* visualization of solid tumor. We developed two-stage UCNP modification method utilizing CA and evaluated their chemical and photophysical properties. Effective accumulation and photothermal therapy of tumor in LLC-bearing mice were observed due to prolonged blood circulation time (up to 3 hours).

MESOTHELIAL-TO-MESENCHYMAL TRANSITION: POTENTIAL THERAPEUTIC TARGET IN POST-SURGICAL PERITONEAL ADHESIONS

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Background Post-surgical peritoneal adhesions (PA) are a leading cause of bowel obstruction, chronic abdominal and pelvic pain and secondary female infertility, which makes them a serious clinical concern. Since they are difficult to diagnose, it is essential to search for prophylactic treatment methods; however, lack of clarity of PA pathophysiology impedes their development. There is a very limited number of studies concerning the role of mesothelial-to-mesenchymal transition (MMT) in PA pathogenesis, which substantiates the significance of this review.

Aim Establishing major steps of PA formation and assess the role of MMT in each of them.

Materials and Methods Systematic review of original research studies from 2001 to 2017 using PubMed, Embase and Scopus databases.

Results Pathogenesis of adhesions can be divided into inflammatory phase, fibrin deposition and matrix remodelling. Upon disruption of mesothelial monolayer, some mesothelial cells migrate from intact surfaces to epithelize the peritoneal defect; however, the absence of contractile proteins in quiescent mesothelial cells points to necessity of MMT for healing of the peritoneum. A decrease in number of functional mesothelial cells due to myofibroblastic conversion leads to insufficiency of anti-adhesive factors, produced by the peritoneum. Additionally, upregulation of fibrinolysis inhibitors, angiogenic factors and collagen I / III aids PA formation at every step. Finally, the presence of fibroblasts positive for epithelial markers on immunohistochemistry within the connective tissue of PAs provides additional evidence for MMT.

Conclusions Current knowledge of PA pathophysiology provides enough to validate MMT inhibition as an appropriate method of preventive therapy.

MOLECULAR BIOMARKER OF THE ACTIVITY OF THE VIRAL PROCESS IN ATYPICAL CHRONIC ACTIVE EBV INFECTION

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Introduction. Nowadays an atypical chronic active infection caused by the Epstein-Barr virus (ACA EBVI) is difficult to diagnose and little-studied disease. Often the ACA EBVI is not diagnosed and/or the degree of active viral infection is not determined.

Aim. Detection of molecular biomarker of the activity of persistent ACA EBVI.

Materials and methods. Under our supervision, there were 98 people of both sexes aged between 23 and 60 years suffering from ACA EBVI.

In addition to traditional clinical methods a PCR method used to detect EBV was using to detect the genome of virus in biomaterials (blood, saliva, urine, scraping from the tonsils and the posterior pharyngeal wall).

Results. We have established the detection rate of EBV genome in various biomaterials in patients suffering from ACA EBVI.

The highest detection rate of EBV DNA is characteristic for such biomaterials as saliva (76.3%), scraping at the back of the pharynx (63.8%) and tonsils (52.7%).

Conclusion. The necessity of compulsory research of these biomaterials is determined by two factors: the place of repeated reproduction of the virus and the fact that the virus circulates less frequently in the blood and a rather limited time. Determination of the activity of a viral infection is necessary to establish a correct diagnosis of ACA EBVI and the appointment of adequate therapy.

MOTOR DEFICITS, ABNORMALSOCIAL BEHAVIOR AND COGNITIVE DISORDERS IN MICE HOUSE ON WESTERN DIET

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Introduction. The "Western diet" is a diet enriched with fat and cholesterol. This type of diet can be an environmental factor that modulates behavior and metabolic processes. Our study has great importance for modern society, since the preference for this diet is increasing in the world.

Aims. We sought to investigate the effects of an insulin sensitizer on the behaviour in female C57Bl6J mice after the consumption of a western diet for three weeks.

Methods. In our study we used the model of non-alcoholic fatty liver disease (NAFLD) that was induced in 3-month-old female C57BL/6J mice by feeding with Western-type diet during 3 weeks.

Social interactions of mice were evaluated in a home cage and in a test for food competition. Cognitive functions were determined by the object exploration test and in the pellet displacement tube-test. The motor function was evaluated using rotarod and wire tests. We also investigated whether dicholine succinate (DS), a mitochondrial complex II substrate that potentiates the effects of insulin on its receptor, can interfere with the effects of Western-type diet.

Results. Housing on Western diet changes normal pattern of social contacts in food competition test and when kept in a home cage, an increase in the indices of locomotor activity. Mice housed on Western diet displayed motor deficits and deficits hippocampus-dependent performance. Dosing with insulin receptor sensitizer dicholine succinate neutralizes the identified changes.

Conclusions. Three-week maintenance of mice on the "Western diet" leads to suppression of social interactions, hyperactivity, strengthens dominant behavior, causes motor disorders, and is accompanied by changes in cognitive functions. These changes can be reversed with an insulin sensitizer.

NOVEL MUTATIONS OF GASTRIC CANCER IN RUSSIAN PATIENTS IDENTIFIED BY NGS

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This study is aimed to identify somatic and germline variants in Russian gastric cancer (GC) patients by NGS. To evaluate GC mutation profile, we conducted NGS for 52 tumor samples using Ion AmpliSeq Cancer HotSpot panel (CHP) and our custom 6-gene (*BMPR1A, SMAD4, CDH1, TP53, STK11, PTEN*) hereditary GC (HGC) panel. Variant alleles were verified by Sanger sequencing to clarify their somatic or germline origin. Prediction of pathogenicity for novel variants was obtained using SIFT, PolyPhen2, SNPs&GO and MutationTaster software. Using CHP detected 38 variants in 22 genes, and 33 variants in 6 genes were found with our HGC panel. We're identified 8 novel mutations, one (*ATM*:p.Glu3022=) is synonymous, 1 is frameshift (*STK11:*p.Ser283fs), 1 affects splicing (*CDH1:*c.1320+2T>G), and the remaining 5 (*SMAD4:*p.Val158Ala, *STK11:*p.Met289Lys, *CDH1:*p.Thr303Pro, *RB1:*p.Leu564Phr, *RB1:*p.His686Asn) are missense. Notably, 2 of the novel variants (*RB1:*p.Leu564Phr & *CDH1:*p.Thr303Pro) are germline and are predicted damaging and disease-causing by bioinformatic tools. Somatic genetic variants profiling will help to identify GC driver mutations. Novel genetic variants may serve as new targets for future therapies and help in understanding the pathobiology of GC.

PLATELET GLUTATHIONE REDUCTASE AND GLUTATHIONE-S-TRANSFERASE ACTIVITY IN FIRST-EPISODE PATIENTS WITH SCHIZOPHRENIA AND SCHIZOAFFECTIVE DISORDER

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Background. First-episode psychosis is the most important time range for antipsychotic treatment and prevention of repeated psychoses, relapses, and further disease progression.

Aim. Comparative assessment of platelet glutathione reductase (GR) and glutathione-S-transferase (GST) activities in first-episode psychoses and in control group, and search for link between these activities and clinical assessments of the patients.

Methods. PANSS scores and platelet GR and GST enzymatic activities in the first-episode patients (men) with schizophrenia (SZ, n=21) or schizoaffective disorder (SZA, n=32) were assessed before and after the treatment course with antipsychotics and recorded in database. Control group consisted of 33 men volunteers. Non-parametric statistics was used for between-group comparisons (Mann-Whitney U-test), and search for correlations (Spearmen rank order correlations).

Results. Significantly reduced GR activities were found in SZ or in SZA group, both before and after the treatment course, as compared with the control group. Significantly reduced GST activities were found in SZA group, both before and after the treatment course, as compared with the control group. Negative correlation was found between GST activity measured before the treatment course and PANSSneg, PANSSpsy, and PANSStotal assessed in SZ group after the treatment course ($R=-0.48$, $p<0.05$).

Conclusion. Reduction of GR and GST activities may evidence for decreased glutathione antioxidant defense in first-episode psychoses, correlation between GST activity and PANSS may have a prognostic value for antipsychotic treatment efficacy.

POSSIBLE OF ATOMIC FORCE MICROSCOPY IN THE BIOMEDICAL RESEARCH

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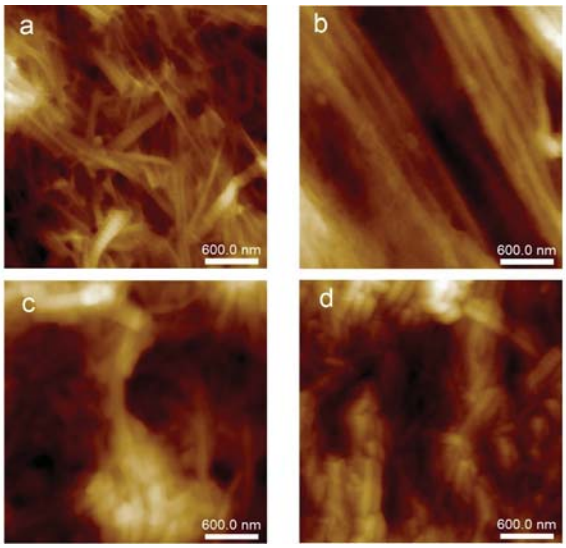
Modern atomic force microscopy (AFM) plays an important role in biomedical research. By selecting different scanning modes and corresponding probes, AFM allows investigation of the structure of both fixed histological sections and living biological objects, as well as very soft adhesive structures, such as natural hydrogels.

Here, we present recently introduced methods in AFM, developed specifically for the biomedical research.

PeakForce Tapping® is a relatively new mode introduced by Bruker. The mode based on the processing of force-distance curves in each point of measurement allows combination of a mild force load on the sample and a high resolution, which is especially important for imaging soft biological samples such as cells.

PeakForce QNM® (Quantitative NanoMechanics, by Bruker) is a scanning mode with the simultaneous measurement of topography and different material properties, such as Young's modulus, adhesion, deformation etc., at the nanoscale. Using PeakForce QNM®, we studied a variety of biological objects using a Multimode 8 atomic force microscope by Bruker. For example, we studied vocal fold tissues of rabbit – normal tissues, scar tissue and the scar tissues after the treatement with autologous mesenchymal stem cells (MSC). We have shown the differences between the normal and scar tissues in the packing of collagen fibrils, their thicknesses and Young's moduli. Besides, we have shown that, after the MSC treatment, both the collagen packing and Young's modulus resemble those of the normal vocal fold tissue that indicates the restoration of the original tissue's elasticity.

The Fast Force Volume regime allows mapping of Young's modulus and stiffness with the preset number of points on the object's surface. It is convenient for very soft materials, such as hydrogels and certain live cells, where imaging with the Peak Force QNM® regime is not possible.



In Figure 1, we display how PeakForce Tapping® - PeakForce QNM® on air was used to study the pathology of rib cartilage in children aged 8-17 years with congenital deformations of the chest – pectus excavatum (PE) and pectus carinatum (PC). We characterized three new types of amianthoid transformation (AT) of the costal cartilage collagen fibers in children: a "classic", a "fine-fibred" and an "intertwined" type. All the AT types represent different stages of extracellular matrix transformation and have different packing and structure of collagen fibers.

Figure 1. AFM of native matrix and the AT types: a) native matrix of rib cartilage; b) "classic" type of AT; c) "fine-fibred" type of AT; d) "intertwined" type of AT. All images have a scan size of 3x3 μm.

In Figure 2, we demonstrate application of PeakForce Tapping® for imaging live cells in their own cell medium, using a Bioscope Resolve AFM (Bruker).

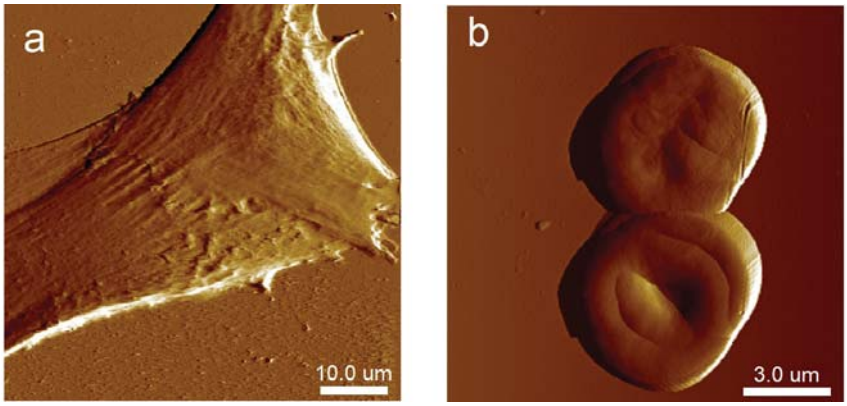


Figure 2. AFM images of live cells in the cell media. a) human MSC, scan size 60x60 μm; b) Two types of human erythrocytes: spherocyte and discocyte, scan size 14x14 μm.

PRO-INFLAMMATORY CNS CHANGES AND ALTERED EMOTIONALITY AS PRODROMAL PHASE OF THE AMYOTROPHIC LATERAL SCLEROSIS SYNDROME: A STUDY ON FUS TRANSGENIC MOUSE MODEL

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Genetic mutations in FUS (*Fused in sarcoma*), a DNA/RNA-binding protein, are associated with inherited and some sporadic forms of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). A line of transgenic mice with neurospecific expression of truncated highly aggregate-prone form of human FUS, FUS[1-359], has been used in this study as a new paradigm of ALS. Taking into account current views that FTLD and ALS can share pathological features and ALS syndrome might affect the brain before the spinal cord, we used this model to study potential changes in emotionality and inflammation during the pre-symptomatic period of ALS. FUS[1-359] transgenic (FUS-tg) female mice and their wild type (WT) littermates were investigated for depression- and anxiety-like behavior, exploration, hippocampus-dependent performance, and expression of markers of inflammation and plasticity under normal conditions, as well as after LPS challenge, prior the manifestation of motor deficits. FUS-tg mice displayed increased scores of behavioural despair in the forced swim test, elevated signs of anxiety in the O-maze and dark-light box, lowered exploration of new environment and new objects, and impaired hippocampus-dependent performance in the food pellet displacement in the tube test. A cohort of mice that was challenged with a bolus intraperitoneal injection of low dose of lipopolysaccharide (0.1 mg/kg) displayed markable increase of floating behavior in the forced swim test and a decrease in sucrose preference, while no such changes in wild type controls were found. Other behaviours, including measures of anxiety, novelty exploration and food displacement in the tube test, as well as expression of inflammatory factors, were not significantly affected in LPS-challenged FUS-tg mice in comparison to naïve transgenic animals, that is likely due to ceiling effects of profound changes in the latter cohort. No signs of motor deficits were found in FUS-tg mice in the rotarod, Pole test, Wire test, thus, confirming that tested animals had the presymptomatic phase of the ALS syndrome. mRNA levels of IL-1β in the prefrontal cortex (PFC) after LPS treatment, when compared to vehicle-treated group, increased only in WT mice. The elevation of COX-1 expression after LPS treatment was found to be significantly higher in WT animals compared with FUS-tg mice. Regardless the genotype, elevation of Timp1/Mmp9 ratio in the PFC was identified after LPS injection compared to vehicle-treated mice. This proteolytic system in WT mice was found to be more sensitive to LPS treatment: the expression of both genes increased after LPS in comparison with vehicle-treated, while in FUS-tg mice no statistically significant change in mRNA levels of these genes was shown, however their ratio is changed in both WT and FUS groups. Together, our study revealed profound emotional deviations and pro-inflammatory changes prior the manifestation of ALS syndrome in employed here animal model and suggest the use of anti-inflammatory therapy for the prevention of familial forms of this disorder.

PROTECTIVE EFFECTS OF TREATMENT WITH THIAMINE (VITAMIN B1) AND BENFOTIAMINE IN THE MODEL OF EMOTIONAL STRESS INDUCED BY ULTRASOUND

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"Emotional stress" is a state of distress which is triggered by perception of saddening or adverse information but not any organic or physical disturbances. It is regarded characteristic for humans leading to depression and associated with increased brain peroxidation. In a new model of emotional stress, mice are exposed to ultrasonic frequencies that mimic their natural sounds of fear/anxiety (20-25Hz) that unpredictably change with "neutral" stimuli (25-45Hz). We studied whether treatment with thiamine (vitamin B1) or its precursor benfotiamine, as antioxidant molecules, counteract behavioral and neurochemical outcome in mouse ultrasound model of emotional stress. Parameters of aggression, anxiety and brain oxidative metabolism were investigated in mice exposed to this paradigm and chronic treatment with thiamine or benfotiamine.

Male BALB/c mice were subjected to a 3-week ultrasound stress and dosing with thiamine or benfotiamine (200 mg/kg/day, p.o.) and studied in the Open field, O-maze and Resident-Intruder tests. Total glutathione and protein carbonyl contents were taken as markers of oxidative stress and determined in different brain structures by the fluorometric assays. Stressed animals showed increased signs of anxiety-like behavior and aggression and elevated total glutathione and protein carbonyl contents in the CNS. Treatment with either drug precluded most of these stress-induced changes. The administration of thiamine or benfotiamine counteract behavioral effects of emotional stress presumably by antagonizing oxidative processes.

STUDY OF INTERACTION OF THE CHAPERONE CELL SYSTEM WITH PRION PROTEIN

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A number of serious diseases are caused by the accumulation of protein aggregates in the nervous tissue, for example, Parkinson's disease or prion diseases. In case of accumulation of erroneously folded proteins, the cellular system of chaperones receives an alarm signal and begins to interact with the assembled protein formations. However, the result of such interaction between chaperones and amyloid proteins has not been fully clarified and can be important for efficient strategies of drug development and treatment of such diseases.

Thus, the aim of our work was to study the interaction of chaperones (eukaryotic chaperonin TriC and bacterial chaperonin complex GroEL14/GroES7) with amyloidogenic prion protein in a normal state and in case of protein glycation taking place in case of diabetes. We also modified prion protein by methylglyoxal in order to show how the metabolic disorder could affect the protein of interest.

Furthermore, we investigated the effect of different forms of prion protein on the activity of chaperonin complex. Chaperonin-dependent reactivation of GAPDH was blocked by the monomeric form of the PrP, more significantly in case of glycated protein. PrP oligomers only slowed down chaperone-dependent reactivation of GAPDH, and the PrP fibrils practically did not affect that process.

The presented data show that both eukaryotic chaperonin TriC and bacterial complex GroEL14/GroES7 bind different forms of prion protein with better affinity than denatured GAPDH. This significantly alters the efficiency of amyloid transformation and can affect the development of amyloidosis.

The work was supported by Russian Scientific Foundation, project No 16-14-10027.

THIAMINE SUPPLEMENTATION ALLEVIATES INFLAMMATON INDUCED BY ALUMINUM CHLORIDE

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Aluminum chloride ($AlCl_3$) has broad applications and became an important environmental risk factor. Since $AlCl_3$ can upregulate glycogen synthase kinase-3 (GSK3), a marker of distress and inflammation, we sought to establish a model of environmental toxicity of $AlCl_3$ and examine potential effects of thiamine as anti-inflammatory agent.

Aim of the study: Investigate anti-inflammation effects of thiamine in models of environmental toxicity associated with $AlCl_3$ in vivo and in vitro.

Methods: Male Sprague-Dawley rats were exposed to $AlCl_3$ (100 mg/kg/day) as well as to thiamine (25 mg/kg/day) for 30 days. Thereafter animals were tested in the open field and Barnes maze. Subsequently, RT-PCR analysis of α- and β- isoforms of glycogen synthase kinase 3 (GSK-3) and interleukin 1 beta (IL-1β) in brain and ELISA plasma corticosterone (CORT) were carried out. We chose tissue protein carbonyl content as marker of oxidative stress. Neuro2a cells in thiamine-deficient media were treated with $AlCl_3$ (1.25 mM) as well as thiamine (50 μM) during 24 hours. Thereafter cell survival rate as well as content of 8-OHdG, a marker of DNA damage, were examined.

Results: Chronically intoxicated rats with a dose 10-fold in excess of the maximum allowed intake, showed memory impairments and increased anxiety as well as a significant increase of GSK-3α/β and IL-1β mRNAs, CORT and protein carbonyl contents. $AlCl_3$ decreased cell survival of Neuro2a cells and increased 8-OHdG content. These $AlCl_3$-induced alterations were mostly antagonized by thiamine in vivo but not in vitro.

Conclusion: Thiamine supplementation of animals could be an effective way to counteract inflammatory processes induced by environmental toxins whose cellular mechanisms of its effects remain to be investigated.

AGING MARKERS IN THE CELLS OF PATIENTS WITH THE COCKAYNE SYNDROME. FEATURES AND DIFFERENCES

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The Cockayne syndrome is a rare autosomal recessive disease, described in the 1930s by Edward Alfred Cockayne. Patients suffer from cachexia dwarfism (when the weight is lowered compared to the norm even more than the growth), photosensitivity, deafness, various visual impairments: optic atrophy, cataracts, degeneration of the corneal epithelium, retinal injuries, as well as neurodegenerative symptoms, such as partial demyelination of subcortical structures, increase in ventricular size, cerebral atrophy, calcification of basal ganglia. The average life expectancy of patients with the Cockayne syndrome is 12 years. In the cells of patients with the Cockayne syndrome, the process of nucleotide excision repair (NER), its branch transcribed coupled with transcription (transcription coupled repair, TCR, TC-NER) is disrupted. We have established that all the aging markers studied are strongly expressed in the cells of patients with the Cockayne syndrome. Thus, the idea of the Cockayne syndrome as a syndrome with expressed cellular signs of accelerated aging is confirmed. This allows us to consider the Cockayne syndrome as a segmental progeria and use cell lines obtained from patients as model objects for studying the processes of aging and testing geroprotectors.

ANALYSIS OF ECTOPIC DYSFERLIN EXPRESSION EFFECT ON THE PROLIFERATIVE ACTIVITY OF HEK293A CELLS AFTER ELECTROPORATION

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Mutations in human dysferlin gene (DYSF) lead to the development of dysferlinopathies. Accumulation of dysferlin protein in the muscle cells-sarcolemma's sites of damage has been described. Therefore, participation of this protein in sarcolemma repair processes can be assumed. Synthesis of a non-functional protein or its insufficiency due to mutations in DYSF gene leads to sarcolemma recovery processes and vesicular transport disruption.

To achieve ectopic dysferlin expression, HEK293A cells were transfected with the previously obtained plasmid vector pCMV-DYSF encoding cDNA of human dysferlin gene. Transfection was performed using TurboFect according to the procedure recommended by the manufacturer. To evaluate the efficiency of transfection and comparative analysis, HEK293A cells were transfected with the plasmid vector pEGFP-N2. Damage to the cell membrane by cell electroporation was performed 48 h after transfection at 200 V and pulse time of 25 ms in a 0.4 m cuvette.

Mitochondrial dehydrogenase activity was evaluated using the MTS test 48 h after electroporation. After electroporation, the proliferative activity of HEK293A cells transfected with plasmid pCMV-DYSF (HEK293A-DYSF) decreased by 20% compared to HEK293A-DYSF cells without electroporation. Interestingly, electroporation decreased the proliferative activity of HEK293A cells transfected with plasmid pEGFP-N2 (HEK293A-EGFP) by 40% compared to HEK293A-EGFP cells without electroporation. Thus, after electroporation, the proliferation activity of HEK293A-DYSF cells was 20% higher than HEK293A-EGFP cells. These results may indicate a positive effect of wild-type dysferlin expression on membrane repair processes in human cells. However, further studies using other cell types and membrane damaging effects are needed to confirm this theory.

ANALYSIS OF THE BIOLOGICAL PROPERTIES AND THE ANTITUMOR ACTIVITY OF MESENCHYMAL STEM CELLS PRIMED WITH CISPLATIN AND PACLITAXEL ANTITUMOR DRUGS *IN VITRO*

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Tumor microenvironment consists of various cell types such as endothelial cells, fibroblasts, immune cells and mesenchymal stem cells (MSCs). MSC tropism to damaged tissues and tumor sites makes them a promising vector for therapeutic agent delivery to tumors and metastatic niches. In this study the biological properties and the antitumor activity of MSCs primed with CDDP and PTX were analyzed *in vitro*.

Nontoxic concentrations of CDDP and PTX have been previously selected for MSC priming. For electron microscopy, hADSCs were primed with CDDP and PTX for 24 h. Ultrastructure of hADSCs were examined with Hitachi HT7700 transmission electron microscope. Native hADSCs demonstrated nucleus of irregular shape and cytoplasm rich in various organelles. Incubation of hADSCs with CDDP at the concentration of 5 mg/ml did not result in substantial changes in the cell ultrastructure. Incubation of hADSCs with PTX at non-toxic concentration (30 mg/ml) resulted in significantly increased number of the cell pseudopodia and the number of autophagic vacuoles in the cytoplasm.

After 48 h of incubation of primed hADSCs conditioned medium was collected and applied on SH-SY5Y and HeLa cells. SH-SY5Y and HeLa cell viability decreased by 20% and 15% correspondingly after incubation with conditioned medium in comparison to control.

It was shown that hADSCs were not significantly affected by PTX and CDDP at studied concentrations and these drugs can be used for MSC priming for potential use in targeted anti-cancer therapy.

This study was supported by grant from the RFBR №18-34-00738.

ASSOCIATION OF LEVELS OF CALCIUM AND PYRIDINE NUCLEOTIDES IN SEMINAL PLASMA WITH QUALITY OF EJACULATE IN IDIOPATHIC INFERTILITY

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Objective: The purpose of the research was to regard the interrelation of the levels of calcium, cAMP and the redox state of pyridine nucleotides in seminal plasma and ejaculate quality in cases of idiopathic infertility.

Design and methods: 170 infertile males and 46 fertile males aged 20-43 were examined. The analysis of ejaculate was undertaken according to the WHO protocol. The content of calcium in the seminal plasma was detected with the help of optical emission spectrometry, the cAMP level was found out by enzyme immunoassay. The redox state of pyridine nucleotides was judged from the ratio of pyruvate to lactate, which was determined by the enzymatic method.

Results: an essential decrease of calcium, cAMP, pyruvate and the oxidation-reduction potential of pyridine nucleotides was detected in the seminal plasma of infertile males with pathospermia. This corresponds to the anaerobic inversion of oxidative conversions and metabolism disadaptation. The level of cAMP reliably correlated with the number of progressively mobile spermatozoa, but not with the number of their pathological forms. A positive correlation between the concentration of cAMP and calcium was discovered as well. Pathospermia was characterized by the positive relation between the value of the $[NAD^+]$ / [NADH] coefficient and the spermatozoa concentration that was not present in fertile donors.

Conclusion: thus, the study revealed distinct changes in the concentration of secondary messengers and redox state of pyridine nucleotides in the seminal fluid that can act as molecular predictors for the development of idiopathic infertility.

FEATURES OF THE INFLUENCE OF TOTAL RNA FROM MONONUCLEAR BMCS ON THE DAMAGED LIVER RECOVERY PROCESSES

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The reprogramming of the BMCs genome at the start of regenerative processes is associated with the recently discovered class of non-coding RNAs and namely, with micro-RNAs. Meanwhile, RNA in BMCs, like in other body cells, is a system of signal molecules of various classes of RNA, regulatory effect of which is achieved by their interaction.

The study aim was the research of the ability of total RNA from mononuclear BMCs to induce the regenerative processes in the damaged liver.

Materials and methods: The liver damage was modeled on male Wistar rats (n=72) by 70% hepatectomy – series 1, and by chronic toxic CCl_4 influence – series 2. Each series included 3 groups: control (introduction of saline solution), introduction of mononuclear BMCs ($2.5\text{-}5.0 \times 10^6$ cells), and introduction of total RNA from mononuclear BMCs (30 μg/100g) of healthy animals. The results of therapy were evaluated: in series 1 - by determining the mitotic and proliferative activity of hepatocytes within 48 and 72 hours; in series 2 – by dynamics of restoration of liver biochemical and morphological indices within 6 months.

Results: In all groups of series 1 the increase in mitotic and proliferative activity of hepatocytes was determined, which was reliable only after the introduction of total RNA. In series 2, the more pronounced positive dynamics of liver recovery processes was also noted after the introduction of total RNA.

Conclusion: The total RNA from mononuclear BMCs provides for the transfer of regenerative signals into the damaged liver tissue, inducing its recovery processes.

MOLECULAR-GENETIC AND MORPHOLOGICAL DIAGNOSTIC OF GRAVITY ENDOMETRIUM AND CHORIAL TISSUE IN EARLY PREGNANSY LOSS

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Summary: The results of clinical, molecular-genetic and morphological diagnostics of gravity endometrium and chorionic tissue in three types of non-developing pregnancy, identified by using ultrasound criteria, are presented. The obtained data can be improved the efficiency of the treatment and pre-conception training after suffering early pregnancy loss.

Non-developing pregnancies account for 45-80% of the total number of early reproductive losses. In order to improve the reproductive health of a woman it is critical to improve the therapy efficiency after early pregnancy loss, taking into account ultrasound examination results and of morphological assessment of gravity endometrium and chorial tissue. We examined and treated 70 women with the diagnosis of non-developing pregnancies during early pregnancy (less than 12 weeks). The patients ages was from 18 to 40 years old admitted to the Gynaecology Department of University Clinical Hospitals No. 4 of I.M. Sechenov First Moscow State Medical University of the Ministry of Healthcare of Russia (Sechenov University). A molecular - genetic research of chorial tissues was carried out in 42 women with AN I for the search for aneuploidy on the chromosomes X, Y, 13, 14, 16, 18, 21 and 22. Test method: multilocus quantitative fluorescent PCR (Amel, DXS6809, DXS6803, DXS8377, SBMA, D13S258, D13S634, D13S742, D18S535, D18S386, D18S391, D21S11, D21S1411, IFNAR, D14S122, D14S127, D14S128, D16S534, D16S476, D16S690, D22S683, D22S691, D22S873) followed by fragment analysis on the genetic analyzer ABI 3100. 43% of patients had embryonic miscarriage (EM) (no heart beat of the embryo), 44% of cases were - Empty sac (ES) (no embryo and yolk sac). 9 patients (13%) had -Yolk sac (YS) (no embryo with yolk sac present) (Kolte A.M., et all.,2015). The average obstetrical stage in EM and ES were diagnosed made 9 ± 2.0 and 8 ± 2.0 weeks, respectively. Upon admission, 45 patients (64%) noted scarce bleeding, 25 patients (36%) did not have any bleeding at all. For surgical treatment of NP, manual vacuum extraction aided by video hysteroscopy was used. After gestational sac extraction, all patients had their diagnosis confirmed morphologically. During gravidy endometrium examination, attention was paid to the nature and rate of inflammatory changes. Chromosomal abnormalities were detected in 40% observations on chromosomes X, Y, 13, 14, 16, 18, 21 and 22. Structure of the revealed pathology: triploidy – 6 (14.2%) of cases, trisomy 16, 21, 22 – 2 (4.7%) each cases, respectively, but trisomy 13, 14, 18, monosomy X, monosomy 21 were detected in 1 (2.3%) each, respectively. Morphological examination revealed that 7 (10%) of patients with ***ES*** *were* without any bleeding. Endometrium was edematous, with focal haemorrhage, small areas of necrosis, slight leukocytic infiltration in 29% and lack of lymphoid cell infiltration. 63% of women with bleeding in **ES** had moderate and marked infiltration with polymorphonuclear leukocytes in their endometrium, even with foul foci formation; 17% of women had focal lymphoid cell infiltration. In **YS,** 3 (33.3%) patients out of 9 had no bleeding, but their endometrium demonstrated slight leukocytic infiltration; 67% of patients had lymphoid cell infiltration. Patients with **EM** had lymphoid cells and did not have any bleeding (33%) or had bleeding (40%); more pronounced the phenomenon was in women with blood type A (II). Where bleeding was present, patients with **EM** had moderate and marked infiltration with polymorphonuclear leukocytes, even with foul foci formation, 1.5 times **rarer** than patients with **ES.** When chorial tissue was assessed it was found out that 47 (67%) women out of 70 patients had dystrophic and necrobiotic changes in their chorionic villi. In **EM**, dystrophic, necrotic and sclerotic changes in villi were more frequent than in patients with ES (33% and 26%, respectively). In general, most marked morphological signs of an acute inflammation process in endometrium (heavy leukocytic infiltration, foul foci formation) were more common for women with bleeding typical of miscarriage. Therefore, in ES where patients *did not complain of scarce bleeding, inflammatory changes in endometrium were less pronounced* compared to those having bleeding. In **YS** and **EM**, most common is *lymphocitic infiltration both with and without bleeding*. Lymphocitic infiltration which is more typical of patients with **EM** was accompanied with inactive viral inflammation in endometrium. Morphological examination are shown the need for ultrasound screening at week 8-9 in order to timely identify non-developing pregnancy. Early hospitalisation into an inpatient unit and timely minimally invasive extraction of dying gestational sac facilitates prevention of complications, e.g. acute and chronic endometritis. Analysis of morphological examinations (taking into account the non-developing pregnancy type) requires personalised approach to antibacterial (fluoroquinolones, macrolides) and antiviral therapy (viferon 1,000,000 IU rectal suppositories, viferon gel 40,000 IU for local therapy) after minimally invasive therapeutic and diagnostic extraction of a pathological gestational sac. Multilocus quantitative fluorescent PCR method is used for research of numerical chromosomal abnormalities of the first trimester and provides a frequency of chromosomal abnormalities in 40% patients with non-developing pregnancy.

PCA3 AND *TMPRSS2:ERG* GENES EXPRESSION ANALYSIS IN URINE SEDIMENT OF THE PATIENTS WITH ADENOCARCINOMA, BENIGN PROSTATE HYPERPLASIA AND OTHER PATHOLOGICAL CHANGES OF PROSTATE

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Prostate tumor cells and nucleic acids slip into the lumen of the urinary tract and are present in the urine sediment, in which mutations and changes of gene expression can be determined. The aim of the our study was to analyze *PCA3* and *TMPRSS2:ERG* expression in urine sediment with benign prostate hyperplasia (BPH) and prostate cancer (PCa) to determine the diagnostic significance of the combined expression level of these genes as PCa marker. The study included 51 patients with BPH and/or prostatitis (control), 59 patients with PCa. After prostate massage, RNA was extracted from the of urine sediment, reverse transcription was performed, then gene expression was analyzed in real-time PCR and the deltaCt value (Ct PCA3-KLK3) was calculated. Medians were 4.09 in the control and -0.20 in the PCa. Using ROC analysis the optimal deltaCt threshold was found to be 1.23. The accuracy of the *PCA3* overexpression was 82, sensitivity 76, specificity 88%. Expression of the chimeric oncogene *TMPRSS2:ERG* was absent in the control and was detected in 59% of the PCa. DeltaCt does not differ in patients with BPH, low and high grade PIN, prostatitis, while significantly increased in PCa with respect to any of the control subgroups listed above ($p<0.01$). Thus, the *PCA3* overexpression and the *TMPRSS2:ERG* are characteristic of PCa. Analysis of these genes expression with the proposed modification of RT-PCR in urine sediment allows to diagnose PCa with accuracy 82%.

PECULIARITIES OF MITOCHONDRIAL MEMBRANE POTENTIAL ASSESSMENT IN C6 GLIOMA CELL LINE

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Studying the mechanisms by which tumor cells in general and their mitochondria in particular retain or eject xenobiotic compounds remains a relevant problem being a basis for development of diagnostics methods and malignant tumor therapy.

The present study was aimed at assessing mitochondrial membrane potential of C6 glioma cells using various fluorescent probes and at comparing their accumulation with that of normal astrocytes. We used C6 glioma cells and rat astrocytes, which were loaded with fluorescent mitochondrial probes: TMRE, JC-1, Mitotrackers Red and Green. Fluorescence was detected with LSM700 laser confocal microscope. Potential independent dye Mitotracker Green demonstrated a classical mitochondrial network with no signs of fragmentation both in glioma cells and in astrocytes. Loading with potential dependent dyes (TMRE, JC-1 and Mitotracker Red) demonstrated high heterogeneity of mitochondrial membrane potential in C6 glioma cells. The most illustrative results were obtain using the JC-1 probe: the culture contained glioma cells with low-energized mitochondria (green) as well as cells with mitochondria with high potential (red). Variation coefficient for JC-1 fluorescence intensity was more than 2-fold higher in glioma cells compared to astrocytes. We also studied the changes in mitochondrial potential in the presence of uncoupler CCCP in concentrations of 0.2, 1 and 5μM. The cells were loaded with 200nM TMRE 10min after adding CCCP. Flow cytometry analysis of TMRE fluorescence intensity in astrocytes showed it's drop in CCCP dose-dependent manner, whereas in C6 cells it rises. We attribute this phenomenon to a more active accumulation of TMRE in glioma cells followed by dye self-quenching. However, when we reduced the TMRE concentration to 50 nM fluorescence intensity also increased. Therefore, glioma cells have certain peculiarities compared to normal astrocytes. They are related to the functioning of mitochondria and accumulation of hydrophobic cationic compounds which possibly underlie the mechanisms of tumor drug resistance.

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THE ROLE OF MAST CELLS IN ALLERGIC REACTIONS OF IMMEDIATE TYPE

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Introduction: Mast cells (MC) are highly specialized immune cells of vertebrate connective tissue. Mast cells play a huge role in the development of an allergic reaction at the location of the pathogen by isolating mediators of inflammation.

Objective: To study the mechanism of IgE-dependent MC activation and inflammatory mediators released by these cells.

Materials and methods: The articles for the period from 2012 to 2017 were analyzed. The search was performed in the databases PubMed, Embase, Scopus. The words used are “mast cells”, “allergic reactions”, “clinical”.

Results: During the analysis of literature data, the mechanisms of IgE-dependent activation of MC were studied. It was found that with IgE-dependent activation, the antigen must bind to at least two IgE molecules on the surface of the mast cell, which leads to the development of an allergic reaction of immediate type. IgE is able to bind firmly to receptors to the Fc fragment on the surface of mast cells and stay here for up to six weeks. Binding of IgE to obese cells leads to their degranulation with the release of mediators (heparin, histamine, prostaglandins and leukotrienes), which cause the symptoms of allergy and inflammation.

Conclusions: The mechanism of IgE-dependent activation depends on the attachment of antigen molecules to the surface of the mast cell. The interaction of IgE with mast cells releases mediators and the appearance of symptoms of an allergic reaction.

CURRENT DRUG DESIGN

KEY ENABLING TECHNOLOGIES IN DRUG DISCOVERY AND PHARMACEUTICAL PRODUCTION

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Introduction

In September 2003, Aboud Leila and Scott Henry wrote an article for the Wall Street Journal that was entitled: "New prescription for drug makers: Update the plants". In a rather eloquent sentence they stated that: "The pharmaceutical industry lags behind potato chips and laundry detergent makers in the use of modern manufacturing systems".

Things have changed greatly over the last decade and that is due, in particular, to the dynamic and cost-effective generic drug market that has increased competition between manufacturers. While the inventor of a socially valuable patented drug may request high prices, according to each country's market, subsequent competition from substitute therapies, even those that are patented, can push these prices down over time. The entry of generics into the market after patent expiration pushes down prices even further and stimulates a thorough revision of production costs and technologies for process intensification.

The transdisciplinary approach taken by pharmaceutical chemists, biotechnologists and engineers is capable of expediting the process of drug discovery and invigorating the design of improved production procedures from the lab scale to pilot tests and industrial production. I envisage that innovation and the ability to demonstrate the huge potential of enabling technologies in pharmaceutical production will be driving forces for competitiveness, business and social impact.

Materials and methods

Of the many enabling technologies for drug discovery and development that are available, microwaves (dielectric heating) have undoubtedly provided the most impressive contribution [1, 2]. This efficient energy source has led to compound libraries for lead generation and optimisation being assembled in a fraction of the time required by classical methods and in higher yields (multi-functionalized structures, long peptides etc.). Microwaves are the technique of choice for the preparation of biocompatible carbon-based materials (carbon nanotubes, reduced graphene oxide etc.) [3], that can influence stem cell attachment, proliferation and differentiation towards specific target cells or tissues.

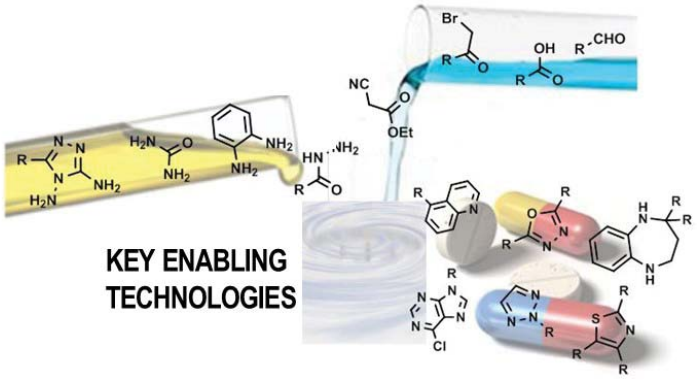
Natural bio- and chemo-diversity offer an inexhaustible library of natural products that can act as sources of new lead bioactive compounds. A fine example of this is the field of cancer, from the 1940s to the end of 2014, in which about 50% of the 175 small molecules approved were either natural or naturally-derived products. Natural product research needs to continually make use of new technologies to quicken the screening, isolation, and structure elucidation processes if it is to stay competitive with other drug discovery methods.

The new concept of green extraction in flow-mode reactors has revolutionized production methods. The use of cavitational reactors (power ultrasound and hydrodynamic cavitation) enables the orthodox concepts of solvent and extract to be bypassed [4]. The fast cell wall disruption caused by intense cavitation in a flow reactor can easily dissolve or simply disperse/emulsify primary and secondary metabolites in a liquid (generally water). These non-thermal techniques guarantee the full natural composition of the original plant by averting degradation, and thus pave the way for large commercial scale-up and significant payback on capital investment. Improvements in pharmaceutical processing that have been granted by new technologies, such as sonocrystallization, ultrasound-aided filtration, dispersion and emulsification under high shear homogenizers and hydrodynamic cavitation are also highly relevant.

Results

Substantial improvements in product quality, process enhancement and cost reduction have been achieved using currently-available techniques, both on the lab scale as well as in, after careful studies, pilot

scale-up and industrial scale applications. Relevant examples are solventless mechanochemical processes with suitable mills, flow-chemistry syntheses in millimetric multichannel mesoreactors and microwave use in flow mode [5]. Key enabling technologies present new ways of synthesizing complex molecules in new reactions that are not possible using conventional methods.



Conclusions

I would like to conclude by citing Albert Szent-Gyorgyi: "discovery is seeing what everybody else has seen, and thinking what nobody else has thought". Many pages about drug discovery and pharmaceutical production can be written thanks to key enabling technologies. Exciting new possibilities and the large potential for high-tech intellectual property will be highly attractive for a wide range of companies and stakeholders.

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A SCALABLE FABRICATION PROCESS OF POLYMERIC DISSOLVING MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY

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Microneedles are designed to create temporary channels on the skin without reaching to the dermis to enhance the drugs transport across the skin barrier. Dissolving microneedle array (DMNA) fabricated from biodegradable polymers can release the encapsulated drugs by dissolving in the skin without any biohazardous waste. In recent years, remarkable progress has been achieved in developing DMNA for successful transdermal delivery of biological small molecules, macromolecular drugs and vaccines.

High-temperature molding, UV photo-polymerization curing, and aqueous solution casting have been used for molding DMNA from dissolving materials. However, these methods are rather complicated involved with several processes, and the radiation source, polymerizing reagents or elevated temperatures may impair the stability of the biomacromolecular drugs and cause skin irritation. Although two-step molding has been widely utilized for the fabrication of DMNA, insufficient drug loading in the needle portion and lack of practicable industrial preparation method have severely impeded their further application.

A modified two-step method using different solvents for needle and base portions under mild conditions was developed by our group, and a series of DMNA containing various model drugs such as levonorgestrel, thymopentin, and salmon calcitonin were successfully prepared. Insertion assessment, stability test, and drug release study were conducted *in vitro*. The therapeutic efficiency was confirmed via *in vivo* pharmacodynamic study. In addition, a novel automatic microneedles array fabrication system (AMAFS 1.0) was initially developed by our group, which can continuously manufacture microneedles with a simple and reproducible operation to control mechanical parameters.

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ASSEMBLING CHEMOMES TO CREATE PHARMACEUTICAL MOLECULES AGAINST DRUG TARGETS

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This talk introduces a *de novo* chemotype (substructure) generation algorithm (DSGA) that derives frequent substructures in order to avoid the subjectivity of empirical method, and avoid the meaningless substructures generated from algorithmic approaches by statistical analyses. DSGA derives frequent chemical substructures (FCS) from a large compound library. In a FCS, substructures are not inter-included. When the library is big enough to represent the chemical diversity, such as ZINC database (27 million medicinal compounds), the resulting FCS is termed as the FCS dictionary (FCSD) for drug-like compounds. For a focused compound library (FL), DSGA can derive a focused FCS (*f*FCS) from FL. *f*FCS can be used as structural descriptors for focus library SAR studies.

Six focused libraries against targets PDE4D, mTOR, HDAC1, DPP4, BACE and ALR2 were tested with DSGA approach. Using the *f*FCSs as structural descriptor sets, six virtual screening models were generated to predict ligands against the targets, the prediction accuracies are greater than 90%.

Three methods were proposed to assembly drug-like molecules from substructures: (1) using the laws in the nature, such as isoprene rule; (2) organic synthesis rules, such as retro-synthon rules proposed by E. J. Corey; (3) pharmaceutical rules derived from a focused compound library against a specific target. We use DSGA to figure out rules that are used to compose privileged scaffolds by assembling FCS.

It can be chemically challenging to make the compounds proposed by these assembling approaches. By combining DSGA method, bioisoterism method and click chemistry, we generated privileged chemome (substructures/chemotypes) from Hsp90 inhibitor library, then find out available chemical fragments with bioisoterism rules. With SPR technology, we confirmed the fragments that interacted with Hsp90. Finally, we used click chemistry to assembly the substructures, and produced nanomolar selective Hsp90 inhibitors.

DEVELOPMENT OF A PHARMACOLOGICAL COMPOSITION TO INDUCE A LONG, STABLE AND REVERSIBLE HYPOMETABOLIC AND HYPOTHERMIC STATE IN RATS IN TERMONEUTRAL CONDITIONS

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One of the most important tasks of modern biology and medicine is a development of technologies for inducing in humans a state of artificial hypobiosis with a possibility to control metabolism and core body temperature. Such technologies may become a solution for such problems as long-term human survival in a confined space under hypoxic conditions, low temperature exposures, deficiencies of a life-support resources, complicated surgeries lasting for many hours, and also prolongation of the "Golden Hour" for wounded soldiers in the field. We have developed an efficient technology for production of a stable liquid mixture of some known pharmaceutical compounds saturated with xenon.

After intravenous injection in rats, the pharmacological composition induced a fast decrease in heart rate followed by 1.5–2 hour stable decline in animal body temperature by about 7˚C-8˚C that may last 8 hours at the ambient temperature of 21-22˚C.

According to our data, after the effect of the composition was declined, the body temperature spontaneously returned to its initial values. At a maximum decrease of the body temperature, oxygen consumption may be two-fold reduced, herewith, blood saturation remained unaltered.

It is shown that the absence in the experimentally-based pharmaceutical composition of one of the components significantly reduces its effectiveness.

When animals exited from hypometabolia and hypothermia, they revealed no changes in their behavior, retaining previously developed skills.

According to the preliminary data, the suggested composition greatly increases resistance of organism to external cooling and hypoxia. It allows to temporarily lower the oxygen and energy demands of the organisms and subsequent recovery of vital functions.

DOPAMINE AND NITROETHANOLAMINE BEXAROTENE DERIVATIVES FOR GLIOMA TREATMENT

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Bexarotene (BXR), a perspective drug candidate for the lethal brain tumor glioblastoma treatment, has a limited cytotoxicity. To enhance it, we modified BXR with a NO donor nitroethanolamine (Bxr-NEA) and dopamine (Bxr-DA), both of which are known to induce NO-dependent cell death for various tumor cell lines, and evaluated their using monolayer cell culture and multicellular tumor spheroids.

Rat glioma C6 and human glioma U-87MG cell lines were used as tumor cells, while human fibroblasts BJ-5ta were proposed as control. Tumor spheroids, which are considered as 3D in vitro model of tumors in vivo, were obtained by RGD-induced cell assembly [Akasov et al, Int J Pharm. 2016; 506(1-2):148-57].

Both Bxr-DA and Bxr-NEA were more toxic for glioma cells than BXR. Thus, after 24 h incubation with monolayer C6 cells, IC_{50} values were 31±1 μM, 23±1 μM, and 122±2 μM for Bxr-DA, Bxr-NEA, and BXR, respectively. Cell death occurred via apoptosis according to annexin and propidium iodide staining. Tumor spheroids demonstrated higher resistance to treatment, and the IC_{50} values for C6 cells in spheroids after 24 h incubation were 51±2 μM, 40±2 μM, and 148±3 μM for Bxr-DA, Bxr-NEA, and BXR, respectively. The selectivity index of the compounds increased from 1.3-1.5 for Bxr to 1.7-3.1 for Bxr-DA.

In summary, BXR modification with NO donors and NO inducers is promising to increase the drug cytotoxicity, while Bxr-DA and Bxr-NEA derivatives could be proposed for glioblastoma treatment. The study in part of spheroids was supported by RFBR (18-04-01087).

FOLATE-ASSOCIATED CATIONIC LIPOSOMES FOR TARGETED DOXORUBICIN DELIVERY

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Targeted drug delivery systems are promising, since they allow to protect drugs as well as to prolong their circulation, to localize and to target them to tumor cells. Liposomes were first nanoparticles proposed as drug delivery systems in the middle of 1960th due to their bilayer lipid structure.

Liposomes can be delivered into cancer cells by so-called Enhanced Permeability and Retention (EPR) effect (passive targeting) as well as using cell-specific ligands that allow them to specifically bind to cell via a complementary membrane receptor (active targeting). Cationic liposomes are supposed to easily penetrate into tumor cells due to their rather high positive membrane charge. To provide targeted drug delivery to tumor cells, cationic liposomes could be modified with folic acid.

The aim of the study was to obtain doxorubicin (DOX)-loaded folate-associated cationic liposomes (FLPs) and to study their cytotoxicity *in vitro* both in monolayer cell culture (2D) and multicellular tumor spheroids (3D).

To prepare FLPs, two types of lipid compositions were used as bases, namely cationic lipopeptide (1) and a mixture of polycationic amphiphile (2) with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). Both bases were combined with two different folate ligands: (3) and (4). Thus, four types of DOX-loaded liposomal dispersions were obtained (Table 1). Physical-chemical FLPs parameters, such as mean diameter, stability and ζ-potential were measured. MTT-test was used for cytotoxicity assay with HeLa (a human cervical cancer), MCF-7 (a human breast adenocarcinoma), U-87 MG (human brain glioma) and C6 (rat brain glioma) cell lines which are known to differ in a number of folate receptors. The FLPs accumulation and localization both in monolayer culture and tumor spheroids were evaluated by confocal microscopy and flow cytometry. Cytotoxicity of various FLPs was studied by MTT-test. The liposomes without DOX (a control) did not possess any cytotoxic effect.

Thus, DOX-loaded folate-associated cationic liposomes were found to be promising for targeted drug delivery.

Table. 1. Physical-chemical parameters of the obtained liposomal dispersions

Lipid composition, % w/w		D, nm	PI, %	ζ- potential, mV
(1) + (3)	98:2	254	87	+47
(1) + (4)	98:2	135	93	+51
(2) + DOPE + (3)	32,5:65,5:2	238	94	+16
(2)+ DOPE + (4)	32,5:65,5:2	270	98	+28

This study was supported by Russian Foundation for Basic Research (grant 18-34-00919 in part of liposomes, grants 17-54-33027 and 18-04-01087 in part of 3D tumor spheroids).

NOVEL APPROACH FOR ROS MEASUREMENTS IN CANCER CELLS TREATED WITH PHOTOSENSITIZERS

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Photodynamic therapy (PDT) is a promising technique for antitumor treatment, which is based on administration of a photosensitizer and subsequent tumor irradiation that leads to generation of reactive oxygen species (ROS). Since the antitumor efficacy of ROS depends on cell-specific antioxidant defense system and tumor microenvironment, it is important to develop methods for high-throughput screening of ROS generation of different photosensitizers inside cells.

We proposed a novel tool for single cell ROS measurements and have shown that this approach has the potential to be used to study the photosensitizer toxicity. We have developed a stable electrochemical probe for measuring intracellular ROS using platinized carbon nanoelectrodes with a cavity on the tip. In the current research, we choose well-known natural photosensitizer riboflavin (RF) as a model agent for photodynamic therapy. Human melanoma A-375 cells were incubated with 100 μM of RF for 30 min, and ROS production under UV light was measured both inside single A-375 cells and outside the cells. We observed that UV photoactivation of RF accumulated in the cells led to the ROS production. Our results show a significant difference for intracellular levels of ROS measured during UV irradiation of A-375 cells treated with RF in comparison with non-treated cells.

In summary, we have developed a label-free method for assessing intracellular activity of photosensitizer using the rapid measurements of ROS with a novel nanoelectrode. The work has been supported partly by Grant RSF No. 16-13-10528, partly by Grants RFBR No. 17-00-00122 (K) (17-00-00118) and No. 16-04-00318\18.

QSAR STUDY OF TOXICICITY OF ORGANIC COMPOUNDS BASED ON 2D SIMPLEX REPRESENTATION OF MOLECULAR STRUCTURE

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During recent decades different theoretical approaches, such as QSAR (Quantitative Structure Activity Relationship), have been used to facilitate and accelerate the process of new drugs creation [1]. The aim of the study is to create QSAR models that allow virtual screening of acute toxicity of compounds in the development of new drugs and identify molecular fragments that steadily increase acute toxicity (toxicophores).In this work, the data set of compounds with acute oral toxicity in rats was obtained from the Toxicity Estimation Software Tool (TEST) [2]. The investigation of influence of the molecular structure of 7205 organic compounds on acute toxicity (LD_{50}) has been carried out with the usage of 2D simplex representation of molecular structure and support vector machine (SVM), random forest (RF), gradient boosting machine (GBM). The developed approach has been implemented in the SPCI software [3] tool, which is publicly available at http://qsar4u.com/pages/sirms_qsar.php.

The obtained QSAR models had reasonable predictive ability estimated by 5-fold cross-validation (Q^2 = 0.54-0.61). Fragment contributions to toxicity were calculated from these models. Known toxicophores were top ranked fragments. We analyzed the contributions of other highly contributing fragments in order to find new potential toxicophores. Additionally, the influence of molecular context of some toxicophores was revealed.

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STRUCTURAL PECULIARITIES AND MOLECULAR MECHANISMS DETERMINING SPECIFICITY OF WHEAT PAPAIN-LIKE CYSTEINE PROTEASE TRITICAIN-A

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Triticain-α is a papain-like cysteine protease (PLCP) from wheat (Triticum aestivum L). Similar to the majority of proteolytic enzymes, Triticain-α is expressed in the form of a zymogen, the autocatalytic activation of which leads to the maturation of an active protease exhibiting pronounced glutenase and collagenase activities at low pH values. Analysis of gluten and collagen cleavage sites revealed that similar to other plant PLCPs, the substrate specificity of Triticain-α is determined by the hydrophobic amino acid within the P2 position of the substrate. However, a comparative study of kinetics of the cleavage mediated by Triticain-α on the pH range 3.6 to 6.5 using distinct fluorogenic peptide substrates showed a pH-dependent specificity determined by the amino acid residue located in the P1 position of the substrate. Molecular docking of substrates in the catalytic domain of Triticain-α obtained by homological modeling allowed to reveal glutamate at position 62 and aspartate at position 160 in the S1 binding pocket, which can interact with substrate through electrostatics. Sequence alignment of the protease domains of Triticain-α and other PLCP detected variability of these residues. Thus, it was concluded that in the papain-like cysteine proteases of plants in addition to the S2 binding site responsible for the protease specificity S1 binding site also contributes in determination of the specificity of enzymes.

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THE PHARMACOLOGICAL DIFFERENCE BETWEEN HUMAN AND RAT ACID-SENSING ION CHANNELS TYPE 3

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Human and rat acid-sensing ion channels type 3 (hASIC3 and rASIC3, respectively) are expressed mainly in peripheral sensory neurons and play an important role in pain perception and inflammation development. In the same time, in our study, we found that hASIC3 and rASIC3 have a number of principal pharmacological differences. Firstly, at physiological resting pH 7.3–7.4 hASIC3 is in the desensitized state, while rASIC3 responds normally to proton stimuli. Secondly, we discovered compounds, such as a bisbenzylisoquinoline alkaloid, lindoldhamine (LIN), from laurel leaves, as well as endogenous isoquinoline alkaloids (EIAs), that have a different effects on human and rat ASIC3 channels: a) at pH 7.4 or higher, LIN and EIAs more effectively activated a sustained, proton-independent, current through hASIC3 than through rASIC3; b) LIN and EIAs potentiated proton-induced transient currents in human, but not rat, ASIC3 channels. These unique, species-selective, ligands of ASIC3 channels, open new avenues in studies of ASIC structure and function, as well as providing new approaches to drug design.

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ANALYSIS OF THE EFFECT OF CONDITIONED MEDIUM HARVESTED FROM MESENCHYMAL STEM CELLS WITH INTERLEUKIN 2 OVEREXPRESSION ON TUMOR CELL VIABILITY *IN VITRO*

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Nowadays, cell and gene therapy are ones of the most promising approaches for cancer treatment. Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells, which can be isolated from different types of adult tissues. Due to their tropism to the tumor niche, MSCs are promising vectors for the delivery of various antitumor agents. One of this agents is IL2 – immunomodulating cytokine, which regulates the activities of white blood cells.

In this study human MSCs were isolated from adipose tissue. MSCs were transduced with recombinant lentiviruses encoding IL2 gene and blue fluorescence protein (BFP) gene. Resulting cell lines were selected with blasticidin S (5 μg/ml) for 10 days. The IL2 gene overexpression was confirmed by quantitative PCR.

The influence of the conditioned medium (CM) harvested from native MSCs, MSC-BFP or MSC-IL2 on SH-SY5Y, SNB19 and A549 tumor cells *in vitro* was investigated. CM was harvested after 24, 48 and 72 hours of cultivation. The viability of SNB19 and A549 tumor cells cultured for 48 h in CM was below the viability of cells cultured in fresh medium. However, there was no significant difference between the SNB19 or A549 samples cultured in CM harvested from native MSCs or MSC-BFP/MSC-IL2. The viability of SH-SY5Y was significantly higher in samples cultured in CM harvested from MSC-IL2 (24 and 72 hours).

Variable effect of MSCs with IL2 overexpression on tumor cell cultures requires further investigations before usage of MSC-IL2 in clinical practice is achieved. This study was supported by grant from the RFBR 18-04-01133.

BIODEGRADABLE POLYELECTROLYTE CAPSULES: ENZYMATIC DESTRUCTION AND THERMO-INDUCED SHRINKING

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Promising direction of nanotechnologies is development of drug delivery systems, where encapsulation plays a leading role due to several advantages, such as protection of encapsulated material, its prolonged release, separation of different bioactive components in one formulation etc.

We propose the biodegradable capsules based on polypeptide (poly-L-arginine) and polysaccharide (dextran sulfate) with encapsulated DNA and evaluate their thermo-driven shrinking and enzymatic destruction. The degradation of the capsules was demonstrated by the influence of the nonspecific enzyme – pronase, which decomposes almost all polypeptides. Scanning electron microscopy (SEM) investigations provide an evidence of the capsule destruction: their shell became thinner and porous. DNA release from the capsules additionally proves the degradation of the system. We demonstrate a possibility of the capsule shrinking by their heating at 90^{O}C for 1 hr. The capsule size decreases from 5 μm to 2 μm, which was shown by SEM and dynamic light scattering (DLS).

This work was performed using the equipment of the Shared Research Center FSRC "Crystallography and Photonics" RAS and was supported by the Russian Ministry of Education and Science". This research was supported by the Federal Agency of Scientific Organizations (Agreement No 007- ГЗ / Ч 3363/26).

CARBON NANOPARTICLES AS NOVEL CARRIERS FOR RADIOPHARMACEUTICALS

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At present, nuclear medicine is becoming more and more relevant in the developed countries of the world and finds new practical applications for the diagnosis and treatment of socially significant diseases. One of the main requirements for radiopharmaceuticals (RFs) is accumulation in the target organ. Directed transport of the radiopharmaceutical is provided by the interaction of two components: transport nanocarriers delivering a radioactive isotope to the target organ; and the isotope. Due to the variety of radionuclides and so much of potential nanocarriers capable of delivering the isotope to the target organ, today we can create the RFs for the diagnosis or treatment of any system of the body. Basic requirements of nanocarriers in RFs are biocompatibility, non-toxicity, selective accumulation in targeted organs, radiation resistance and a significant ability to complexation with radionuclides.

In this work, to solve the problem of delivering a radioactive isotope to a specific organ, it is promising to use new carriers – carbon nanomaterials: nanodiamond, multiwall carbon nanotube and graphene oxide. They possesses suitable physicochemical properties: nanoscale, developed free surface (which is likely to determine high sorption capacity), chemical and radiation resistance, biocompatibility, non-toxicity and the possibility of surface modification by creating certain functional groups by gas and liquid-phase chemical reactions, as well as grafting of various substances to hold radionuclides of different nature.

Sorption experiments of radioactive ions ^{99}Tc and ^{211}Pb on the surface of carbon nanomaterials were carried out under strictly controlled conditions, in which the time to achieve the mobile equilibrium, the dependence of sorption on pH, the total sorbate concentration, etc. were investigated. Sorption parameters and capacity of carbon nanocarriers were determinated.

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ENZYMATIC SYNTHESIS OF NEW NUCLEOSIDES - FLEXIMERS

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Drugs based on modified nucleosides have proved to be effective antiviral (Ribavirin, Azidotimidin, Vidarabin) and antitumor (Cladribine, Fludarabine, Citarabine) drugs in medical practice. One of classes of modified nucleosides is so called “fleximers”.

In fleximers, nitrogen-containing heterocyclic bases are connected via a single C–C bond (see **Fig. 1**). As a result, a rigid fused bicyclic system transforms into flexible heterocyclic system. Those compounds were designed as bioprobes for enzymes, but showed good activity against corona- and filoviruses [1, 2].

We developed an approach to synthesis of ribo- and 2’-deoxyribonucleosides by a transglycosylation reaction using purine nucleoside phosphorylase (PNP) *E. coli*. This enzyme transfers a carbohydrate moiety from natural nucleoside to modified heterocyclic base (**Fig. 1**). This method of synthesis is regio- and stereospecific and allows obtaining new fleximer nucleosides, which usually cannot be synthesized by classical chemical methods.

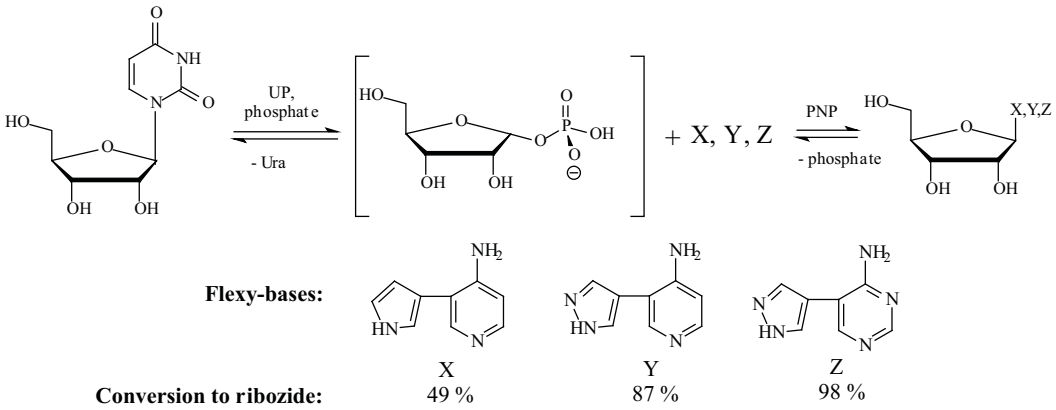

Fig. 1. Transglycosylation reaction
(Ura - uracil; UP - uridinephosphorylase; PNP - purinenucleosidephosphorylase)

We have found that conversion of fleximer base into corresponding nucleoside depends on number of nitrogen atoms in a base. The conversion grows from 49% to 98 % while increasing of this number.

All compounds were characterized by HPLC, LC-MS, and NMR-spectroscopy.

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IDENTIFICATION OF NOVEL HUMAN DIPEPTIDYL PEPTIDASE-IV INHIBITORS OF PEPTIDE ORIGIN

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Nowadays drug discovery is based on the analysis of information about pathological mechanisms, macromolecular targets and their ligands [1, 2]. Our study aimed to identify short peptide as a potential natural inhibitor of dipeptidyl peptidase IV (DPP-IV) to optimize the control of type 2 diabetes. We present the results of computational prediction of biological activity spectrum for new peptide lysyl-α-glutamyl-tryptophan (Lys-Glu-Trp), followed by the subsequent molecular docking for analysis of peptide binding with its protein target.

Computer system PASS 2014 (Prediction of Activity Spectra for Substances) predicts several thousand kinds of biological activity with average accuracy about 95%. Predicted activity spectrum for Lys-Glu-Trp was analyzed by computer program PharmaExpert, which contains data on more than 12,000 established relationships between the pharmacotherapeutic effects and mechanisms of action. According to the PASS prediction, Lys-Glu-Trp may exhibit the following biological activities: Metabolic syndrome treatment (Pa=81%), DPP-IV inhibition (79%) and Glucagon-like peptide (GLP-1) inhibition (79%).

For molecular docking, DPP-IV structure was taken from the PDB database (PDB code 3F8S). The co-crystallized ligand (PF2) in the DPP-IV structure was removed. Molecular docking calculations were carried out to simulate the interaction mode between some of the FDA approved drugs (Sitagliptin, Vildagliptin, Alogliptin, Denagliptin, Gemigliptin, Saxagliptin, Linagliptin) and DPP-IV using the AutoDock Vina. The top low energy structures of all DPP-IV inhibitors had docking energies ranging from – 8.91 to –6.3 kcal/mol. A more negative score indicates that a molecule (ligand) is more likely to dock with the structure (enzyme) and achieve more favorable interactions. Docking score for Lys-Glu-Trp -7.6 kcal/mol that reflects the plausible high inhibitory potential of present studied Lys-Glu-Trp compound with DPP-IV domain.

Thus, the results of molecular docking confirmed the activity against DPP-IV for Lys-Glu-Trp initially predicted by PASS. Results obtained by in silico analysis suggest that Lys-Glu-Trp may modulate activities of essential regulatory incretin peptide hormones DPP-IV and GLP-1, which was confirmed by the subsequent biological testing.

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IN VITRO CYTOTOXICITY OF DOXORUBICIN-LOADED POLYSACCHARIDE/POLYPEPTIDE MULTILAYER CAPSULES

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Polymer-based containers became very promising carriers proposed for drug delivery, during the last decades especially nanoscale systems are in demand. The aim of the research is to develop polymer capsules using combination of layer-by-layer shell assembly and its subsequent thermo-induced shrinking, and to study their interaction with tumor cells.

Capsules were composed of 3 bilayers (dextran sulfate/poly-L-arginine)$_3$ and loaded with doxorubicin (DOX). Heating of the capsules suspension under the optimised conditions leads to a decrease in the average size of the capsules (280 ± 90 nm) together with simultaneous sterilization of the sample. Cell internalization and accumulation of shrunken capsules exhibit a clear time-dependent manner for both, MCF-7 and drug resistant MCF-7/ADR cell lines. DOX-loaded 300 nm capsules are localised mainly in cell membrane and partly in cytoplasm. After 72 h of incubation IC_{50} values for free DOX and DOX-loaded 300 nm capsules are 0.22 and 31.49 μM, respectively. The result of MTT-test proves the DOX activity in the shrunken capsules.

This work was performed using the equipment of the Shared Research Center FSRC "Crystallography and Photonics" RAS and was supported by the Russian Ministry of Education and Science". The study was supported by the Presidential Research Stipend, awarded by the President of the Russian Federation in part of shrinking procedure development, Russian Foundation for Basic Research (Project No. 17-33-80141 mol_ev_a) in part of *in vitro* studies and capsule characterization.

MECHANISMS OF POSSIBLE ANTIBIOTICS ACTION POTENTIATING USING SECONDARY METABOLITES FROM PLANTS

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Background: Multidrug resistance is worldwide problem of 21st century. It occurs in microbial cell and results from activation of different mechanisms in cell structures. Secondary metabolites from plants are synthesized during the photosynthesis. Their release results from activation of intrinsic protective mechanisms.

Aim: To clarify possible mechanisms of antibiotic action potentiating using secondary metabolites from plants.

Materials and methods: We analyzed experimental papers and scientific reviews on the subject using NCBI MedLine, Scholar.Google, and Elsevier databases.

Results: Main antibiotic potentiating mechanisms of secondary metabolites from plants aimed on disturbing structures which are responsible for multidrug resistance forming. In bacterial cell secondary metabolites are able to inhibit β-glucane synthesis, depress efflux. Along with that in fungal cells much more mechanisms occur. For instance, secondary metabolites can inhibit ergosterol synthesis.

In the other hand, secondary metabolites can depress different life processes in pathogen cell: inhibition of nucleinic acid synthesis, DNA-gyrase and glycosidase activity, spindle apparatus forming and mitochondrial electron transport chain.

Conclusion: Wide biological activities of plant secondary metabolites makes them be considered as an effective aid for antibiotic action potentiating in the case of multidrug resistance. Our plan calls for further experimental research on optimal proportions of secondary metabolites for that.

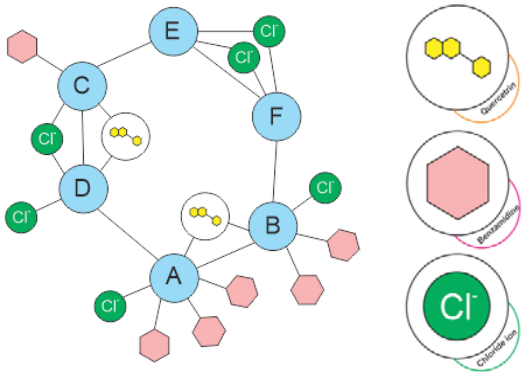

Fig 1. HpFabZ enzyme inactivated with Quercetrin

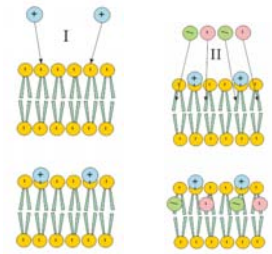

Fig 2. Stages of Membrane structure destructing by Saponines and Tannines

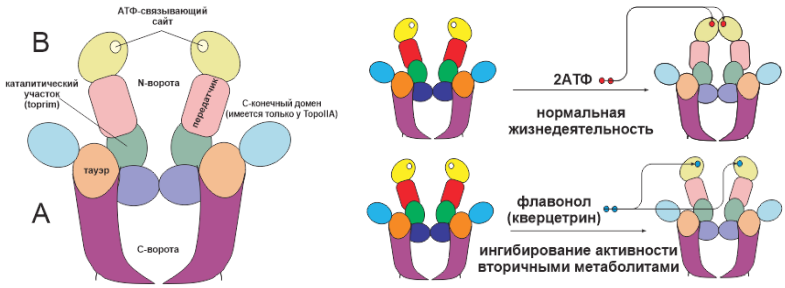

Fig 3. DNA gyrase competitive inhibition using Quercetrin

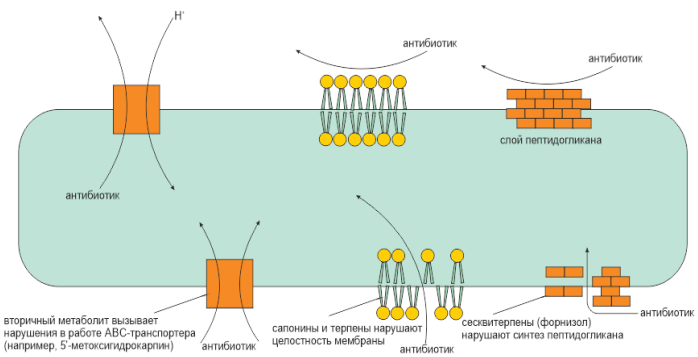

Fig 4. Possible antibiotic potentiating mechanisms of secondary metabolites from plants

All figures created using CorelDraw™

METHODOLOGICAL FLAWS OF RESEARCH ARTICLES ON CELL-BASED THERAPIES COMPLICATE PERFORMANCE OF HIGH-QUALITY SYSTEMATIC REVIEW

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Systematic review and meta-analysis of research articles in the new emerging fields of science, such as cell-based medicine or clinical molecular diagnostics face particular obstacles. In course of performing systematic review dedicated to the cell-based therapies used for the treatment of diabetic foot ulcers (DFU), the most general flaws of the research papers were detected.

Methods. Comprehensive ("shotgun") search query was formulated to find all studies relevant to the subject of systematic review. The database of 5455 abstracts was created. After primary examination relevant studies were selected and submitted to a full-text review (Fig. 1).

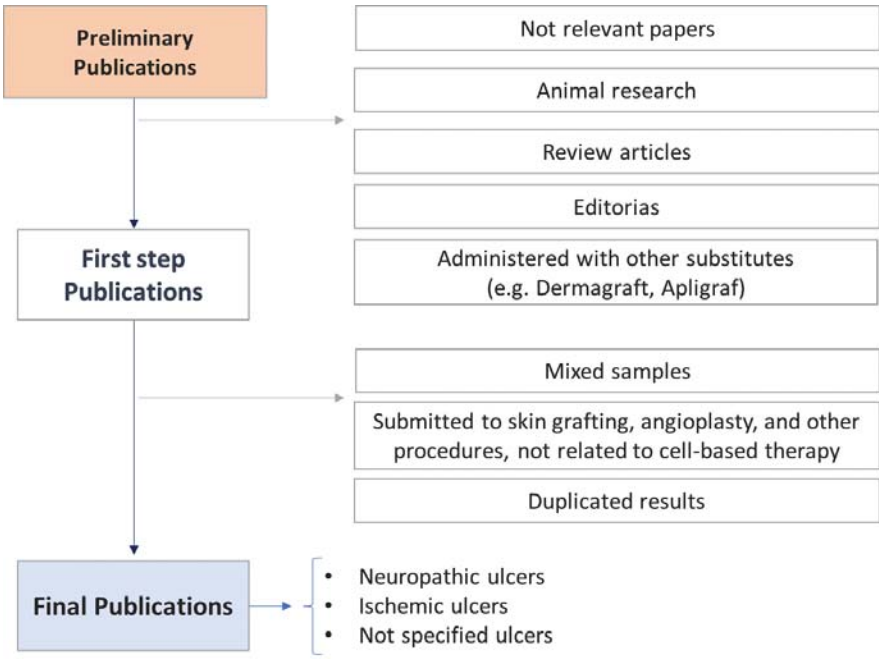

Fig. 1. Sample of Systematic Reviews and Meta-Analyses (PRISMA) scheme.

Results. Reviewing displayed the most general omissions in articles devoted to the cell-based therapies used for DFU treatment:

1. Unspecified nature of treated DFU (neuropathic/ischemic/neuroischemic);
2. Lack of measurement data regarding wound area, number of administered cells;
3. Results of DFU healing are not separated from other chronic wound healing results.
4. Failure to report adverse events, related to the cell transplantation.

Conclusion. Omissions peculiar to the articles on cell-based technologies aggravate data collection/analysis and substantially complicate performance of a high-quality systematic reviews.

CONFORMATIONAL POLYMORPHISM AS NEW METHOD CREATION HIGHT EFFECTIVE DRUGS

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The end of the XXth century is celebrated by the active structural changes in the worldwide pharmaceutical industry. Since 2000 the volume price of transactions on merging and mutual absorption of pharmaceutical companies, creation of strategic scientific alliances as well as conclusion of commercial agreements on the joint product promotion, has exceeded USD more than 100 bln. No doubt, active implementation of structural reforms, as a mechanism for the enterprises' efficiency enhancement, testifies to a certain loss of such efficiency by industrial sectors involved in «New Quality» development of the products under manufacture. The studies carried out on the basis of fifty major pharmaceutical companies for the dynamics of new chemical/molecular entity (NCE) introduction for the market, testifies to the enormous volume of material resources, purposed for the pharmaceutics RD-sector, not providing the branch for the steady growth rate. That seems to be a serious argument in favour of the specialists' position, requiring new interpretation of traditional approach to drug creation, and getting over the difficulties by change of scientific paradigm. The pharmaceutics of the XXth century is an extensive type pharmaceutics. Since the beginning of 1940s the larger number of medicinal substances have been introduced into medical practice compared to that of the multithousand-year history of the mankind. Such kind of approach has been providing for the solution of medical problems by putting into therapeutic practice the newer and newer xenobiotics. Notwithstanding the fact, that data about more complex and subtle structure of a substance, as well as on biological effects arising from the use of molecules of specific geometry have been available since 1950s, a long period of time the research of the new molecules, but not the new molecular conformations, was considered to be economically proved. Starting since 1980s, the research dominants of the new substances and original medicinal forms are beginning to tend towards the study of dispersity effects area, use of polymorphic modifications, selection of molecules by their optical activity and so on.

The proposed original principle of NCE creation, based on the study of conformational polymorphism effects, opens the new technological, medical, patenting, as well as financial opportunities. Introduction of physically firm metastable modifications of biologically active substances consisting of conformationally distorted molecules into the sphere of industrial operation will make it possible not only to obtain drugs of a new high-quality level by their pharmacological and toxicological properties, but will also celebrate a revolutionary turn from the extensive principles to the intensive ones in NCE research industry.

RADICAL-CATCHING ACTIVITY OF FLAVONOIDS

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Comparison of the experimental data on the amount of radicals trapped by one molecule (N) of dihydroquercetin, quercetin, naringenin, apigenin, rutin, kaempferol, luteolin and morin with the results of computer chemistry calculations, modeling of the most probable ways of radical-catching activity of flavonoids. The N values are obtained *in vitro* using decolorization, kinetic methods and spectrophotometric titration with respect to the radical cations 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS•+), extinction of the radical cation ABTS• + at a wavelength of 730 nm (solutions of PBS) or 747 nm (ethanol solutions) was assumed equal to 15000 l/(mol * cm). The N values for the above methods is in the range of 4.6-12.0, 2.7-4.7 and 0.9-8.0 respectively. Geometric optimization calculations carried out in the Jaguar package (program Schrodinger) in a gas

phase and in an water phase with the PBS model and the method M06-2X with a basic functions set 6-31+G** . Calculations are obtained: a) homolytic cleavage of the first O-H bond (these calculations were carried out both in the gas phase and in the water, further calculations were carried out only in gas phase); b) homolytic cleavage of the second bond O-H; c) homolytic cleavage of the third O-H bond; d) formation of quinoide structure; e) stabilization of quinoide structure due to dimerization with the formation of a new CC-connection with the ring A or ring B; f) stabilization due to the solvent attachment. The most probable ways of radical-catching activity by flavonoids are shown.

THERMO-INDUCED SHRINKING OF POLYSACCHARIDE/POLYPEPTIDE MULTILAYER CAPSULES DECORATED WITH MAGNETIC NANOPARTICLES

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Design of drug delivery systems is the one of a priority task for biomedical science. One of the most promising strategies is the development of targeted delivery vehicles. Magneto-responsive capsules could be effective for targeting the drug of the interest to a specific area of the organism, and to promote its controlled release. The purpose of our study was to investigate the effect of heating on capsule size, stability, and loading of biodegradable capsules modified with magnetic nanoparticles.

Capsules assembled from poly-L-arginine/dextran sulfate (PArg/DS) and magnetic nanoparticles (NP) exhibit size reduction and profound compaction regardless of number of polymer layers and polymer layer sequence. Modification of capsule shell with magnetic nanoparticles does not affect the shrinking efficiency: the average capsule size is reduced by 62±7% and 60±9% for $(PArg/DS)_3$ and $(PArg/DS)_{1,5}/NP/(DS/PArg)_{1,5}$ capsules, respectively. The compaction of capsule shells as a result of heating was used to load capsules with a low molecular weight model compound - rhodamine 6G (479 Da). The loading procedure was optimized, and achieved loading efficiency estimated as 79±5% and 96±5% for intact and shrunken capsules, respectively. Difference in shell density of the intact and shrunken rhodamine-loaded capsules was observed by confocal microscopy. Well-dispersed capsules indicate good colloidal stability of the system in water medium even with quite small Z- potential (-18 and -15 mV for intact and shrunken capsules, respectively).

This work was performed using the equipment of the Shared Research Center FSRC "Crystallography and Photonics" RAS and was supported by the Russian Ministry of Education and Science". The study was supported by the Russian Foundation for Basic Research (Project No. 17-33-80141 mol_ev_a) in part of capsules design and by the Federal Agency of Scientific Organizations (Agreement No 007-ГЗ/Ч3363/26) in part of samples characterization.

BIOMATERIALS IN REGENERATIVE MEDICINE

A COMPREHENSIVE IN VITRO STUDY OF STRUCTURAL AND FUNCTIONAL ALTERATIONS OF ACELLULAR COLLAGENOUS MATRICES FOLLOWING CROSS-LINKING

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Bioplastic materials made of decellularized tissues are widely used in reconstructive surgery. In most cases these materials require chemical treatment to suppress immunogenicity and biodegradation. Bovine pericardium tissue is of particular interest in virtue of its facile accessibility and attractive hemodynamic properties. Several modifications of bioplastic materials based on decellularized bovine pericardium have manifested themselves in tissue engineering therapy. However, the consequences of these modifications have not been systematically investigated. In our work we have performed a complex *in vitro* study of structural and functional alterations of decellularized bovine pericardium following cross-linking with different chemical agents including genipin, isocyanate, carbodiimide and epoxy compound.

Ultrastructural studies employing a set of cutting-edge techniques indicated the diversity of the structural reorganization following cross-linking. According to the results of mechanical trials all the cross-linkers reduced the value of Young's Modulus to a different extent. Carbodiimide and genipin-treated matrices exhibited the lowest anisotropy of mechanical properties. In turn, the epoxy compound group demonstrated the lowest shrinkage temperature regardless of the highest cross-linking index. The collagenase susceptibility was an order of magnitude higher for the carbodiimide group compared to the others. The most protective cross-linker in regards to the collagenase stability was the epoxy compound. The genipin and isocyanate protection against collagenase digestion was remarkable, yet significantly lower compared to epoxy compound. Cytotoxicity studies demonstrated satisfactory biocompatibility of the matrices suitable for clinical implementation.

Acknowledgements. This work is supported by the Russian Foundation for Basic Research under grant #18-33-00982.

BIOFABRICATION AND PHENOTYPING OF CHONDROSPHERES FOR CARTILAGE REPAIR

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Introduction. The effective treatment of cartilage defects is an essential clinical problem for which a solution has yet to be found. The most promising strategy for cartilage repair represents scaffold-free approach, based on the use of self-assembling living building blocks, also known as chondrospheres (CSs). In order to optimize and improve this technology we have developed the reproducible method for scalable biofabrication of viable chondrospheres of standard size, shape, and material properties.

Materials and methods. Primary bovine chondrocytes, Cytochalasin D, Nocodazole, immunohistochemical and biomechanical analyses were used for biofabrication and phenotyping of chondrospheres.

Results. The increasing elasticity of chondrospheres correlates with the growing deposition and accumulation of extracellular matrix, confirmed by expression of collagen type II and aggrecan. Moreover, perturbation of cytoskeleton using Cytochalasin D dramatically reduces material properties of chondrospheres, whereas Nocodazole does not affect their rigidity.

Conclusions. Our data demonstrate the role of cytoskeleton and gradually accumulating extracellular matrix in the material properties of chondrospheres. Tissue spheroids rigidity determines their tissue fusion kinetics but does not correlate with their spreading kinetics. Such estimation will be useful in the prediction of post-printing behavior and integrative capacity of chondrospheres after implantation in vivo.

BONE TISSUE ENGINEERING: THE ROLE OF SEEDED CELLS

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Currently there are three key elements in tissue engineering to treat bone defects: cells, scaffolds and growth factors [1]. The cell approach consists of the preliminary seeding of cells onto scaffolds before these matrices are implanted. Several cell types can potentially be used as cellular material, but mesenchymal stem cells (MSCs) are thought to be the most attractive for making a bone repair [2,3]. However there is much that is unknown about MSCs and which needs to be established before this treatment can be widely applied in clinical situations. The purpose of the present work was to study the involvement of seeded allogeneic MSCs in bone formation, in vivo, using the model of transgenic mice and genetically labeled cells.

The scaffolds were sterilized, individually seeded with MSCs from the bone marrow of male 5-week-old GFP(+) transgenic C57/Bl6 or GFP(-) male C57/Bl6 mice. Critical-sized defects were created on the calvarial bone of each animal. Scaffolds with or without seeded cells were implanted into the injury sites. The cranial bones were harvested at either 6 or 12 weeks after implantation. All samples were stained with Hoechst and observed using fluorescence microscopes. GFP(+) transgenic mice having scaffolds with non-labeled MSCs were used for the observation of host cell migration into the scaffold. GFP(-) mice having scaffolds with GFP(+) MSCs were used to assess the functioning of the seeded MSCs. The control group comprised GFP(+) transgenic mice having scaffolds without any cells.

The data demonstrated that allogeneic MSCs were found on the scaffolds 6 and 12 weeks later. Moreover by week 12 there was newly formed bone tissue from the seeded cells without requiring osteogenic pre-differentiation. What is more important, host cells did not appear, and the control scaffolds without seeded cells remained empty. Also the possibility was shown for vessel formation from seeded MSCs without preliminary cell cultivation under controlled conditions. Although the exact mechanisms of the involvement of the seeded allogeneic MSCs in bone formation need further investigation, our data contributes to the understanding of the positive results of MSC transplantation.

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EXTENSION OF MAXIMAL LIFE SPAN AND HIGH BONE MARROW CHIMERISM AFTER NONMYELOABLATIVE SYNGENEIC TRANSPLANTATION OF BONE MARROW FROM YOUNG TO OLD MICE

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The increase in MLS is the most significant indicator of hitting the basic mechanisms of aging, in particular, with regard to age-related loss of stem cells. At old age, the content of stem cells in the bone marrow falls by more than 10 times, and their substitution by the transplanted material can occur without the myeloablative conditioning of the recipients. The aim of this work was to determine the effect of nonablative syngeneic transplantation of young bone marrow (BM) to laboratory animals (mice) of advanced age upon maximum duration of their life. To do this, transplantation of 100 million nucleated cells from bone marrow of young syngeneic donors to an old nonablated animal was performed at the time when half of the population had already died (15 months old). Cells were injected in a volume of 200-300 ml per animal via the tail vein six times within 3 months, with 10-20 day intervals.

As a result, the maximum life span (MLS) defined as the average life span of 10% of the longest-living mice, increased by 31+-3% in the experiment comparably to control group, and the survival time from the beginning of the experiment increased 3+-0.3-fold (fig.1). This significant effect on maximal life span, unlike the median life span fluctuations, indicates that BM transplantation affects the intrinsic aging mechanism. The life-extending effect was significantly stronger than in earlier works with similar design (no irradiation or chemotherapy, no hereditary pathologies in recipients, advanced age at the start of the BM administration) because of (i) the larger amount of transplanted material and (ii) a close relation of the donors and recipients.

The chimerism of the bone marrow 6 months after the transplantation was 28% , as we determined using fluorescent donors of age 3-15 weeks, heterozygous for the green protein transgene (expressing GFP), of the same B10-GFP line and the same mice family as non-fluorescent old mice recipients. This high and permanent chimerism indicates, that rejuvenation is caused not only by paracrine effect, but also by direct cell replacement.

The observed life span extension is accompanied with the extension of active and healthy life period. Transplanted mice were active, had an even spine and shiny even hair (video https://www.dropbox.com/s/zbxnf9vqyxxrczd/ExperimentSDC11916.AVI?dl=0) at the same age of 19 months when the last mouse of the control group was dying), sedentary almost immobile and hunchback with poor hair: https://www.dropbox.com/s/54s35sx6gopbm4y/Contr20160617_191830.mp4?dl=0

The result is encouraging for clinical adaptation for aged humans (70-80-years old). With good and excellent histocompatibility, not only freshly isolated but also cryopreserved bone marrow and peripheral blood cells could be effective. The richest sources of highly proliferative mesenchymal stem cells are the umbilical cord and menstrual blood [Kovina M.V., Krasheninnikov ME., Dyuzheva TG., Danilevsky MI., Klabukov ID., Balyasin MV., Chivilgina OK., Lyundup AV. (2018) Human endometrial stem cells: high-yield isolation and characterization. Published on-line in *Cytotherapy* https://www.sciencedirect.com/science/article/pii/S1465324918300045]. Mass cryobanking of stem cells of young people would solve the issue of donation, which is very acute already now and might become even more complicated in the future, especially in view of geriatric application of stem cells.

Key words: bone marrow transplantation; mesenchymal stem cells; longevity; life extension; cryobank.

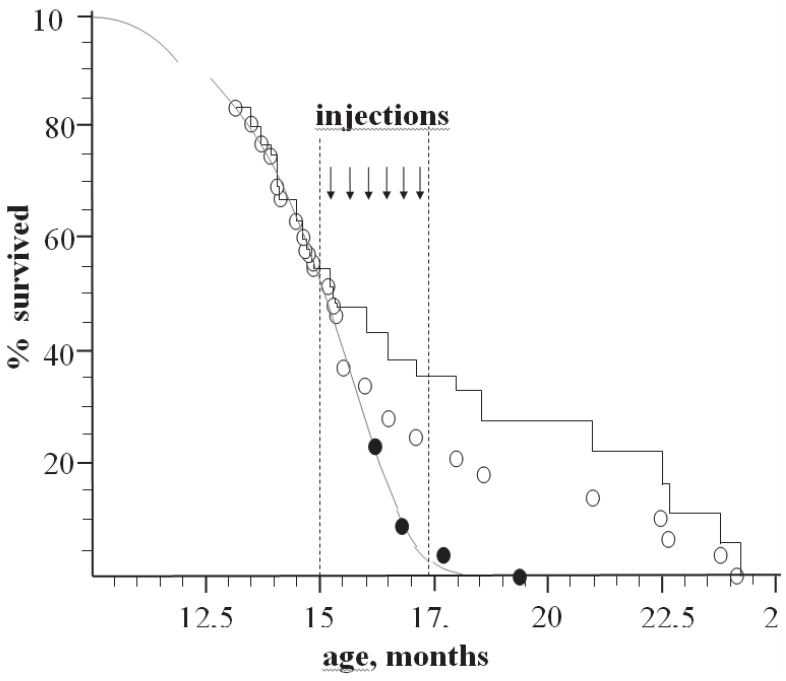

Figure 1. Effect of nonablative transplantation of syngeneic BM of young donors on the population dynamics of aging recipients. Ages of death of 25 mice from the experimental group who died before BM injections were added to the statistics of the control group for $\dot{I}LS_{\hat{E}_2}$ calculation. Open circles (○) - experimental group, closed circles (●) - control group, gray curve - Gompertz–Makeham curve, black curve - experimental group corrected for embolism.

SHP-1 REGULATES HEMATOPOIETIC STEM CELL QUIESCENCE BY COORDINATING TGFB SIGNALING

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Cell cycle quiescence is critical for hematopoietic stem cell (HSC) maintenance. TGFβ signaling in bone marrow niche has been identified in regulating HSC quiescence, however the intrinsic regulatory mechanisms remain unclear. This study reports that *Shp-1* knockout HSCs have attenuated quiescence and impaired long-term self-renewal. SHP-1 activated HSCs are surrounded by megakaryocytes, which regulate HSC quiescence by producing TGFβ1. Mechanistically SHP-1 interacts with the immunoreceptor tyrosine-based inhibition motif (ITIM) on TGFβ receptor 1, and is critical for TGFβ signaling activation in HSCs. Functionally *Shp-1* knockout HSCs do not respond to TGFβ enforced HSC quiescence regulation, both *in vitro* and *in vivo*. Therefore, we identify TGFβ-SHP-1 as a novel intrinsic regulatory mechanism for HSC quiescence maintenance.

SOLUTIONS FOR CELL TECHNOLOGIES IN CLINICAL PRACTICE: DEVICES FOR ADIPOSE TISSUE PROCESSING AND CELL FRACTION EXTRACTION

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Adipose tissue is the most convenient and rich source of cell material for regenerative medicine due to the high content of progenitor cells, which number exceeds in many times the number of them in the bone marrow and other tissues. The stromal-vascular fraction of adipose tissue (SVF), containing different populations of stem progenitor cells, can be easily isolated by an enzymatic method and used for various pathological conditions.

Isolation of SVF from adipose tissue is a multi-stage laboratory process that requires qualification and certain skills in working with cell cultures and biological objects. However, all manual methods are characterized by high time and organizational costs and assume the presence of the so-called “human factor”. Therefore, the separation technologies are evolving towards the automatization of the process. Automatic and semiautomatic systems of SVF extraction have been created in last decade: PNC’s Multi Station, CHA Biotech Cha-Station, Cytori Celution 800 / CRS System, Medi-Khan’s Lipokit MaxStem, JTC’s miniSTEM and NeoGenesis’s UNiStation.

Based on the requirements for clinical efficacy and safety, it is possible to formulate the basic principles on which an optimal protocol for the production of stem cells must be built in strict compliance with safety requirements (no possibility of contamination of a sample of biological material); adequate criteria of efficiency and preservation of the regenerative potential of the cell fraction; simplicity of use (the possibility of conducting a stage of allocation by clinical specialists, minimum requirements for the qualification of personnel in the field of cell technology and maintenance) and economic efficiency. Solving this problem will increase the confidence of specialists in the use of cell technologies on a wider scale, and they will become more accessible to patients.

SYNTHESIS OF LINEAR AND STAR-SHAPED BIODEGRADABLE (CO)POLYMERS FOR BIOMEDICAL APPLICATIONS

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In this work, the strategy for the synthesis of methacrylate-terminated star-shaped polyesters (poly(D,L-lactide), poly(ε-caprolactone) or its copolymers) and preparation of biodegradable 3D scaffolds from these polymers will be presented. This strategy consists of bulk ring-opening (co)polymerization of corresponding cyclic ester using multifunctional initiator followed by end-capping of resulting star-shaped polymer with methacryloyl chloride. The influence of the catalyst nature and monomer to initiator ratio on the resulting polymers properties will be reported here. These star-shaped polyesters were then used for the preparation of biodegradable scaffolds with a 3D microscopic architecture using method of two-photon polymerization. The relationship between the nature of polyester and scaffold properties will be also discussed. It has been demonstrated that the fabricated scaffolds are able to provide a beneficial microenvironment for the osteogenic differentiation of mesenchymal stem cells *in vitro* and support *de novo* bone formation *in vivo*, which shows them as very promising 3D microstructured implants for bone regeneration applications.[1] Finally, the synthesis of the series of random copolymers of D,L-lactide and ε-caprolactone and their application for the preparation of scaffolds by foaming in supercritical carbon dioxide will be also reported.

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CHONDROGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN A PERFUSION BIOREACTOR

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In vitro tissue-engineered constructs (TECs) are created using a perfusion bioreactor – a multicomponent system which provides the conditions for cell differentiation and proliferation to form tissue equivalent.

The aims of the present work were to culture TEC of human cartilage in a dynamic environment of a perfusion bioreactor during culturing of human adipose-derived mesenchymal stem cells (hADSCs) on the biopolymer microstructured collagen-containing hydrogel (BMCH) in a chondrogenic medium.

Materials and methods. Each cell-engineered construct (CEC) of cartilage tissue consisting of BMCH (ZAO «Biomir service», Krasnoznamensk, Moscow Region) and hADSCs was placed in a culture chamber of a bioreactor of an original design. The chambers with CEC were cultured for one day in the growth medium under static conditions, then for three days in the flow conditions at a speed of 1.0 ml/min, after which the growth medium was replaced with a differentiation medium. Cell viability was monitored by means of fluorescent staining with «Live/Dead». The analysis of morphology was carried out using histological methods.

Results. A significant increase in cell mass and cell incorporation into the bulk of BMCH were observed after 72 hours of CEC culturing in the growth medium. The cells with lacuna-like structure, young chondrocytes, were present in the histological specimens on the 25th day of the experiment. The spontaneous generation of microsphere-like structures was detected. The generation of ECM with positive stain for glycosaminoglycans was accompanied by the BMCH resorption.

Conclusion. The possibility of TEC development from the appropriate CEC in a perfusion bioreactor was demonstrated.

DEPOSITION OF CHITOSAN AND COLLAGEN ONTO POLYLACTIDE FILMS

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Polylactide is biodegradable polymer which is widely used for fabrication of scaffolds for tissue engineering, but the surfaces of such scaffolds are hydrophobic and have a lack of specific sites for cell adhesion and growth. Deposition of bioactive components, such as proteins and polysaccharides, onto polylactide surfaces is highly desired to control their biocompatibility. This work is aimed to explore various methods of deposition of collagen (type I) and chitosan onto polylactide films, such as chemical entrapment (pre-treatment of polylactide by solvent mixture); plasma surface activation of polylactide prior incubation in collagen or chitosan solutions; and electrospray deposition. The coated films were studied in terms of surface morphology (scanning electron and atomic-force microscopy), chemical structure (FTIR and X-ray photoelectron spectroscopy) and hydrophobic/hydrophilic balance (goniometry). Coating of hydrophobic polylactide with collagen and chitosan led to a decrease of angles of wettability in all cases, except chemical entrapment method, which led to significant changes in surface morphology. According to X-ray photoelectron spectroscopy electrostatic deposition method was found to be more effective in terms of content of nitrogen (i.e. chitosan) in surface layer.

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DESIGN OF A MICROFLUIDIC DEVICE FOR THE INVESTIGATION OF AXO-AXONAL INTERACTIONS *IN VITRO*

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Complex neuronal connectivity between different brain regions is one of a key aspects of information processing and cognitive functions. It is important to understand how neurons in different layers and areas of the cerebral cortex can form very precise networks in the brain and send its axons to distant target areas such as spinal cord. Neurites of neighboring cells are used by neurons as guiding support for their migration and axon navigation. Here we propose a new method of using to adapt the known methods of using microfluidics to study axon navigation.

Polydimethylsiloxane (PDMS) microfluidic chips contained four chambers (A-D) and microchannels intersected at right angles. Cortical neuronal cells from E18 mice were plated in one chamber A of the microfluidic chips. The axons had to grow straight into an opposite empty chamber B without turning to the neighboring chambers D and C. After 7 days new cells from another mice were plated in neighboring chambers D and C.

We evaluated the axonal navigation of neocortical cells when they met the axons of other cells of the same area of the brain. Axons grew along the axons of other cells both toward the bodies of the cells, and from the bodies of the cells.

The reported study was funded by RFBR according to the research project № 18-34-00639.

DIRECT COMPARISION OF CHONDRO-GUIDE® AND CHONDROTEK COLLAGEN MEMBRANES REVEALED NO DIFFERENCES IN RELATION TO RESTORATION OF ARTICULAR CARTILAGE DEFECTS IN RATS

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Background: Cartilage has a poor regenerative capacity and currently there is no an effective treatment for promoting cartilage repair. Accordingly, large trauma-associated cartilage defects are often treated surgically by covering with synthetic collagen membranes.

Purpose: To compare the healing capacities of two types of synthetic collagen membranes employing a rat model.

Materials and methods: Full thickness cartilage defects were made surgically in rat patellofemoral groove and immediately covered with one of the two collagen membranes: Chondro-Gide® (Switzerland) or Chondrotek (Russian Federation). Control group was left without coverage. The International Cartilage Repair Society (ICRS) score and histological analysis were carried out in 2 and 4 months after implantation.

Results: Both collagen membranes have a positive effect on cartilage repair since the thickness of newly formed tissue was significantly higher than in control group. However, formation of fibrocartilage but not hyaline cartilage was observed in all groups. No significant difference was observed between Chondro-Guide® and Chondrotek membranes repair capacity.

Conclusion: Both collagen membranes have comparable repair capacity and both failed to facilitate formation of hyaline cartilage.

MITOCHONDRIA TARGETED ANTIOXIDANT SKQ1-CONTAINING EYE DROPS PREVENTS ANESTHESIA-INDUCED DRY EYE SYNDROME

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Dry eye syndrome (DES) is an age-related condition increasingly detected in younger people of risk groups, including patients who underwent ocular surgery or long-term general anesthesia. Being a multifactorial disease, it is characterized by oxidative stress in the cornea and commonly complicated by ocular surface inflammation. Polyetiologic DES is responsive to SkQ1, a mitochondria-targeted antioxidant suppressing age-related changes in the ocular tissues. Here, we demonstrate safety and efficacy of topical administration of SkQ1 at a dosage of 7.5 μM for the prevention of general anesthesia-induced DES in rabbits. The protective action of SkQ1 improves clinical state of the ocular surface by inhibiting apoptotic and prenecrotic changes in the corneal epithelium. The underlying mechanism involves the suppression of the oxidative stress supported by the stimulation of intrinsic antioxidant activity and the activity of antioxidant enzymes, foremost glutathione peroxidase and glutathione reductase, in the cornea. Furthermore, SkQ1 increases antioxidant activity and stability of the tear film and produces anti-inflammatory effect exhibited as downregulation of TNF-α and IL-6 and pronounced upregulation of IL-10 in tears. Our data suggest novel features of SkQ1 and point to its feasibility in patients with DES and individuals at risk for the disease including those subjected to general anesthesia.

Acknowledgments: This study was supported by the Russian Science Foundation (Project no. 161500255).

MORPHOLOGICAL COMPARATIVE STUDY OF OPEN-WOUND AND SKIN POCKET RABBIT MODELS FOR IMPLANTATION OF BIOMATERIALS

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Introduction. Modern morphological studies of tissue reaction to implantation of biomaterials lack information on differences between experimental models.

Aim. To study and compare tissue reaction to biomaterial implantation in open-wound and skin pocket rabbit models.

Materials and methods. Polycaprolactone meshes were implanted into rabbit ears for 30 and 60 days. Meshes were fixated on open wounds (d = 1cm) or surgicaly inserted into skin pockets on ventral ear surface. Autopsy materials of four experimental rabbits (each carried twelve implanted meshes) and six control rabbits with intact ears were fixated in 10% neutral formaline. Histological slides were stained with haemotoxilyn-eosin and picrosirius red and studied with LEICA DM4000 B LED microscope, equipped with a LEICA DFC7000 T digital camera. LAS V4.8 software (Leica Microsystems, Switzerland) was used for the examination and imaging of the samples. Morphological study was performed with semi-qualitative (score system) and qualitative (morphometry) methods. Results were analyzed with 2-way ANOVA in GraphPad 7.

Results. Polycaprolactone meshes implanted in rabbit ear open wounds lose structure integrity and cause more excessive inflammation and capsule formation in comparison with skin pocket models. The capsule's width in open-wound model is larger than in skin pocket model.

Conclusions. Experimental model should be considered as a causing factor for some histological findings in tissue reaction to implanation.

OPTICAL PROPERTIES OF MATERIALS BASED ON CHITOSAN AND CARBON NANOTUBES

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The creation of three-dimensional tissue-engineered structures using laser printing methods is a promising line of research in the modern medicine. Such structures can be used as materials for replacing damaged areas of biological tissues. One of the most frequent and dangerous diseases relate to cardiovascular system. Great number of works are devoted to the use of chitosan for accelerating the healing process of damage to the heart tissue. The use of carbon nanotubes enhances the electrically conductive and mechanical properties of the resulting materials. These properties are especially important in the restoration of muscle tissue.

To study the binding of chitosan with single-walled carbon nanotubes, IR and Raman spectra were obtained and analyzed. Also, to study the nonlinear characteristics of dispersions, a scheme was developed with a nanosecond pulsed laser operating at a wavelength of 532 nm. According to the results of the study by spectroscopy, it was found that components were bound due to noncovalent interactions that appeared as a winding of the amino sugars chain on carbon nanotubes.

With an increase in energy, there is a sharp decrease in the past energy of a single pulse due to the optical nonlinear effects appearance near the nanotubes.

The stability of the dispersion components to heating by laser radiation makes it possible to use them for the fabrication of tissue-engineered structures using laser printing methods.

PHOTO-CROSSLINKED HYDROGEL SCAFFOLDS BASED ON HYALURONIC ACID DERIVATIVE FOR TISSUE ENGINEERING

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3D hydrogel-based scaffolds with predesigned structures and functionality has gained a lot of interest in tissue engineering. Scaffolds should imitate physical-chemical properties of replaced tissue for the best implant survival rate. This fact requires prerequisite scaffold material properties, such as biocompatibility, biodegradability, adhesion properties of surface, high porosity. Hyaluronic acid is considered to be one of the most advantageous material. However, scaffolds based on non-modified hyaluronic acid possess relatively limited mechanical properties and can be vulnerable to rapid degradation and contraction.

Here, we demonstrate a method of scaffold production based on hyaluronic acid modified with glycidyl methacrylate (HAGM). UV or visible light irradiation of solution, containing HAGM and photoinitiator, induced photo-crosslinking, resulting in production of tough and insoluble hydrogel. Photoinduced gelation is possible due to radical reaction of double bond moieties of glycidyl methacrylate in HAGM. The role of photoinitiator played endogenous compound: riboflavin mononucleotide (vitamin B2). Scaffolds were fabricated via 3D printing by using home-built 3D printer.

HAGM-based scaffolds were produced for *in vitro* and *in vivo* applications. The lack of cytotoxicity and good cell adhesion were demonstrated in MTT assay and in proliferation of immortalized human fibroblasts BJ-5ta on hydrogel surface. Biocompatibility was confirmed by the lack of significant inflammation processes at subcutaneous positioning of scaffold in mice.

RELEASE OF IMPREGNATED PROTEIN FROM POLYLACTIDE AND CHITOSAN GRANULES, DEPENDING ON THEIR SIZE, POROSITY AND THE PRESENCE OF A CROSS-LINKING AGENT

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Background. The ability to set the peak of growth factors release from osteoplastic materials at the end of an inflammation stage (3-5 days after implantation), is an important task for modern pharmacology and bioengineering.

Aim. Determine the parameters of chitosan and polylactide beads provide optimal kinetics of rhBMP-2 release.

Materials and methods. Chitosan and polylactide (PLA) particles were produced by spray freeze-drying method. Chitosan beads were treated with glutaraldehyde to form covalent bonds between the polymer chains. rhBMP-2 was impregnated in situ in chitosan particles, in PLA particles - after fabrication. The concentration of released rhBMP-2 at the DMEM /F-12 culture medium with 10% bovine serum was measured by ELISA. The experiment was carried out for 5 days in 3 replicates.

Results. Chitosan large size particles provided the maximum release of rhBMP-2 (80 ± 13%) to the 3rd day (minimum sufficient time for effective osteoinduction in the postoperative period). The small particles left 100% of the protein at the 2nd day. The crosslinked chitosan beads released up to 18 ± 10% of the impregnated proteins with the maximum peak (10 ± 3%) on the 6th day. A statistically significant difference in the release rhBMP-2 between large and small polylactide particles with different porosity was not detected. The release of 100% protein was revealed at 2nd day. The effectiveness of impregnation in polylactide granules was 15% of the total protein weight compared to chitosan beads, where this index was 92 ± 7%.

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STUDY OF COMPOSITE MATERIALS BASED ON CARBON NANOTUBES ON THEIR VOLUME STRUCTURE USING X-RAY MICROTOMOGRAPHY

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Recently, 3-D composites have been widely used in tissue engineering. To replace lost biotissues, composites must have a certain porosity (pore sizes, specific pore volume, ratio of voids to the solid part of the material). The porous structure of the composites ensures the germination of blood vessels when the structure of the tissue is restored.

To study the structure of 3-D composites, a complex analysis was performed by X-ray microtomography. Complex analysis of the structure of 3-D composites consisted of scanning, reconstruction of images of shadow projections, two-dimensional and three-dimensional visualization of reconstructed images and quantitative analysis of solid and hollow samples. Samples of the composites were formed by laser evaporation of an aqueous dispersion of proteins and single-walled (SWCNT) / multilayer (MWNT) carbon nanotubes. A laser system was developed, which moves a continuous laser beam (800-970 nm) along three axes. In the manufacture of composites, technological parameters varied: the time, power, and the path of irradiation when the laser beam was moved along the layer of the initial liquid dispersion of proteins and nanotubes.

It was found that 3-D composites based on SWCNT and MWNT are uniform throughout the volume. The 3-D MWNT-based composite has a higher porosity (28.31%) than the 3-D composite based on SWCNT (16.44%). The average pore size of 3-D composites based on SWCNT and MWNT is 45 μm and 93 μm, respectively. Created 3-D composites will be used in the tissue engineering of bone and cartilaginous tissues.

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THE PATTERING OF BIOSTRUCTURES WITH CARBON NANOFRAME IN PROTEIN MATRIX

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The results of pattering of biostructures with a carbon nanoframe in a protein matrix are presented. The mechanism of binding of single-walled carbon nanotubes under the influence of laser radiation is revealed. It was shown that the insertion of defects into the nanotubes structure makes it possible to obtain a strong heating in these regions. Uneven absorption of laser radiation in defective areas promotes the binding of nanotube surfaces. Synthesized samples of a frame nanomaterial based on the branched structure of carbon nanotubes are presented. The change in energy during the formation of o bonds in time (within 8 ps) varies in the range -7.43 - 7.36 eV / atom. The creation of a tree-like branched structure of a nanoframe from carbon nanotubes in a protein matrix under the influence of laser radiation has been demonstrated experimentally. It was also experimentally confirmed that laser pulse of nanosecond duration has a structuring effect on an array of CNTs. The dependence of the alignment degree of the carbon nanotubes array on the energy of the laser pulse was observed.

BIOCOMPATIBILITY EVALUATION OF ACELLULAR DERMAL MATRICES OBTAINED FROM PORCINE DERMIS

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Dermal equivalents are widely admitted around the world as a safe and effective method for treating extensive skin defects. This was the reason for the development of a method for obtaining a dermal matrix from the porcine skin.

Materials and methods. Fresh porcine dermis was harvested from 3 months old pig using the dermatome. Dermis was cut into fragments with the size of 10x10 mm. Samples were washed with PBS buffer for 4 hours. To decellularize porcine dermis we've developed detergent-enzymatic protocol consisting of: 24 hours - 1M NaOH, 4% sodium deoxycholate (Sigma Aldrich, USA), 2 hours - PBS buffer, 2 hours - DNase-I (2,000 Kunitz units). After decellularization process samples were rinsed with PBS buffer for 4 hours.

DNA in scaffolds was quantified using DNeasy Blood and Tissue Kit (Qiagen, Germany) and spectrophotometer (Nanodrop, Saveen&Werner, Sweden). The cytotoxic properties and the viability of the rat MSC cells on recellularized scaffolds were evaluated by XTT test. Subcutaneous tests were also performed with heterotopic transplantation to 6 Wistar rats. Samples were transplanted subcutaneously. The explantation was performed on 7, 14, 21, 28 days after transplantation.

Results. The results obtained indicate the effectiveness of the developed protocol and the relatively low cytotoxicity of the samples (CI = 10%). This protocol provides satisfactory metabolic activity and a high proliferation factor (255.67%). Histological evaluation of explants revealed a weak immune response and development of single blood vessels at the periphery of the sample by 14 days.

COMPARATIVE MORPHOLOGICAL AND BIOCHEMICAL METOD DECELLULARIZATION PARENCHYMAL ORGANS ANIMALS WITH NORMAL AND OPPRESSION OF LIPOPEROXIDATION

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Introduction: Recent studies ongoing intravascular coagulation do not contain information on the impact of oppression lipoperoxidation (LPO) on the process and quality of decellularization of parenchymal organs by the method of perfusion.

Objective: To investigate and compare the morphological and biochemical methods decellularization parenchymal organs animals with normal and depressed lipid peroxidation.

Materials and methods: The experiment was carried out on 10 laboratory rats . The first test group (6 rats) for 15 days along with the viscousconsistency feed was given Mercazolil (daily, 12 mg / kg). The second group (4 rats) did not receive any supplements to the food. At 3, 7, and 15 days of the experiment in rats, LPO was determined for the ratio of tiroborbituric acid and the activity of thrombocytes n about the level of P3 and P4 in blood plasma . Decellularization was carried out according to a standard protocol. A morphological and biochemical evaluation of the resulting decellularized extracellular matrix was carried out. The results were statistically processed with a 2-way ANOVA in GraphPad 7.

Results: On day 15, the first group had a significant inhibition of LPO and reduced platelet activity. After evaluation of the obtained material, morphological and biochemical differences between the first and second groups were found . According to the main criterion of decellularization in the first group, the quality of the material obtained was higher than in the organs of the second group.

Conclusions: Oppression of lipoperoxidation affects the quality of the resulting extracellular matrix after decellularization method of perfusion.

EVALUATION OF A NEW NON-HUMAN HEART DECELLULARIZATION PROTOCOL

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One of directions of tissue engineering is development of methods for inner organs bioengineered scaffolds creation similar to native organs in major biological characteristics.

The objective of the research is specification of modified detergent-enzymatic protocol involving sodium deoxycholate and DNAse for non-human primate heart decellularization. The total duration of decellularization was 120 hours.

Morphological methods of research demonstrated total absence of cells and cells nuclei in heart and intactness of the major proteins of the extracellular matrix after decellularization. Immunohistochemical study demonstrated absence of intracellular contractile protein tropomyosin but positive staining of collagen I, collagen IV, laminin, elastin and fibronectin, suggesting preservation of the extracellular matrix in the decellularized heart tissue and coronary vessels. Immunofluorescence negative staining of vWF and MHC I showed absence of endothelial and muscle cells in decellularized matrix.

Our modified detergent-enzymatic protocol of decellularization allowed to save the basic proteins of the ECM of the heart and intact vascular architecture. This protocol may be used in researches for recellularization of biological scaffolds with allogenic and autologous stem cells of non-human primates.

HYALURONIC ACID IN TISSUE REGENERATION: REDISCOVERING A MAJOR PLAYER

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Introduction: Tissue regeneration has always been a specific focus point of the medical science. Nowadays hyaluronic acid is considered as not only a bulk component of dermis, but as the main mediator during all stages of tissue regeneration due to it's unique properties in high- and low-polymeric forms.

Aim: To elucidate the modern concept of wound healing process providing the pivotal role of hyaluronic acid in each particular stage.

Materials and methods: We have carried out the review of scientific papers using NCBI Medline and Scopus and conducted immunohistochemical tests of skin samples with their further analysis.

Results: Now hyaluronan is considered to be a very biologically active substance. High molecular weight hyaluronic acid as an integral component of extracellular matrix has anti-inflammatory properties by itself. The most striking observation to emerge after skin damage is that low molecular weight fragments of hyaluronic acid, on the contrary, tend to induce immune response. In these conditions hyaluronic acid gets involved in various metabolic and signaling pathways thus modulating neovascularization process and attracting immunocompetent cells.

Conclusions: Hyaluronic acts as a "major player" in tissue regeneration due to it's wide spectrum of biological activities in high- and low- polymeric forms. This observation is critical for searching new concepts of effective wound healing.

IMMUNOLOGICAL MARKERS OF REMODELING OF NERVOUS TISSUE AT FOCAL TRAUMATIC INJURIES OF THE BRAIN

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Objective: To study of dynamics of the content of markers remodeling of neural tissue in patients with focal traumatic brain injury.

Material and Methods: Research object was 40 patients both males and females aged 43±7,5 years with traumatic injuries of the brain and 40 conditionally healthy persons correlated according to sex and age. Quantitative content of the protein S-100, CNTF in blood serum were determined by ELISA test. Statistical analysis of the findings was carried out with the help of the software packages IBM SPSS 20 Statistics.

Results. Protein S-100 level on the first day from the moment of obtaining an injury increased by 27,05 times in comparison with the control ($p<0,001$). In the rest observation periods index concentration gradually decreased ($p_{1-4}<0,001$), but was authentically higher than the control ($p<0,001$).

CNTF level on the first day from the moment of obtaining an injury increased by 64,67 times in comparison with the control ($p<0,001$) and remained high in the rest examination period.

Conclusion: Dynamics of the content of neurospecific proteins in patients with focal traumatic brain injury allows to estimate separate mechanisms of neural tissue remodeling process selectively.

IN VIVO EVALUATION OF POLYCAPROLACTONE AND VATERITE SCAFFOLD BIOCOMPATIBILITY

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Particularities of bone tissue cause the necessity of several additional properties of the scaffolds for stimulation its regeneration including the relevant mechanical characteristics. The modification by inorganic materials, particularly $CaCO_3$, imparts osteoconductive properties to a polymeric scaffold and will be beneficial for designing bone reconstruction materials.

The aim of the study was to examine the biocompatibility of the Polycaprolactone (PCL) and Vaterite (CaCO3) scaffold after its subcutaneous implantation in experimental animals.

The study was carried out on 10 white rats, in which PCL/CaCO3-scaffolds were implanted subcutaneously. For histological study the rats were withdrawn from the experiment on the 21st day. The sections were stained with Mayer's Hematoxylin and Eosin solution.

It was found that, the PCL/CaCO3 scaffold was intensively populated by fibroblasts 21 days after subcutaneous implantation. On the background of fibroblastic elements predomination in cellular populations only single lymphocytes and macrophages were detected in the scaffold area. Another important hallmark was a great number of well blood-filled microvessels in the scaffold area.

Thus, the results of histological study demonstrated the capability of the PCL/CaCO3 scaffold to be colonized by fibroblastic elements and vascularized without promoting an inflammatory response in the surrounding tissues in the course of subcutaneous implantation tests in white rats that proved its biocompatibility.

MORPHOLOGICAL EVALUATION OF TISSUE REACTION TO SUBCUTANEOUS IMPLANTATION OF DECELLULARIZED MATRICES

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The purpose of this work - a comparative study of biocompatibility, biodegradability, local tissue reaction to the implantation of decellularized extracellular matrices of intrathoracic organs.

Methodology. The work was performed on 10 male Wistar rats weighing 210 ± 40 g. Decellularization of the diaphragm, lung, rat heart was performed by modified protocols using sodium deoxycholate and DNase. The samples were implanted subcutaneously in the interscapular region. Rats were derived from experiment on days 7 and 14. We made a qualitative assessment of the composition of the cellular infiltrate around the implant using immunohistochemistry. Reactions were performed with the following primary antibodies: anti-CD3 (ab16669, Abcam, USA), anti-CD20 (ab85809, Abcam, USA), to the Mannose receptor of macrophages (ab64693, Abcam, USA).

Results. Based on the morphological analysis, a histological evaluation of rat tissue response to subcutaneous implantation of the decellularized heart matrices was performed. The qualitative cellular composition of the inflammatory infiltrate was studied with an assessment of the dynamic changes in the macrophages, T- and B-lymphocytes amount on days 7 and 14 after the beginning of the experiment. It is shown that the tissue response to implantation depends not only on the quality of decellularization and the efficiency of antigen molecules removal, but also on the initial histological architectonics and quality of preimplantation preparation of the sample.

The results obtained allow considering subcutaneous implantation of decellularized matrices as an important step in vivo assessment of tissue response reactions of the recipient organism in response to the implantation of tissue-engineering structures.

POLYLACTIDE MICROPARTICLES VIA OIL/WATER SOLVENT EVAPORATION TECHNIQUE: COMPARISON OF EMULSIFICATION METHODS

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Polylactide-based nano- and microparticles are widely proposed as drug and/or cell delivery systems. However, the biomedical application of polylactide particles requires a well-defined control over their size distribution during the fabrication process as well as advanced surface chemistry to promote cell adhesion and growth. This work was aimed to evaluate an effectiveness of chitosan modified by 2,2-bis(hydroxymethyl)propionic acid as emulsifier in aqueous phase as well as to investigate an effect of various emulsification methods on size distribution of polylactide microparticles fabricated via oil/water solvent evaporation technique. The microparticles were prepared by long-term emulsification of oil phase, i.e. polylactide solubilized in methylene chloride as core material, and an aqueous phase, which consisted of water solutions of poly(vinyl alcohol) or modified chitosan as stabilizing agents. The emulsification was carried out using either (1) mechanical four-blade propeller stirrer or (2) ultrasonic bath (FisherBrand FB 15046). The microscopic observations of microparticles stabilized with poly(vinyl alcohol) showed that mechanical stirring provided bigger mean particle size, than ultrasound process: 150 μm and 16.5 μm, respectively. Application of modified chitosan allowed to control total yield and size distribution of prepared microparticles as a function of amino group degree substitution. Thus, variation of emulsification methods and chemical structure of emulsifier in aqueous phase allows us to control mean size and size distribution of the prepared microparticles.

REGENERATION OF RAT SKELETAL MUSCLE INDUCED BY MSC-POPULATED COLLAGENOUS SCAFFOLDS

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Application of mesenchymal stem cells (MSCs) for tissue regeneration is a well-developed practice in regenerative medicine. Delivering MSCs on biocompatible scaffolds provides the best survival rates of MSCs after implantation. Many scaffolds are made of collagen that is non-immunogenic and biodegradable. This study aims to compare the influence of three collagen-based scaffolds on skeletal muscle regeneration in the presence of adipose-derived MSCs.

MSCs obtained from rat adipose tissue were cultivated on 3 different collagenous scaffolds for 14 days in 10^6 cells/ml concentration. Each scaffold was placed into *musculus soleus* laceration site immediately after the injury and sealed with sutures. Histological examination was performed 14 days later. The scaffolds used were Belkosin (BLK), porous collagen scaffold (PCS) and small intestinal submucosa (SIS). For comparison MSCs were delivered by injection of the cell suspension into the injury site.

All the grafts caused inflammation at the implantation site. SIS+MSC and PCS+MSC seemed to induce slightly more active muscle regeneration and vascularization than Belkosin and MSC suspension. Muscles regenerating with engrafted PCS+MSC exhibited the most positive result. BLK showed the least biocompatibility, as it barely disintegrated in the tissue. SIS and PCS allowed better permeation by recipient cells. Evidently, PCS provides the best structural organization for tissue regeneration due to its porous structure.

BIONIC TECHNOLOGIES AND ENGINEERING

WEARABLE ARTIFICIAL KIDNEY

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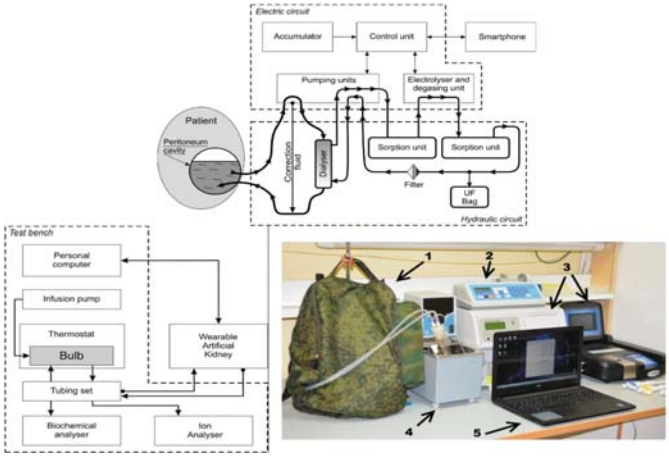
Aim. Renal replacement therapy is the main way to maintain the lives of patients with chronic renal failure. At present time, in the world the work is underway to create a prototype of wearable artificial kidney (WAK).

Materials and methods. Experimental WAK sample has been developed, manufactured and tested. The apparatus implements the method of peritoneal dialysis with the regeneration of spent dialysate in the extracorporeal circuit and consists of a hydraulic circuit that implements recirculation and regeneration of the dialysate and an electrical circuit that implements the control of the procedure, the system as a whole, and communication with the smartphone displaying the user interface.



Functional scheme of WAK

The dialysate solution is regenerated by combining the sorption method and electrolysis. FAS is used as a sorbent; two sorption columns are used in the circuit, each of which contains 35 g of sorbent. The electrolyzer includes 24 titanium electrodes with platinum coating with a total area of 1200 cm^2.

Results. Medical and biological tests of WAK were performed on the animal model. Tests on a healthy animal showed that the apparatus does not have a pathological effect on the biochemical parameters of blood, which makes it possible to eliminate metabolites from the solution for peritoneal dialysis. During the tests on the animal with acute renal failure, the following parameters of dialysate purification were achieved: the mass rate of urea removal of from the consumed solution for peritoneal dialysis was 0.15 g/h, creatinine and uric acid – 0.3 mg/h.

Discussion. Despite the good test results, need for additional studies should be noted. In particular, it is necessary to conduct additional in-depth studies of the electrolysis of the consumed dialysate solution, to continue the search for materials for the preparation of electrodes for precious metals replacement, and to continue research of the possibilities of greater pH stabilization and ionic composition of the solution for peritoneal dialysis.

This work was partly financed by the Ministry of Education and Science of the Russian Federation (project ID RFMEFI57917X0152, Contract № 14.579.21.0152, 26.09.2017).

ELECTRICAL STIMULATION OF GROWTH OF HUMAN CONNECTIVE TISSUE CELLS ON LAYERS OF COMPOSITE BIOCOMPATIBLE NANOMATERIAL

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The aim of the work is to research the effect of electrical stimulation on the growth of cells of human connective tissue on layers of a composite biocompatible nanomaterial (CBN).

The main components of CBN were single-walled carbon nanotubes (SWCNTs), bovine serum albumin (BSA) and distilled water. The formation of layers of CBN was carried out by evaporation of the liquid component from the aqueous dispersion of SWCNTs and BSA under the action of laser radiation in the near infrared region of the spectrum.

The main elements of the developed installation for electrical stimulation were a culture plate, silver U-shaped electrodes and a power unit. The architecture of the installation was constructed in such a way that:

- Silver electrodes were in contact with the layers of CBN.
- An electric field was formed between two differently polarized silver contacts.
- The electrical signal passed directly through the layers of CBN on the surface of which the cells are adhered.

The time of the experiment was divided into two time intervals: the first 24 h the initial incubation period without the electric signal, the next 5 h the time of continuous electrical stimulation. The voltage between the electrodes was 0,1 V.

The developed technique of electrical stimulation of cell growth on the layers of CBN made it possible to improve the adhesion of cells to the layers of CBN, to increase proliferative cellular activity.

HIGH-SPEED VISUALIZATION IN AXIAL FLOW VENTRICULAR ASSIST DEVICE WITH MISROSHPERES

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Introduction. Investigation of fluid flow in the systems of auxiliary blood circulation allows to detect potentially dangerous zones such as areas with high risk of thrombus formation inside the pump and determine the flow characteristics for a given mode of operation. One of the progressive methods for estimating fluid flow is the method which based on tracer visualization of flow by adding to the investigation liquid different particles which can luminesce at a certain wavelength.

Aim of study. To get a picture of velocities distribution and construct various histograms of the main parameters such as speed and vectors of luminescent particles and choose the best one luminescent particles with right liquids.

Materials and methods. Different algorithms for particle image visualization in the programming environment MATLAB, luminescent microspheres with diameters 5, 20 and 50 μm which correspond to the uniform elements of the blood: erythrocytes, platelets and monocytes, different liquids for this research, high-speed camera for detection and registration particles in liquid.

Results. The best one algorithm for MATLAB is fast Fourier transform because of more analyzed data. In the one hand, researching liquid must be similar to human blood, in the other hand this liquid must be fully transparent for better registration by high-speed camera. The best one liquid for investigation is glycerol solution with alcohol inasmuch as its density which can be compared to density of human blood and distribution of particles occurs throughout the volume.

Conclusions. This research can help people to find areas in ventricular assist devices which can be predisposed to the appearance of thrombus. Results of this research can provide how to change construction of this devices to prevent this problem which appears because of movement of the pump blades.

INVESTIGATION OF THE ELECTROMAGNETIC INTERACTION OF THE STATOR AND THE ROTOR IN THE DEVICE OF MECHANICAL REPLACEMENT OF THE HEART FUNCTION

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Introduction. Due to the high rate of heart disease and a shortage of donor hearts, every year the need for more reliable and durable mechanical replacement of the heart grows. Despite the fact that the donor heart transplant is the "gold standard" in the treatment of chronic heart failure, every year the demand for blood circulation support devices (CSP) only increases.

Modern trends in the development of CSP put before the engineers the task of increasing the efficiency, reliability and durability of implantable devices. To achieve the best performance in the design of modern CSP as a working part used electrical machines. The principle of their work is based on the technology of brushless DC motors. The efficiency of these types of motors is determined by such parameters as the number of slots in the stator, the size of the air gap between the rotor and the stator, the maximum value of current and voltage.

Aim of study. The aim of the work is to increase the efficiency and optimize the electric motor used in the apparatus of the auxiliary blood circulation "Sputnik". The chosen goal has a high practical value, because as a result of optimization, it is possible to increase the efficiency of the engine, which will favorably affect a number of parameters, such as, for example, energy consumption and weight.

Result. As a result of the work, the selected electric motor was optimized. The dependence of the output parameters on the geometry of the stator and rotor was determined, the number of windings and the method of winding them. Simulation was performed as a result of which the values of the efficiency of each calculation model were obtained.

MECHANICAL TRAUMA OF BLOOD COMPONENTS IN THE PEDIATRIC VAD OF THE AXIAL TYPE

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Background. The ventricular assist device (VAD) is designed to maintain the circulatory system until the moment of donor heart transplantation. There are cases when implantation of VAD leads to restoration of heart functions. Despite the effectiveness, there are side effects associated with damage to blood components, for example hemolysis. These complications are associated with shear stress (SS) and exposure time (ET).

Aim. Investigation of numerical modeling of the flow of blood through the VAD. Using the program of computational fluid dynamics ANSYS 16.2 Inc., it is necessary to analyze the results of the main mechanical parameters of hemolysis and calculate the hemolysis index (HI).

Methods. Internal elements of the VAD: static straightener at the input; impeller with internal magnet; stationary diffuser at the output. The design mesh of the model is divided into three zones connected through interface zones. The flow under the given conditions corresponds to the regime of turbulent flow. Blood is accepted as a Newtonian fluid with constant parameters: viscosity – 0,0045 (Pa•s); density – 1 057 (kg/m^3). The boundary conditions are determined with respect to the physiology of the patients: the inlet pressure is 0 Pa, the excess static pressure is output, while the blood flow is calculated.

Results. In the simulation of the blood flow in a stationary setting, H-Q curves of the VAD were obtained for the impeller rotation speed range. According to the H-Q curves graph, the working point of the VAD is determined: the impeller rotation speed is 13 000 rpm; head pressure - 80 mm Hg; the mass flow rate is 2,5 l/min. It is established that the SS decreases linearly on all surfaces of the VAD elements with an increase in pressure, but the regions of the blades of the straightener and the diffuser remain critical. The maximum values of SS are observed on the impeller blades, which can cause damage to RBC up to the rupture of the membrane and the release of hemoglobin into the blood plasma. It was found that a significant volume of blood is damaged by von Willebrand factor (9 Pa). A slightly lower proportion of damage, corresponding to the platelet activation (50 Pa) and the potential level of hemolysis (150 Pa). The flow field inside the VAD is also modeled. It was found that with increasing head pressure the flow velocity decreases, hence, the ET increases. After obtaining the values of SS and ET, HI is calculated. It was found that with increasing values of SS and ET, the probability of hemolysis increases.

Conclusion. The range of HI values was 0 to 14 %, the average HI value was 4 %, the standard deviation was 3 %. As expected, the HI increased nonlinearly. The study showed the dependence of HI on SS and ET. Visualization of the blood flow through the VAD revealed problem zones in the pump design: vortex zones in the region of the rectifying apparatus, stagnant in the impeller region. The obtained values of flow characteristics allowed to study erythrocyte damage and formation of thrombi, and also to obtain HI values.

RESEARCH OF THE PROBLEM OF HEAT GENERATION AND RECORDING OF BLOOD CLOTS IN THE ROTARY PUMP OF BLOOD

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Key words: HEART FAILURE, LEFT VENTRICULAR ASSIST DEVICE, THROMBUS FORMATION, TEMPERATURE DISTRIBUTION.

Work objective–to research of the problem of heat generation and recording of blood clots in the rotary pump of blood.

An increase in the number of patients with heart failure (HF) and limitation of donor hearts led to the spread of left ventricular assist devices (LVADs). The application of these pumps has a positive dynamics of survival, improvement of functionality and quality of life. However, there are unpredictable consequences: infections, bleeding and thrombus. One of the limitations of LVADs is the risk of blood clots, the probability of formation of which depends on the heat generation in the pump.

Identification of the pattern of heat generation in axial LVADs has been studied by realization experiments at the mock circulatory loop. Rotary LVAD Sputnik was used to perform the experiments. The mock consisted of a hydraulic circuit, a control unit and devices for fixing the temperature. During the experiments the pump housing was placed inside a viscoelastic Ecoflex silicone with a volume of 1400 cm^3, which limited the spread of heat to the environment during the tests. A different working liquid was used: distilled water (0.85 cP at 23 ° C), a water solution of glycerol with a blood viscosity (3 cP at 23 ° C), a water solution of glycerol with a twice viscosity of blood (6 cP at 23 ° C). The reference speed of the pump was set at 5000, 7000 or 9000 rpm. The hydraulic fluid flow rate was 6.15 L. The experiment was conducted for 90 minutes with a fixed temperature every 2 minutes.

The experiments were carried out at room temperature and with heating of the working fluid to 37 ° C. A thrombus with a volume of 1 cm^3, located directly on the diffusing or on straightening, was made of silicone The graphs of the temperature variation in the stator and output regions are obtained for different system configurations. Thermal cards of the surface of the heart pump were obtained using an infrared thermal imager. Plots of temperature distribution along the pump axis, processed in the TNT Link software, are constructed.

Graphs of temperature distribution depending on the temperature of the working fluid have a similar structure, which allows to determine the presence or absence of thrombus formation, as well as its location. The value of the power consumption corresponds to a certain viscosity of the working fluid. This feature allows you to determine the viscosity of the patient's blood without special sensors.

Application area - software development for LVAD.

ARTIFICIAL MUSCLES: POSSIBILITY OF APPLICATION IN MEDICAL PRACTICE

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From the analysis of the results obtained by us and other authors we can draw the following conclusions:

- artificial muscles (AM) based on the laws of the dynamics of gases and liquids develop the highest values of specific power ($P_m \geq 4$kW/kg) and are suitable for robotics, as well as for external prosthetics of humans limbs;
- AM from ionic electroactive polymers (EAP) give the following indicators: power ($P_m \sim 1.0$ kW/kg), energy ($E_m \sim 530$ J/kg) and efficiency (CE ≤ 20 %) at the level of human muscle tissue ($P_m \sim 0.33$ kW/kg, $E_m \sim 39$ J/kg, CE ~ 20);
- AM based on the laws of conversion of electrical energy into mechanical (nylon-new and polyethylene fibers, nio-fiber nanofibres, materials for shape memory effects have operating temperatures ≥ 60 °C and, undoubtedly, their use as a medical invasive implant is unacceptable;
- carbon nanotubes (CNT) or layers of them in the electrolyte due to chemical reaction deform and generate a mechanical movement similar to what happens in muscles of mammals.

Thus, active research and development, especially in the field of improving the characteristics of drives based on ionic EAP and composite nanomaterials in the composition of CNT, will make it possible to create invasive muscle for medical invasive purposes. The solution of this question is claimed by the modern state of medicine and especially in the treatment of heart diseases.

MAGNETIC PARTICLES IN BIOLOGICAL OBJECTS AND OPPORTUNITIES FOR THEIR REGISTRATION

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At present, magnetic particles (MPs) and their systems are being actively investigated for their application in biological research and medical practice. Usually MPs are spheres of iron oxides (magnetite or hematite), the surfaces of which are coated with different substances. Often they are used to isolate DNA and RNA from biological materials. Also, a new non-invasive method is developing dynamically with the use of MPs. The so-called magnetic partial imaging.

The possibility of noninvasive detection of magnetic particles in biological objects has been investigated. It has been found that magnetic particles, including magnetite, hematite, and catalytic iron particles in carbon nanotubes, can be detected by high-sensitivity magnetic field sensors (MFS) with resolutions of 10^{-8}-10^{-15} T.

It was established that MPs with a specific magnetization of 50 A·m^2/kg, concentration 10^{12} m^{-3} and an average diameter of 50 nm can be detected by SQUIDs or combined MFS at distances of ≤ 0.1 m. Here SQUID is Superconducting Quantum Interference Devices and combined MFS consists of a superconducting magnetic flux concentrator and a magnetosensitive element based on a structure of spintronics.

It was mentioned that superparamagnetic iron particles and carbon nanotubes containing catalytic iron particles can only be detected by SQUIDs or nanostructured combined MFS with their resolution of about 10^{-15} T.

Thus, the high-sensitivity magnetic field sensors with a resolution of $\leq 10^{-11}$ T make it possible to detect magnetic particles in biological objects and can be used for noninvasive control of organs, implants, prostheses, and other elements in biological objects.

PERSONALIZED MEDICINE

APPLICATION OF BAYESIAN NETWORKS FOR PREDICTING THE CHRONIC HEPATITIS C DEVELOPMENT

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There are 130–170 million people infected with hepatitis C in the world, and annually re-infected 3 – 4 million people. The development of the chronic form of the infection and the disease outcome are subject to considerable individual differences. We have developed probabilistic forecasting models based on Bayesian networks for individual prognosis of the disease. In these studies, we used the database containing 253 patients with chronic hepatitis C and the liver cirrhosis which were observed during 5 years. There are about 40 clinical and genetic parameters for each patient. Bayesian networks with naïve topology are used for forecasting of the end point state. The Bayesian networks optimization with respect to the number of nodes is performed to increase the reliability of the forecasting and to decrease the number of prognostic parameters. The area under the ROC-curve (AUC) is the target function in this optimization.

It is shown that reliability of the forecast (the AUC value) is increased in the course of the optimization up to 0.90 and the number of Bayesian networks nodes (prognostic parameters) determining the probability of prediction of patient states decreases after the optimization from 40 to 14. The risk histograms are plotted. These histograms connect the conditional probability of the patient prognosis with a priory probability of prediction for a certain patient risk group. The reliability of predictions is tested at the control patients group. The work was financially supported by the Russian Science Foundation, Agreement no. 14-50-00029.

COMPUTATIONAL MODELING OF HEMODYNAMICS IN INTRACRANIAL ANEURYSM: IMPORTANCE OF BOUNDARY CONDITIONS

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Intracranial aneurysm (IA) is a common vascular disease. In recent years, computational hemodynamic methods were increasingly being applied to provide biomechanical information for assessing the risk of aneurysm rupture or optimizing clinical treatments. Given that high-fidelity geometrical models of IAs can be readily reconstructed from medical images, the prescription of boundary conditions (BCs) becomes a key factor determining the reliability of hemodynamic computation. In this talk, we compared intra-aneurysmal hemodynamic parameters predicted by models whose BCs were prescribed in different ways. Results showed that three-dimensional (3-D) modeling of the entire cerebral arterial network provided the most reliable representation of the inflow/outflow conditions of a local aneurysm model. Applying flow waves predicted by a reduced-order model of the cerebral arterial network to prescribe the outflow BCs of a local aneurysm model brought some improvements over traditional free outflow BC, but did not guarantee a complete reproduction of the results obtained by the global 3-D model. Moreover, we examined how flow waveform variations during normal aging affect blood flow patterns in IAs. Obtained results revealed that aging-associated flow waveform variations had a tendency of elevating oscillatory shear index at the aneurysm wall, but induced little changes in time-averaged wall shear stress. Finally, the hemodynamic impacts of the dynamic motion of aneurysm wall were investigated by integrating wall motion parameters derived from 4D-CTA into hemodynamic models. It was found that incorporating wall motion did not alter the major features of intra-aneurysmal hemodynamics, although moderate hemodynamic changes were observed in some local regions.

EFFECTS OF CYP2D6 GENETIC POLYMORPHISMS ON THE EFFICACY AND SAFETY OF FLUVOXAMINE IN PATIENTS WITH DEPRESSIVE DISORDER AND COMORBID ALCOHOL USE DISORDER

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Background: Fluvoxamine therapy is often ineffective and some patients suffer from dose-dependent adverse drug reactions, reducing the efficacy of the therapy of depressive disorder comorbid with alcohol use disorder. The presence of some polymorphic markers of CYP2D6 increases the amount of isoenzyme to be expressed or enhances its activity, and some polymorphisms reduce the isoenzyme activity resulting in the changes biotransformation and elimination rates of medication.

Objective: To investigate the effects of CYP2D6 genetic polymorphisms on the efficacy and safety of fluvoxamine in patients with depressive disorder and comorbid alcohol use disorder.

Methods: The study involved 45 male patients (average age: 36.44±9.96 years) with depressive disorder and comorbid alcohol use disorder. A series of psychometric scales were used in the research. Genotyping of CYP2D6 (1846G>A) was performed using real-time polymerase chain reaction.

Results: According to results of U-test Mann-Whitney, statistically significant differences between the efficacy and safety of fluvoxamine were obtained on 9th and 16th days of therapy in patients with GG and GA genotypes (The Hamilton Rating Scale for Depression: 10.0 [10.0; 23.0] vs 25.0 [24.0; 16.0] ($P<0.001$) on 9th day and 4.0 [2.0; 5.0] vs 6.0 [6.0; 7.0] on 16th day; Udvald for Kliniske Undersogelser Side Effect Rating Scale: 6.0 [4.0; 6.0] vs 9.0 [9.0; 10.0] ($P<0.001$) on 9th day and 5.0 [1.0; 9.0] vs 19.0 [18.0; 22.0] on 16th day.

Conclusion: This study demonstrated the lower efficacy and safety of fluvoxamine in patients with depressive disorder and comorbid alcohol use disorders with GA genotype in CYP2D6 1846G>A polymorphic marker.

Keywords: *pharmacogenetics, SSRIs, fluvoxamine, biotransformation, personalized medicine, CYP2D6, depressive disorders, alcohol addiction.*

INDUCTION OF MYOGENIC DIFFERENTIATION IN SPHEROIDS FROM ORAL MUCOSA DERIVED MESENCHYMAL STROMAL CELLS

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Three-dimensional cell cultures (3D cultures) are more stable and demonstrate a longer lifespan than 2D cell cultures. 3D cultures can be maintained in culture for a long time - more than a month, unlike monolayer cultures, which quickly reach high confluence and require permanent replating. Therefore, 3D cultures are used in long-term studies, for example, when studying the delayed effects of drugs [1]. Currently, cellular spheroids are most widely used model of 3D cultures ex vivo. Spheroids are self-organizing spherical clusters of cells that can be obtained from one or several types of cells. During the formation of spheroids from the suspension of single cells, not only intercellular contacts are formed, but also contacts with the newly synthesized extracellular matrix, resulting in a organized structure which is close to the tissues in vivo [2]. Many cell types have a natural tendency to aggregate. Due to aggregation cells in spheroids can restore intercellular contacts and create a microenvironment that supports their native phenotype.

When cultured under non-adherent conditions, cells form a sphere, synthesize the extracellular matrix and stop proliferation. Extracellular matrix helps the cells to move within the spheroid, like the movement of cells in the living tissue. Spheroids, therefore, are an adequate model of cell migration, differentiation, survival and growth in vivo.

In our previous studies the possibility of myogenic differentiation of oral alveolar mucosa derived multipotent mesenchymal stromal cells (AMC) was demonstrated for the first time at both early and late passages [3].

In accordance with developed earlier protocols for the production of spheroids from human somatic cells [4, 5], optimum conditions and terms of cultivation were obtained to create spheroids from the AMC and from the attached gingiva stromal cells (AGC) taken as control. The terms of 3D cultivation varied from 1 to 10 days, the optimal time for the formation of spheroids with given properties was 5-7 days. Spheroids were successfully formed in 3D cultures of both AMC and AGC. Different cell concentrations for production of spheroids were tested (0,5-5×10^6 cells/ml). Loose and non-compact spheroids were formed when concentrations of cells were less than 1×10^6 cells/ml. Concentration greater than 4×10^6 cells/ml led to the formation of highly compact spheroids with signs of necrotic changes in the central region, probably due to a lack of nutrient diffusion. Therefore, to obtain spheroids from AMC with subsequent differentiation in the myogenic direction, the optimal cells concentration was 2-3×10^6 cells/ml. The optimal time for the formation of compact spheroids was 5-7 days. Until the fifth day the spheroids were loose, did not have time to undergo compaction. After 5-7 days of cultivation in 3D culture, the degree of compactization of the formed spheroids and their structure remained practically unchanged. Therefore, 5-7 days in 3D culture were chosen as the minimum acceptable time interval for obtaining compact spheroids from cells of different anatomical regions of the oral mucosa (AMC and AGC).

For induction of myogenic differentiation, a differentiation medium was used: DMEM low glucose + 2% Horse Serum (BioInd). Spheroids from the AGS neither spontaneously nor under induction did not differentiate in the myogenic direction and served, as in the case of monolayer cultures, negative control. The spheroids from AMC successfully differentiated in the myogenic direction with the formation of myotubes. In the differentiated AMC spheroids there were no early progenitor cells, MyoD was not synthesized. Moreover not only myotube-like structures were present in AMC spheroids, but more differentiated well-formed myofibers with specific nuclei location at the periphery and the transverse striations, detected by antibodies against sarcomeric alfa-actin.

Thus, the possibility of 3D cultivation of stromal cells of the oral mucosa is shown. It was found that the main difference of this method of cultivation from a monolayer culture is the greater efficiency of myogenic differentiation with the fusion of myotubes into myofibrils.

Our results indicate that AMC cultured in spheroids and differentiated in myogenic direction are perspective candidates for bioprinting and clinical usage in patients with muscle disorders.

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OPTICAL COHERENCE TOMOGRAPHY OF MALIGNANT BRAIN TUMORS: PERSPECTIVES FOR INTRAOPERATIVE DIAGNOSIS

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We demonstrate experimental study *in vitro* of brain malignant tissues by means of optical coherence tomography (OCT). Applying image processing and statistical analysis, we studied differences between OCT signals for intact and malignant glioma tissues of various grades. All the examined samples were excised according to the initial medical diagnosis. They were explored no later than 4 h after its resection. We used gelatin films for covering samples in order to fix them, prevent hydration/dehydration, and sustain structure and composition. We suggested using signal slope as a characterized feature for our analysis. Moreover, to mitigate scattering noise and retain signal artifacts, we applied signal de-noising algorithm based on wavelet analysis. Our pilot experimental results demonstrate perspectives for using OCT technique for differentiation of brain tissues and types of malignant tissues, including intraoperative diagnosis.

PERSONALIZATION OF DRUG WHICH HAS NOT BEEN INVESTIGATED IN THIS WAY YET: IN SILICO, IN VITRO AND CLINICAL PHARMACOGENETIC STUDIES OF PHENAZEPAM

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AIM. To estabilish pathways of phenazepam's liver metabolism. To determine associations of adverse drug reactions to Phenazepam with polymorphisms of CYP3A5, CYP2C9, CYP2C19, CYP2D6 and ABCB1.

MATERIALS AND METHODS. Metabolic pathways of phenazepam were investigated by in silico (PASS, GUSAR software) and in vitro (cell cultures) studies. 102 male patients with non-comlicated alcohol withdrawal syndrome (F 10.3 by ICD-10) were involved into the study in 24 hours after their admission to hospital and were prescribed Phenazepam for 6 days. 5 ml of venous blood was collected from each participant for genotyping to detect CYP3A5*3, CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*17, CYP2D6*4, ABCB1 3435C>T polymorphisms by real-time polymerase chain reaction. CYP3A isoenzyme activity was measured by cortisol/6-beta-hydroxycortisol urine test.

RESULTS. According to in silico and in vitro analysis results, the most probable metabolizer of phenazepam was CYP3A4. By the in vivo study results, CYP3A activity decreased sufficiently (from 3.8 (95%CI - 2.94-4.65) to 2.79 (95%CI - 2.02-3.55), p=0.017) between start and finish of treatment in patients which were prescribed just phenazepam.

Polymorphisms CYP3A5*3, CYP2C9*3, CYP2C19*2 and ABCB1 3435C>T were significantly associated with several adverse reactions.

CONCLUSION. Experimental in silico and in vivo studies confirmed that Russian original benzodiazepine phenazepam was the substrate of CYP3A4 isoenzyme. Results determined leading role of CYP3A5*3, CYP2C9*3, ABCB1 3435C>T polymorphisms as biomarkers of phenazepam's safety in patients with alcohol withdrawal syndrome.

THE INFLUENCE OF THE POLYMORPHISM ABCB1 GENE ON THE EFFICACY AND SAFETY OF BROMODIHYDROCHLORPHENYLBENZODIAZEPINE (PHENAZEPAM) IN PATIENTS WITH ANXIETY DISORDER AND COMORBID ALCOHOL DEPENDENCE

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Introductions. Benzodiazepines are used in the therapy of anxiety disorder and comorbid alcohol dependence. Benzodiazepines have a sedative, anxiolytic and anticonvulsant effect. These effects are due to the interaction of drugs with GABA–receptors. In order to avoid cross-tolerance with alcohol, it is recommended to appoint benzodiazepines in doses exceeding the average therapeutic. In this regard, Phenazepam therapy may lead to an adverse drug reaction.

Glycoprotein P - is an ATP-phase pump, localized on cytoplasmic membranes of various cells and carrying out release into the extracellular space of various xenobiotics, including more than 80% of drugs. Glycoprotein-P is coded by the ABCB1 gene, which has a high degree of polymorphism, which affects the degree of glycoprotein-P activity and, as a consequence, the release rate of drug substrates from the body. This can affect the patient's individual response to drugs, change the profile of effectiveness and safety.

The aim of our study was study the effect of the polymorphism of the ABCB1 gene on the efficacy and safety index of bromodihydrochlorphenylbenzodiazepine (Phenazepam) for patients with anxiety disorder and comorbid alcohol use disorder.

Materials and methods. The study was conducted on 58 patients suffering anxiety disorder and comorbid alcohol dependence.

Efficiency and safety assessment was carried out using psychometric scales and scale of adverse drug reaction (ADR): 1. Scale VAS. 2. Scale HADS. 3. Hamilton Anxiety Rating Scale (HARS). 4. The Zung Self-rating Anxiety Scale (ZARS). 5. UKU Side-Effect Rating Scale (UKU).

Patients were tested the day before the start of therapy, including phenazepam, and after 5 days of therapy. Genotyping ABCB1 gene was carried out by the method of polymer chain reaction in real time with allelespecific hybridization. For determination of differences between statistical data of patients without polymorphism 3435C^{o}T of a gene of ABCB1 and with its existence used the Kruskal — Wallis test.

Results. On the scale VAS on the first day of therapy were calculated: 44,0±9,9 (CC), 44,8±11,0 (CT), 50,2±9,6 (TT), p=0,19. And also on the 5th day: 6,07±2,7 (CC), 6,7±2,3 (CT), 6,0±2,8 (TT), p=0,56. On the scale HADS on the first day of therapy the scores were calculated: 25,5±4,4 (CC), 27,3±3,4 (CT), 27,58±3,6 (TT), p=0,22. And also on the 5th day: 3,6±22 (CC), 4,9±1,3 (CT), 4,0±1,39 (TT), p=0,11. On the scale UKU on the first day of therapy the scores were calculated: 1,28±0,46 (CC), 1,3±0,48 (CT), 1,17±0,39 (TT), p=0,53. And also on the 5th day: 8,7±0,6 (CC), 8,5±0,5 (CT), 8,5±0,5 (TT), p=0,59.

Conclusions. Based on the results of the study, there were no statistically significant differences in the efficacy and safety of bromodihydrochlorphenylbenzodiazepine (Phenazepam) in patients with anxiety disorder and comorbid alcohol dependence. Thus, it has been shown that the polymorphism of the ABCB1 gene can not influence the safety and effectiveness indices of Phenazepam therapy. Probably there is no statistical significance due to insufficient research volume. It is necessary to continue research to solve this problem.

THE INFLUENCE OF THE POLYMORPHISM ABCB1 GENE ON THE EFFICACY AND SAFETY OF MIRTAZAPINE IN PATIENTS WITH ALCOHOL DEPENDENCE

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Introductions. Mirtazapine is used in the treatment of patients with alcohol dependence who are in a state of exacerbation of the pathological desire by the affective type. Glycoprotein P - is an ATP-phase pump, localized on cytoplasmic membranes of various cells and carrying out release into the extracellular space of various xenobiotics, including more than 80% of drugs. Glycoprotein-P is coded by the ABCB1 gene, which has a high degree of polymorphism, which affects the degree of glycoprotein-P activity and, as a consequence, the release rate of drug substrates from the body. This can affect the patient's individual response to drugs, change the profile of effectiveness and safety.

The aim of our study was study the effect of the polymorphism of the ABCB1 gene on the efficacy and safety index of mirtazapine in patients with alcohol dependence.

Materials and methods. The study was conducted on 58 patients with alcohol dependence. Efficiency and safety assessment was carried out using psychometric scales and scale of adverse drug reaction (ADR): 1. Scale VAS. 2. Scale PACS. 3.UKU Side-Effect Rating Scale (UKU). Patients were tested the day before the start of therapy, including mirtazapine , and after 16 days of therapy. Genotyping ABCB1 gene was carried out by the method of polymer chain reaction in real time with all elespecific hybridization. For determination of differences between statistical data of patients without polymorphism 3435C°T of a gene of ABCB1 and with its existence used the Kruskal — Wallis test.

Results. On the scale VAS on the first day of therapy were calculated: 41,0±11,2 (CC), 47,7±11,5 (CT), 51,2±8,9 (TT), p=0,18. And also on the 16th day: 6,08±2,5 (CC), 6,3±1,9 (CT), 5,9±2,3 (TT), p=0,56. On the scale PACS on the first day of therapy the scores were calculated: 8,31±1,55 (CC), 8.27±1,27(CT), 7,92±1,7 (TT), p=0,22. And also on the 16th day: 1,6±2,2 (CC), 2,3±1,3 (CT), 1,8±1,39 (TT), p=0,11. On the scale UKU on the first day of therapy the scores were calculated: 1,22±0,43 (CC), 1,3±0,46 (CT), 1,16±0,36 (TT), p=0,53. And also on the 16th day: 8,5±0,6 (CC), 8,3±0,5 (CT), 8,6±0,6 (TT), p=0,59.

Conclusions. Based on the results of the study, there were no statistically significant differences in the efficacy and safety of mirtazapine in patients with alcohol dependence. Thus, it has been shown that the polymorphism of the ABCB1 gene can not influence the safety and effectiveness indices of mirtazapine therapy. Probably there is no statistical significance due to insufficient research volume. It is necessary to continue research to solve this problem.

CASE REPORT: CLINICAL APPLICATION OF NONINVASIVE ESTIMATION OF FRACTIONAL FLOW RESERVE WITH A ONE-DIMENSIONAL MATHEMATICAL MODEL BASED ON ROUTINE COMPUTED TOMOGRAPHY ANGIOGRAPHY

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We developed a method allowing noninvasive assessment of fractional flow reserve (FFR_{CT}) by a one-dimensional mathematical model construction.

Aim: For the first time in Russia, to apply the developed method of FFR_{CT} in a patient with severe coronary artery calcification.

Methods: A 76-year-old male patient with Canadian Cardiovascular Society class III angina underwent coronary CT angiography performed on a 640-slice CT scanner Toshiba Aquilion ONE. CT revealed pronounced coronary calcification, Agatston score – 1256, which made it impossible to assess stenotic lesions at the CT laboratory level. CT images were processed by our working group with further FFR_{CT} assessment. The patient was admitted to the cardiac surgery department for the invasive assessment of FFR as a reference standard. Stenoses were considered functionally significant for FFR/FFR_{CT} <80. Examination revealed functionally significant stenosis of the left anterior descending artery (LAD), so the patient underwent percutaneous coronary intervention with drug-eluting stent «Synergy» 4.0x20 mm.

Results: FFR_{CT} was assessed for two stenoses by means of mathematical modeling. Stenosis of the middle segment of the LAD – up to 90%: FFR_{CT} – 0.31; stenosis of the proximal segment of the right coronary artery (RCA) – up to 60%: FFR_{CT} – 0.80. According to the invasive FFR assessment: stenosis of the middle segment of the LAD – up to 90%, FFR – 0.42; stenosis of the proximal segment of the RCA – up to 60%: FFR – 0.83.

Conclusion: The developed technique can reproduce invasive FFR measurements in patients with high calcification index with sufficient precision, which significantly improves the diagnostic value of CCTA.

CHONDROGENIC POTENTIAL OF MESENCHYMAL STROMAL CELLS FROM VARIOUS SOURCES AND THEIR APPLICABILITY FOR ARTICULAR REGENERATION UTILIZING SCAFFOLD-FREE TISSUE ENGINEERING TECHNIQUES

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Cartilage tissue engineering remains a challenging problem. Optimal protocols for treating or replacing damaged articular joints are yet to be developed. Limitations include: the need to culture cells quickly in large amounts and to organise and shape cells. Our aim was to evaluate the chondrogenic potential of three mesenchymal stem cell (MSC) cultures within various scaffold-free tissue engineered constructs.

MSCs from dental pulp, Wharton's jelly of umbilical cord and adipose tissue were initially expanded in 2D cultures. Cells were then subjected to chondrogenic differentiation in 3D cultures formed by pellet culture and spheroid culture methods.

Pellets were prepared by centrifuging cells in conical bottom 96-well plates (250,000 cells per well). Spherical aggregates were formed in 96-well ultra-low attachment plates (8,000 cells per well). Primary human chondrocytes served as positive controls.

The three MSCs types showed the ability to form spheroids and pellets. Chondrogenesis was evaluated by the presence of glycosaminoglycan-rich extracellular matrix (ECM) and cartilage-specific markers including aggrecan and collagen II. Cell proliferation was measured by lactate dehydrogenase activity. Spheroid fusion assay was performed to assess the viability and motility of cells after prolonged 3D culture.

The data obtained suggest that both the pulp of deciduous teeth the Wharton's jelly are efficient and easy accessible sources of chondrogenic progenitors. Commonly used 3D culture techniques allow cells to differentiate into functional ECM-producing chondrocytes. Combination of various 3D culture approaches provides great adaptability of cartilage bioprinting techology.

This work was supported by the Russian Foundation for Basic Research (grant №16-29-07322).

ELECTROPHYSIOLOGICAL ASSESSMENT OF HUMAN INDUCED PLURIPOTENT STEM CELLDERIVED SEROTONERGIC NEURON MODEL

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The serotonergic system of the brain plays a crucial role in regulating brain functions and has been implicated in various psychiatric disorders, including ADHD and other neurodevelopmental disorders. Thus, studying the role of risk genes in functional properties of human serotonergic neurons is of fundamental importance for understanding their pathogenesis. However, human serotonergic neurons are not readily available for *in vitro* studies. Here, we electrophysiologically assessed previously established *in vitro* serotonergic neuron model derived from human induced pluripotent stem cells (hiPSC). This model can be useful for evaluation of the impact of risk genes in a patient-specific manner.

Serotonergic neurons were differentiated from a hiPS cell line. Whole-cell patch clamp recordings were performed weekly within 5 weeks and typical electrophysiological properties of the serotonergic neurons were determined, e.g., half-high width (HHW) > 1.2 ms, firing rate < 12 Hz and absent/low after-hyperpolarisation.

We recorded repetitive firing elicited by current injections, as well as spontaneous firing in neurons over the whole measurement period. Starting with a maximum frequency on week one (2.08 ± 0.64 Hz), repetitive firing decreased significantly and reached characteristic frequencies on weeks three to five (from 0.45 ± 0.24 to 0.77 ± 0.42 Hz). Action potentials with amplitude and HHW typical for serotonergic neurons were recordable during all five weeks.

The hiPSC-derived serotonergic neurons exhibited an electrophysiological signature characteristic for raphe serotonergic neurons as previously reported from *in vivo* studies. However, further validation e.g. by using specific serotonergic agonists/antagonists is ongoing.

FUTURE OF HEALTHCARE: TOWARDS IMPLEMENTATION OF DIGITAL MEDICINE

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Digital medicine is an integral part of digital economy which will revolutionize healthcare. Digital medicine is not just a paperless healthcare. Digital medicine in essence is everything related with healthcare information. However, implementation of the digital medicine could not be based only on more optimal use of medical information. Moreover, medical information must be also generated in the new most effective way maximally optimal for its sequential digitalization and real time integration into databases. It requires the development of new types of digitalized diagnostic devices and biosensors, novel therapeutic modalities based on patient specific medical information and sophisticated softwares. The systematic analysis of existing ways of providing healthcare service demonstrates that digitalization could be implemented on practically all traditional steps of providing healthcare starting from patient registration, diagnostics, medical treatment and sequential rehabilitation.

Using infographics we presented the integrated concept of implementation of digital medicine in national healthcare. The development of so-called "virtual patient" integrating all personalized medical information and medical data and records instead of traditional paper-based history of diseases is the the central idea of proposed integrated concept of implementation of digital medicine. The seamless integration of personalized medical information and medical records in "virtual patient" will need, of course, the development of novel sophisticated softwares. The certain examples of using digital medical information integrated into virtual patients for novel modalities of medical treatment using robotic 3D bioprinting as well as *in vivo* robotic bioprinting are also provided.

Finally, we formulated 5 basic principles of successful implementation of digital medicine in Russian healthcare system which include:

1. The Russian government must lead healthcare reform based on implementation of digital economy by formulating vision and execution the integrated approach including the development of national standards, regulations, laws and, ideally, by providing the sufficient financial support.

2. The foreign experience in ongoing successful implementation of digital medicine in different national healthcare systems and especially international standards and best practice in this field must be carefully investigated, monitored and maximally used.

3. Collaborations with world leading research and development organizations as well as with foreign and national private companies which demonstrate strong expertise in the area of information technology and digital medicine is essential and critically important.

4. Russian private companies and start-ups must be maximally involved in the development of necessary infrastructure for implementation the concept of digital medicine including both hardwares (digitalized sensors, devices, biomonitors and so on) and softwares.

5. Medical educational institutions and organizations such as national medical universities and academies must develop new educational programs, courses and may be even new departments for training specialists in new profession - IT-medics.

MATHEMATICAL MODELING OF THE INTERACTIONS OF THE CARDIOVASCULAR SYSTEM WITH THE VENTRICULAR ASSIST DEVICE

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Background. Modeling allows studying the biotechnical system in various physiological states, to shorten the development time of the technical system and to reduce costs, by partially replacing in-vivo tests.

Aim. Modeling aims to reproduce the circulatory features with a given accuracy: the distribution of pressure and flow as a function of time, the zone of stagnation, the zone of increased pressure, the distribution of volumes in the vascular system.

Methods. An important role in the development of the ventricular assist device (VAD) is played by mathematical modeling of the cardiovascular system. According to the requirement of the international standard for moving implants, VAD must undergo tests not only on animals, but also on models.

The model represents the cardiovascular system in the form of one or several sections, depending on the purposes of modeling, to simulate the operation of the diseased heart and the help provided by the VAD.

Results. Models are needed both at the design stage of the device, and already immediately before its implantation for a successful adjustment to the characteristics of each patient. Thanks to the personified model, it is possible to select the optimal pump operation mode for each patient individually, taking into account the level of heart failure, which will favorably affect the patient's quality of life.

Conclusion. For reliable accuracy in the mathematical model of the cardiovascular system, it is necessary to take into account the laws: Poiseuille, Frank-Starling, baroreflex and ventilation of the lungs and the Navier-Stokes equation.

NEUROVAZAL UNIT IN THE PERIVENTRICULAR AREA OF THE BRAIN WITH PATIENTS WITH IDIOPATHIC NORMAL PRESSURE HYDROCEPHALUS

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Idiopathic normal pressure hydrocephalus (iNPH) is characterized by a slow (chronic) expansion of the ventricular system of the brain against a background of formally normal intracranial pressure and a gradual development of the clinical manifestations. Researches shows that hydrocephalus causes changes at different levels of brain structure, including the blood-brain barrier.

Aims. Identify ultrastructural changes and features of neuro-glial and neuro-vasal interrelations in the periventricular zone of the brain in patients with iNPH.

Methods. Subjects of the study were brain biopsies of patients with iNPH, obtained during a ventriculosurgery operation, conducted on the basis of the Military Medical Academy S.M. Kirov. Using the FEI Tecnai G2 Spirit BioTWIN transmission electron microscope (Netherlands), provided by the IEFC Center for Collective Use I.M. Sechenov, morphological changes of neurons, glial cells and elements of the blood-brain barrier were studied. Sample preparation was carried out by traditional method for electron microscopy.

Results. Among the researchers there is a popular theory of neurovascular units, the primary purpose is to maintain homeostasis of the brain microenvironment (M. Zonta, et al 2003; C. Iadecola, 2004; G.J. DelZoppo, 2009;). It is based on the structural and functional relationship of neurons, perivascular glia, and microvessel structures that feed these cells. Exploring the periventricular region of the brain of patients with iNPH, we found the destruction in all parts of the neurovascular unit. The most of the neurons were hyperchromic with necrotic and apoptotic signs. The role of perivascular glia in this area of the brain was more often performed by oligodendrocytes, and sometimes by microgliocytes. These cells in 80% of cases were at different stages of apoptosis and aslo often hypertrophic. The nuclei of the oligodendrocytes were irregular in shape and contained clumps of condensed heterochromatin distributed throughout the entire karyoplasm. The cytoplasm is often vacuolated and, as in neurons, could contain large clusters of lipofuscin and hemosiderin. The basal membrane of the vessels is unevenly thickened, sometimes encrusted with calcifications, sprouted with collagen and other fibrous structures. Endothelium, as a rule, is dystrophic, locally edematous. Pericytes also with pathology signs, contained destructively altered organelles and granules of toxic granularity. Local edemas of vascular wall structures were supplemented with often expressed edemas of the perivascular space.

Conclusion. The results of the study showed a lot of ultrastructural changes in the neurovascular relationships, which have specific features in the periventricular zone. Thus, brain dysfunction in iNPH is caused by destruction of the neurovascular unit of the periventricular zone of the brain in addition to the causes of its causing.

TISSUE-ENGINEERED BIOEQUIVALENT FOR URETHRAL RECONSTRUCTIVE SURGERY

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To date, the common way to treat urethral stricture is substitute urethral reconstruction using a buccal mucosa graft or an acellular matrix seeded with cell suspensions. However, many studies showed that the use of these approaches could lead to the development of fibrosis, recurrent stricture, necrosis, and graft rejection. This occurs because of insufficient cell number, possible changes in cell phenotype (due to epithelial-mesenchymal transition), and absence of blood supply in a graft. Therefore, we sought to develop a tissue-engineered urethral wall bioequivalent, which can be used to treat urethral strictures longer than 2 cm.

To do this we fabricated a bioequivalent of the urethral wall using spheroids from autologous buccal epithelial cells and a hybrid matrix that consisted of reconstituted collagen and reinforcing polylactoglycolide fibers, and showed no cytotoxic effects. Using buccal epithelial cells from the oral mucosa, we developed a novel approach to recover and maintain the stable cell phenotype and to form a multilayered epithelial lining *in vitro* via the 2D/3D cell self-assembling. The multilayered epithelial lining formation was achieved after placing spheroids for 7 days onto a hybrid matrix due to cell migration and proliferation. This autologous tissue-engineered bioequivalent showed its safety and efficiency on rabbit animal model and allowed us to reconstruct the urethra. While achieved these successful results of preclinical trials, we initiated clinical trials (NCT03205670) and performed first operation on human to treat the urethral stricture.

ANALYSIS OF CELL VIABILITY IN A THREE-DIMENSIONAL CO-CULTURE UNDER THE ACTION OF AN ANTITUMOR DRUG CISPLATIN

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In this work we are investigate the effect of cisplatin on the joint culture of tumor and stromal cells in co-culture on the extracellular matrix.

Materials and methods. To create co-culture, cells mixed in a 1:1 ratio (10×103 cells/well) in 96-well plates. DMEM medium with 10% FBS at 37 °C 5% CO2 was used for cultivation. Each well of the 96-well plate placed with 50 µl of Matrigel. To analyze the viability of MSCS and SH-SY5Y, after 24 h of incubation the medium was replaced and cisplatin was added in the concentration from 0.04 µg/ml to 10 µg/ml (n=5) After 24 h of incubation with cisplatin, MTS-test performed using CellTiter 96 AQueous Cell analyzer.

Research result. Analysis of results showed that the concentration of cisplatin in 10 µg/ml, show 77 % viability of MSCS, 70 % SH-SY5Y, 81% co-culture compared with untreated cells. At a concentration of cisplatin 5 µg/ml, the viability of MSCS was 89 %, SH-SY5Y 83 %, co-culture 99% compared to untreated cells. At this concentration, a statistically significant ($p < 0.01$) increase in the viability of co-cultured cells was also determined, in contrast to the MSC and SH-SY5Y monocultures, by 10% and 16%, respectively.

Discussion. cisplatin concentrations from 0.04 to 2.5 µg / ml stimulated viability of co-cultured cells by an average of 13% compared to untreated co-cultured cells.

Summary. MTS test analysis showed that the viability of tumor cells was the lowest in all groups, MSCS were slightly higher and the highest rates were in co-culture. This work was supported by the RFBR grant No 16-34-60201

LIVER REGENERATION

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Introduction: Liver transplantation remains the only option for treating liver failure but is only available for a small number of patients. Alternative methods of treatment can expand the number of patients receiving effective treatment.

Objective: To study the features of the process of liver regeneration.

Materials and methods: The articles for the period from 2012 to 2017 were analyzed. The search was performed in the databases PubMed, Embase, Scopus. The words used are “liver regeneration”, “stem cell–derived hepatocytes”, “engineered liver”.

Results: tumor necrosis factor (TNFα), interleukin-6 (IL-6), hepatocyte growth factor (HGF) cause hepatocytes to pass from G0 to the S-phase of the cell cycle. Chronic liver damage causes a canal reaction in which liver progenitor cells (LPC) participate. Normally LPC show the ability of bipotential differentiation to both hepatocytes and cholangiocytes. Cell therapy by repopulation with transplanted hepatocytes is a safe and effective method, but only leads to a short-term partial correction of metabolic disorders, it is necessary to optimize engraftment and spreading. Liver diseases at the final stage are incompatible with cellular therapy due to the lack of a suitable environment for cell engraftment and repopulation. It is important to prove that the bioengineering liver is clinically safe, the network of the vasculature is not damaged to provide functional vascularization.

Conclusions: Replication of hepatocytes is dependent on the effect of growth factors and cytokines - TGF-a, HGF and IL-6, LPC can differentiate to both hepatocytes and cholangiocytes, the best solution is the use of induced pluripotent stem cells.

METHOD OF 3D BIOPRINTING OF TUMOR MODEL

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3D bioprinting is a promising method for biofabrication and tissue engineering with further applications in regenerative medicine and personalized medicine. To fabricate a three-dimensional tumor model, we used human neuroblastoma cells SH-SY5Y and stem cells isolated from human adipose tissue (ADSCs). We put these cells in two different types of hydrogels. The tumor cells were mixed with sodium alginate-based CELLINK A hydrogel ($7x10^6$ cells in 1ml gel), ADSC were mixed with alginate and highly hydrated cellulose nanofibrils based CELLINK gel (3×10^6 cells in 1 ml gel). Blender software was used to create 3D STL model, then the model was pre-processed for printing in the SLIC3R program. Tumor model itself is basically tumor cylinder encapsulated in ADSC cylinder. This type of model was chosen to study the processes of invasion of tumor cells in nearby matrix, as well as direct intercellular interactions. The model was fabricated with CELLINK INKREDIBLE bioprinter. To start the process, a number of manipulations were carried out in accordance with the manufacturer’s instructions. During the printing process, we encountered several problems of regarding the calibration of the printer, the working out of the model, and nozzle obstructions. We also found that precise pressure calibration is crucial for tissue construct quality. Ultimately, 6 samples with different size, shape, and metabolism were fabricated. Potentially, this tissue construct could be used for anti-tumor drug screening, but the method requires further calibration.

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PERSONALIZED MEDICINE AS A NEW DAWN IN THE BATTLEFIELD

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For years medicine has being practice under general rules of symptoms and disease classification that looked at a general population rather than people as individuals. Late discoveries and breakthroughs have shown that we can not use the "one size fits all" approach anymore. With the approach of genome sequencing, data and informatics, and wearable technology, we have come to a concept of "personalized medicine".

Our health is determined by our genetic make up, lifestyle, and environment.By combining and analyzing information about our genome, with clinical and diagnostic information and then comparing that with data from others, patterns can be identified. Those patterns can be used to evaluate one's risk of developing disease earlier, provide accurate diagnosis, and formulate a specific-targeted treatment plan.

For the longest time, diseases have been formulated around general symptoms and organs system, and diagnosis were formulated through such approach and process. The genome analysis have shown that the disease pathway is deeper than organs. The Slightest DNA change can cause serious problems.

The current blockbuster approach to drug development assumes that all patients with a particular condition respond similarly to a given drug. All patients with the same condition receive the same first line treatment even though it may be only 30 to 60% effective.

With personalized medicine we can appreciate the disease subtype and treat accordingly. This new dawn in medicine will enhance patient care.

PRODUCTION OF MESENCHYMAL STEM CELL CULTURES WITH LUCIFERASE AND GFP REPORTER GENE EXPRESSION

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Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells. MSCs exhibit a homing behavior toward sites of inflammation, damaged tissues and tumor sites. Thus MSCs can be used for directed delivery of chemotherapeutic drugs or other anti-cancer agents. Labeling MSCs with reporter gene genetically provide a method for tracking the fate of MSCs *in vivo*.

In this study mouse MSCs that express the luciferase reporter gene from the firefly *Photinus pyralis* (ffLuc) or Green Fluorescent Protein (GFP) were produced. Mouse MSCs were isolated by enzymatic digestion with Crab Collagenase. The cells were largely positive for mesenchymal stem cell surface markers including CD44, CD90, CD29, CD105, CD73 and Sca-1 and negative for hematopoietic stem cell surface markers.

Recombinant lentiviruses LV-ffLuc and LV-GFP were produced by co-transfection of the HEK293T packing cell line with three plasmids: encoding gene vector plasmid (pLX302 Luciferase-V5puro or pWPT-GFP, Addgene); encoding *gag/pol* genes and additional viral genes packaging plasmid (psPAX2, Addgene); and encoding glycoprotein G of the vesicular stomatitis virus gene plasmid (pCMV-VSV-G, Addgene). The produced lentiviruses were concentrated by ultracentrifugation for 2 hours at 26,000 rpm. The viral titer was determined by flow cytometry of cells transfected with lentiviruses carrying *gfp* gene. Mouse MSCs were transduced with recombinant lentiviral vectors encoding *ffLuc* or *gfp* gene. Resulting cell line was selected with puromycin for 10 days.

The resulting cell culture will be further primed with various chemoitherapeutic drugs *in vitro*. This study was supported by grant from the RFBR 18-34-00738.

КЛОНАЛЬНО-НАПРАВЛЕННАЯ ПЕРСОНАЛИЗИРОВАННАЯ ХИМИОТЕРАПИЯ ПРИ РАКЕ МОЛОЧНОЙ ЖЕЛЕЗЫ

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Актуальность. Согласно результатам предыдущих исследований, было показано, что при проведении неоадъювантной химиотерапии (НХТ) под влиянием химиопрепаратов происходит клональная эволюция опухоли, при которой происходит изменение опухолевых клонов (ОК) – полное/ частичное исчезновение или появление новых клонов. Новые клоны, образованные под действием НХТ, содержат амплификации в следующих локусах: ***5p, 6p, 7q, 8q, 13q, 9p, 9q, 10p, 10q21.1, 16p, 19p, 18chr.*** При этом, выявлено, что появление в остаточной резидуальной опухоли в процессе НХТ любых двух и более амплификаций этих регионов, приводит к 100% гематогенному метастазированию, у всех остальных больных с элиминацией ОК, отсутствием действия химиотерапии на клоны или у больных с появлением делеционных клонов не было гематогенных метастазов в 5-ти летний период наблюдения (по методу Каплана-Майера, p = 0,00001 Log-rank test). **Целью** настоящего исследования явилась оценка эффективности НХТ и 2-летней безметастатической выживаемости больных с диагнозом рак молочной железы (РМЖ) на основе изучения наличия амплификационных клонов в первичной опухоли больных и оценке экспрессии генов сомато-стволового перехода.

Материал и методы. Материалом для исследования служили образцы биопсийного материала до лечения и опухолевого материала после проведения НХТ для каждого из пациентов. Наличие амплификаций определялось в вышеуказанных регионах с использованием микроматрицы CytoScan HD Array (Affymetrix, USA). Было сформировано 3 группы пациентов. 1-ая: больные, в опухоли которых определялось наличие любых 2-х амплификаций. Им назначалась персонализированная НХТ (в соответствии с патентом RU 2594251). Во 2-ю группу были включены пациенты, у которых изначально в опухоли было 0-1 амплификационный клон и лечение начинали с оперативного этапа. Пациентам 3-й группы также исходно имеющим 0-1 клон, лечение начинали с персонализированной НХТ. Остальные этапы лечения были стандартными. По основным клинико-морфологическим параметрам группы не различались.

Результаты. 86% пациентов 1-й группы (n=29) достигло полных или частичных регрессий после проведения НХТ, 2-летняя выживаемость в группе составила 97% (28/29).

Во 2-й группе выживаемость составила 93% (13/14). В 3-й группе эффективность НХТ составила 84% (16/19), но при этом показатели выживаемости были статистически значимо ниже, чем в 1-й группе - 68% (13/19) (p=0,011). Отталкиваясь от доказательства происхождения опухоли из опухолевых стволовых клеток, нами было выдвинуто предположение о существовании программы сомато-стволового перехода при возникновении ОК. Следующим этапом работы было изучение экспрессии генов сомато-стволового перехода (TERT; OCT3; SMO; MYC; SNAI2; MOB3B; TGFBR1; KLF4; BMI1; VIM; FLT3; LAT; SMAD2; LMNB2; KLF1; TGFb). Было показано, что у больных без метастазов до лечения гиперэкспрессированны 5 генов - OCT3; BMI1; LMNB2; TGFb1 и FLT3;, у больных с метастазами до лечения гиперэкспрессированы 7 генов - OCT3; BMI1; LMNB2; TGFb1; TERT; SNAI2; TGFbR1. После проведения НАХТ в остаточной резидуальной опухоли больных без гематогенных метастазов частота гиперэкспрессированных генов не меняется. У больных с метастазами после НХТ в остаточной резидуальной опухоли гиперэкспрессированы 14 из 16 изученных генов – кроме KLF1 и SMAD2. При этом, было показано, что при гиперэкспрессии в остаточной резидуальной опухоли трех генов OCT3, LAT и LMNB2 у 69% больных (11/16) зарегистрировано возникновение гематогенных метастазов. При гипоэкспрессии хотя бы одного из этих генов 5-ти летняя безметастатическая выживаемость составляет 94% (34/36).

Выводы. 1. Первые полученные результаты свидетельствуют о предиктивной и прогностической значимости наличия амплификационных клонов в первичной опухоли у больных РМЖ. Назначение НХТ наиболее целесообразно у пациентов с наличием в первичной опухоли 2-х и более клонов, в то время как при 0-1 клоне проведение химиотерапии в предоперационном режиме не показано. 2. Получена модель прогнозирования возникновения гематогенного метастазирования на основе анализа экспрессии 16 генов сомато-стволового перехода. Чувствительность прогноза метастазирования составляет 69%, специфичность 94%, диагностическая точность 82%.

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ПОИСК ПОДХОДОВ К ПОВЫШЕНИЮ ТОЧНОСТИ ДОКИНГА

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Разработка лекарств основывается на парадигме: молекула лекарства должна связаться со специфическим местом биологической макромолекулы, чаще всего с активным центром белка-мишени, и вызвать эффект в организме, блокируя развитие заболевания. Молекулярное моделирование, и особенно докинг, всё более широко используется в процессе разработки лекарств – на начальной её стадии. С помощью докинга молекула (лиганд) испытуемого вещества (кандидата в лекарство) позиционируется в активном центре белка-мишени и оценивается энергия её связывания с белком: чем больше эта энергия, тем эффективнее лекарство. Чтобы расчеты были эффективным инструментом разработки лекарств, точность предсказания энергии связывания белок-лиганд должна быть лучше 1 ккал/моль. Программы докинга востребованы, и в недавних обзорах [1-3] упоминаются более 50 программ докинга и около десятка интернет ресурсов, постоянно устраиваются соревнования по докингу. В этих программах реализовано большое многообразие различных методов и аппроксимаций. Наиболее популярные [1] программы AutoDock и AutoDock Vina [4] для докинга лигандов используют стохастические методы и эмпирические силовые поля, причем Vina использует очень грубые упрощения, в том числе вообще не учитывает электростатические взаимодействия атомов. В AutoDock для позиционирования лигандов применяется генетический алгоритм глобальной оптимизации и используется гораздо более аккуратное, но всё же эклектическое эмпирическое силовое поле, а также сильно упрощенная модель для эффекта десольватации и подгоночные параметры, чтобы наилучшим образом воспроизвести эксперименты для тестового набора комплексов. Предварительно рассчитывают сетку потенциалов взаимодействия пробных атомов лиганда с белком. Все эти особенности и упрощения в той или иной степени присутствуют и в большинстве других программ докинга, ведь многие десятилетия все программы докинга разрабатывались и развивались в парадигме «быстрее и ещё быстрее», чтобы провести докинг многих тысяч лигандов за короткое время на laptop'е. Но расчеты «на коленке» в русском языке имеют и другой смысл: грубо, тяп-ляп и т.п., и это проявляется при широком использовании программ докинга для реальных разработок лекарств. Одно можно сказать с уверенностью: позиционирование лигандов осуществляется многими из этих программ удовлетворительно, но все программы, как правило, дают большие ошибки в энергиях связывания белок-лиганд, хотя за счет применения подгоночных коэффициентов эти ошибки часто скрываются и программы докинга выдают «разумные» величины энергии связывания. Трудность достижения высокой точности таких расчетов обусловлена их сложностью, и источников ошибок много. В докладе рассматриваются основные упрощения, приводящие к ошибкам докинга, и подходы для их устранения, недавно реализованные в суперкомпьютерных программах FLM и SOL-P; представлены результаты тестирования. Программа SOL-P осуществляет успешный докинг гибких лигандов в белки с несколькими десятками подвижных атомов в конформационном пространстве с беспрецедентно большим числом измерений за счет применения нового метода глобальной оптимизации – метода тензорных поездов. Делается вывод о возможности преодоления практически всех существующих сегодня упрощений, ограничивающих точность докинга, и создания программ докинга повышенной точности, применение которых для разработки лекарств должно существенно повысить эффективность разработки новых лекарств.

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ВЛИЯНИЕ ПОЛИМОРФИЗМА ГЕНА ABCB1 НА ПРОФИЛЬ ЭФФЕКТИВНОСТИ И БЕЗОПАСНОСТИ ПРИМЕНЕНИЯ ФЕНАЗЕПАМА У ПАЦИЕНТОВ С ТРЕВОЖНЫМИ РАССТРОЙСТВАМИ, КОМОРБИДНЫМИ С АЛКОГОЛЬНОЙ ЗАВИСИМОСТЬЮ

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Актуальность. Бензодиазепиновые транквилизаторы (БДТ) применяются для лечения больных с тревожными расстройствами, коморбидными с алкогольной зависимостью.

БДТ обладают седативным, анксиолитическим и противосудорожным эффектами. Эти эффекты обусловлены взаимодействием препаратов с ГАМК-рецепторами. Чтобы избежать перекрестной толерантности с алкоголем, при которой обычные дозы препаратов не эффективны, рекомендовано назначать БДТ в дозах, превышающих средние терапевтические. В связи с этим при приеме БДТ могут возникать нежелательные лекарственные реакции со стороны различных органов и систем организма.

Гликопротеин Р – представляет собой АТФ-фазный насос, локализованный на цитоплазматических мембранах различных клеток и осуществляющий выброс во внеклеточное пространство различных ксенобиотиков, в том числе и более 80% лекарственных средств (ЛС). Гликопротеин-Р кодируется геном ABCB1, который обладает высокой степенью полиморфизма, что выражается в разной степени активности гликопротеина-Р, а как следствие, в изменении скорости выведения ЛС-субстратов из организма. Это в свою очередь, может оказывать влияние на индивидуальный ответ пациента на ЛС, меняя показатели профиля эффективности и безопасности.

Целью данной работы является оценка взаимосвязи полиморфизма гена ABCB1 с профилем эффективности и безопасности бромдигидрохлорфенилбензодиазепина (Феназепама), у пациентов с тревожными расстройствами, коморбидными с алкоголизмом.

Материалы и методы. В исследовании принимало участие 58 пациентов, страдающих тревожными расстройствами, коморбидными с алкоголизмом, находившихся на стационарном лечении в ГБУЗ «МНПЦ наркологии ДЗМ».

Для оценки эффективности бромдигидрохлорфенилбензодиазепина (Феназепама) применяли международные психометрические шкалы: 1. Визуально-аналоговая шкала оценки влечения к алкоголю (ВАШ). 2. Госпитальная шкала тревоги и депрессии (HADS). 3. Шкала тревоги Гамильтона (HamiltonAnxietyRatingScale (HARS)). 4. Шкала самооценки тревоги Цунга (TheZungSelf-ratingAnxietyScale (ZARS)). Профиль безопасности исследовали с помощью шкал оценки побочного действия (UKUSide-EffectRatingScale (UKU)).

Исследование пациентов проводилось за день до начала терапии, включавшей бромдигидрохлорфенилбе нзодиазепин (Феназепам), и через 5 дней терапии.

Проведение генотипирования по выбранному полиморфизму ABCB1 3435C^{o}T, производили с использованием полимеразной цепной реакции в режиме реального времени с аллельспецифической гибридизацией (Real-TimePCR). Для определения различий между группами количественных данных пациентов без полиморфизма 3435C^{o}T гена ABCB1 и с его наличием использовали H-тест Крускалла-Уоллиса.

Результаты исследования

По шкале VASв 1-ый день лечения баллы получены следующие: 44,0±9,9 (CC), 44,8±11,0 (CT), 50,2±9,6 (TT), p=0,19.На 5-ый день лечения: 6,07±2,7 (CC), 6,7±2,3 (CT), 6,0±2,8 (TT), p=0,56.По шкале HADS в 1-ый день лечения были получены баллы: 25,5±4,4 (CC), 27,3±3,4 (CT), 27,58±3,6 (TT), p=0,22. На 5-ый день лечения: 3,6±22 (CC), 4,9±1,3 (CT), 4,0±1,39 (TT), p=0,11. По шкале UKU в 1-ый день лечения получены следующие баллы:1,28±0,46 (CC), 1,3±0,48 (CT), 1,17±0,39 (TT), p=0,53. На 5-ый день лечения: 8,7±0,6 (CC), 8,5±0,5 (CT), 8,5±0,5 (TT), p=0,59.

Выводы. По результатам исследования не было получено статически значимой разницы в показателях безопасности и эффективностибромдигидрохлорфенилбензодиазепина (Феназепама) у пациентов с тревожными расстройствами, коморбидными с алкоголизмом. Исходя из этого можно говорить об отсутствии влияния полиморфизма гена ABCB1 на профиль эффективности и безопасности применения Феназепама. В тоже время необходимо отметить, что, вероятно, отсутствие статистически значимой разницы в показателях не выявлено, ввиду недостаточного объема выборки. Необходимо продолжение исследования для решения данного вопроса.

ПЕРСОНАЛИЗИРОВАННАЯ МАТЕМАТИЧЕСКАЯ МОДЕЛЬ ОЦЕНКИ ИЗМЕНЕНИЙ КРОВОТОКА В ЭКСТРАКРАНИАЛЬНЫХ ОТДЕЛАХ БРАХИОЦЕФАЛЬНЫХ АРТЕРИЙ НА ПРЕДОПЕРАЦИОННОМ ЭТАПЕ КАРОТИДНОЙ ЭНДАРТЕРЭКТОМИИ

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Цель и задачи работы. Атеросклероз сонных артерий является одной из наиболее частых причин развития ишемических инсультов и выступает этиологическим фактором в 20-30% случаев. Учитывая высокий периоперационный риск (смерть, острые нарушения мозгового кровотока (ОНМК)), каротидная эндартерэктомия показана только пациентом с тяжелым атеросклеротическим поражением брахиоцефальных артерий (стеноз более 70% при УЗДГ или более 50% при ангиографии). Частота ОНМК при каротидной реваскуляризации составляет от 1.5 до 9.0%. Прогнозирование подобных осложнений возможно посредством оценки церебральной гемодинамики. Для этого, как правило, требуются дорогостоящие и доступные лишь в высокоспециализированных центрах аппараты. Целью данного исследования является разработка персонализированной математической модели церебрального кровотока, основанной на данных типовых неинвазивных клинических исследований, доступных в большинстве профильных медицинских организаций, позволяющей с удовлетворительной точностью на предоперационном этапе индивидуально проанализировать изменение церебральной гемодинамики после проведения каротидной эндартерэктомии у группы пациентов.

Материалы и методы. Математическая модель церебрального кровотока, использованная в данной работе, основана на уравнениях движения вязкой несжимаемой жидкости по сети эластичных трубок. Основными расчетными параметрами модели течения в каждом из сосудов являются поперечное сечение и осредненная по поперечному сечению линейная скорость кровотока, которые рассчитываются вдоль длины сосуда с помощью законов сохранения массы и импульса. Объединение трубок в сеть производится за счет требования выполнения условий сохранения массы и непрерывности интеграла Бернулли. На входе в сеть задается типичная временная кривая сердечного выброса. На выходе из сети задаются условия согласованности с параметрами венозного бассейна: сохранение массы и артериовенозный градиент давления.

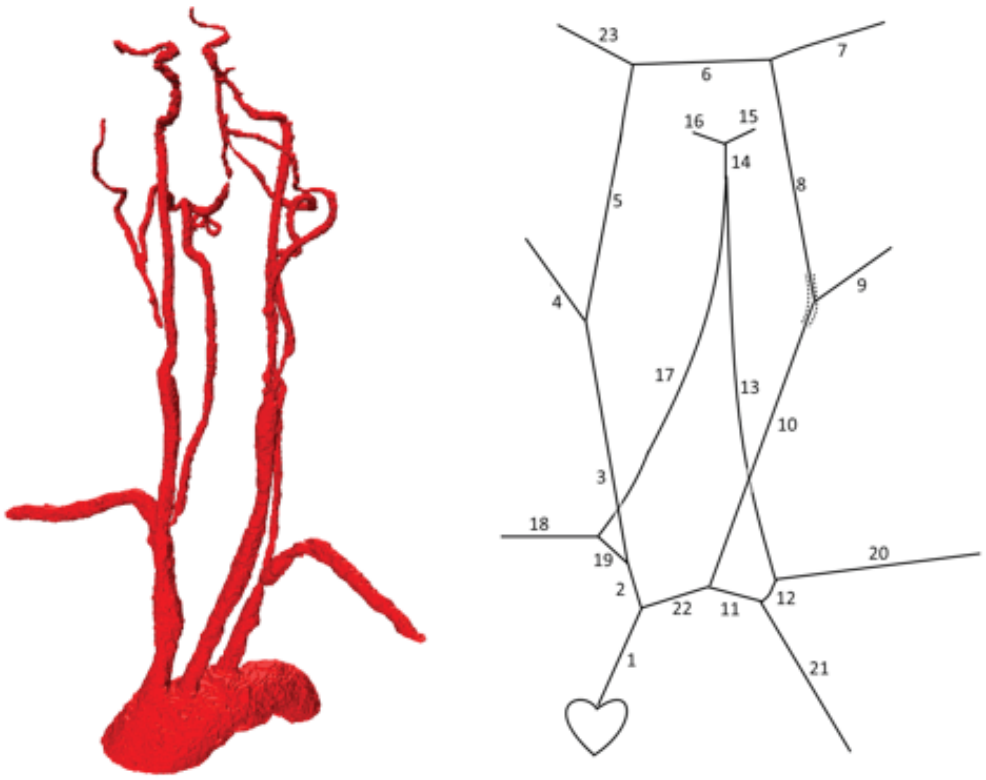

Рисунок 1. Пример реконструкции БЦА по данным МСКТ

Объем наблюдений. В исследование включались пациенты с тяжелым атеросклерозом (более 70%) каротидных артерий, госпитализированные в отделение сосудистой хирургии УКБ №1 ФГБОУ ВО Первого МГМУ им. И.М. Сеченова с октября 2015 по март 2016 для проведения плановой каротидной эндартерэктомии. Случайным образом было отобрано 5 историй болезни. Всем пациентам до и после оперативного лечения выполнялись УЗДГ брахиоцефальных артерий (БЦА) и МСКТ БЦА с контрастированием. Исследование выполнялось на 320-спиральном компьютерном томографе Toshiba Aquilion ONE.

Результаты. Для построения одномерных сетевых структур артериальной системы использовались данные МСКТ БЦА с контрастированием. На рисунке 1 приведен пример результатов реконструкции, полученных на основе алгоритма автоматической сегментации данных КТ. При калибровке моделей кровотока значения параметров подбирались так, чтобы разница между рассчитанными и измеренными до операции значениями систолической скорости кровотока не превышало 6 см/с. При этом среднее относительное отклонение во всех точках составило 4%, максимальное — 16%. При сопоставлении рассчитанной с помощью математического моделирования систолической скорости кровотока с измеренной после операции было получено, что среднее абсолютное отклонение не превосходит 3 см/с, максимальное — 9 см/с. При этом, среднее относительное отклонение составило 6%, максимальное — 20%.

Выводы. Предложен принцип построения персонализированной математической модели для предоперационного анализа изменения гемодинамики в экстракраниальных отделах БЦА после каротидной эндартерэктомии. Модель учитывает и воспроизводит фактическую анатомию и количественные показатели гемодинамики по данным КТ-ангиографии и дуплексного исследования сосудов шеи. Разработанная математическая модель изменения гемодинамики брахиоцефальной системы продемонстрировала хорошую сопоставимость с фактическими результатами, полученными после каротидной эндартерэктомии. Важными характеристиками модели являются ее базирование на прямых методах оценки анатомии и количественных показателей кровотока с одной стороны, и отсутствие необходимости проведения дополнительных диагностических манипуляций пациентам с другой. Естественным ограничением результатов данного исследования выступает небольшое количество наблюдений, включенных в работу. Исследование на большем объеме пациентов позволит дать более объективную информацию и, возможно, уточнить предложенные алгоритмы.

Благодарности. Работа выполнена при поддержке гранта РНФ 14-31-00024. Авторы выражают благодарность сотрудникам Сеченовского университета, особенно Н. Гагариной, Е. Фоминых и А. Дзюндзе, за предоставление данных.

ОЦЕНКА ФРАКЦИОНИРОВАННОГО РЕЗЕРВА КРОВОТОКА С ПОМОЩЬЮ ГЕМОДИНАМИЧЕСКОГО МОДЕЛИРОВАНИЯ И КТ-АНГИОГРАФИИ

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КЛЮЧЕВЫЕ СЛОВА: фракционированный резерв кровотока, модель гемодинамики, сегментация изображений, стеноз

Цели работы. Фракционированный резерв кровотока (ФРК), измеряемый при инвазивной коронарной ангиографии, позволяет оценить степень тяжести стеноза коронарных артерий [1]. На данный момент ФРК является золотым стандартом среди показателей, определяющих необходимость коронарной реваскуляризации. Для оценки гемодинамической значимости стенозов предлагается использовать одномерную сетевую математическую модель течения крови [2]. Модель использует характеристики сосудов, которые извлекаются из данных КТ-ангиографии пациентов. Основной целью работы является демонстрация работы методики оценки ФРК на ряде случаев, а также сравнение показателей ФРК, вычисленных с помощью модели, с измеренными инвазивно.

Методы. У семи пациентов выполнялось КТ ангиографическое исследование коронарных сосудов. Из полученных КТ данных с помощью алгоритмов сегментации и скелетонизации извлекалась структура коронарных артерий, используемая в гемодинамических расчетах модели кровотока. С помощью модели кровотока проводилась оценка показателя ФРК, который затем сравнивался с инвазивно измеренным ФРК.

Результаты. Сравнение рассчитанных с помощью моделей $ФРК_{КТ}$ и измеренных инвазивно ФРК представлено в таблице 1. Обозначения сосудов: ПКА — правая коронарная артерия, ЛКА — левая коронарная артерия, ОА — огибающая артерия, ПНА — передняя нисходящая артерия.

Таблица 1. Измеренный ФРК и рассчитанный с помощью модели $ФРК_{КТ}$

Пациент	Сосуд	Стеноз	Длина, мм	$ФРК_{КТ}$	ФРК	Ошибка
1	ПНА	50%	40	0.51	0.58	+14%
	ЛКА	55%	2	0.72	0.84	+17%
	ОА	80%	10	0.59	0.61	+3%
2	ПНА	80%	20	0.74	0.78	+5%
	ПКА	55%	2	0.93	0.87	-5%
3	ПНА	70%	20	0.81	0.77	-5%
4	ПНА	60%	40	0.6	0.57	-5%
	ОА	50%	10	0.88	0.97	+14%
5	ПНА	50%	10	0.91	0.86	-5%
6	ОА	50%	10	0.89	0.83	-7%
	ПКА	50%	10	0.91	0.93	+2%
7	ПКА	50%	3	0.85	0.89	+5%

Выводы. Полученные данные демонстрируют способность разработанной методики с достаточной точностью воспроизводить показатели инвазивного измерения ФРК, что значительно повышает диагностическую ценность КТ ангиографического исследования.

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ВЛИЯНИЕ ПОЛИМОРФИЗМА ГЕНА ABCB1 НА ПРОФИЛЬ ЭФФЕКТИВНОСТИ И БЕЗОПАСНОСТИ ПРИМЕНЕНИЯ ФЕНАЗЕПАМА У ПАЦИЕНТОВ С ТРЕВОЖНЫМИ РАССТРОЙСТВАМИ, КОМОРБИДНЫМИ С АЛКОГОЛЬНОЙ ЗАВИСИМОСТЬЮ

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Актуальность. Бензодиазепиновые транквилизаторы (БДТ) применяются для лечения больных с тревожными расстройствами, коморбидными с алкогольной зависимостью.

БДТ обладают седативным, анксиолитическим и противосудорожным эффектами. Эти эффекты обусловлены взаимодействием препаратов с ГАМК-рецепторами. Чтобы избежать перекрестной толерантности с алкоголем, при которой обычные дозы препаратов не эффективны, рекомендовано назначать БДТ в дозах, превышающих средние терапевтические. В связи с этим при приеме БДТ могут возникать нежелательные лекарственные реакции со стороны различных органов и систем организма.

Гликопротеин Р – представляет собой АТФ-фазный насос, локализованный на цитоплазматических мембранах различных клеток и осуществляющий выброс во внеклеточное пространство различных ксенобиотиков, в том числе и более 80% лекарственных средств (ЛС). Гликопротеин-Р кодируется геном ABCB1, который обладает высокой степенью полиморфизма, что выражается в разной степени активности гликопротеина-Р, а как следствие, в изменении скорости выведения ЛС-субстратов из организма. Это в свою очередь, может оказывать влияние на индивидуальный ответ пациента на ЛС, меняя показатели профиля эффективности и безопасности.

Целью данной работы является оценка взаимосвязи полиморфизма гена ABCB1 с профилем эффективности и безопасности бромдигидрохлорфенилбензодиазепина (Феназепама), у пациентов с тревожными расстройствами, коморбидными с алкоголизмом.

Материалы и методы. В исследовании принимало участие 58 пациентов, страдающих тревожными расстройствами, коморбидными с алкоголизмом, находившихся на стационарном лечении в ГБУЗ «МНПЦ наркологии ДЗМ».

Для оценки эффективности бромдигидрохлорфенилбензодиазепина (Феназепама) применяли международные психометрические шкалы: 1. Визуально-аналоговая шкала оценки влечения к алкоголю (ВАШ). 2. Госпитальная шкала тревоги и депрессии (HADS). 3. Шкала тревоги Гамильтона (HamiltonAnxietyRatingScale (HARS)). 4. Шкала самооценки тревоги Цунга (TheZungSelf-ratingAnxietyScale (ZARS)). Профиль безопасности исследовали с помощью шкал оценки побочного действия (UKUSide-EffectRatingScale (UKU)).

Исследование пациентов проводилось за день до начала терапии, включавшей бромдигидрохлорфенилбе нзодиазепин (Феназепам), и через 5 дней терапии.

Проведение генотипирования по выбранному полиморфизму ABCB1 3435C^{o}T, производили с использованием полимеразной цепной реакции в режиме реального времени с аллельспецифической гибридизацией (Real-TimePCR). Для определения различий между группами количественных данных пациентов без полиморфизма 3435C^{o}T гена ABCB1 и с его наличием использовали Н-тест Крускалла-Уоллиса.

Результаты исследования. По шкале VASв 1-ый день лечения баллы получены следующие: 44,0±9,9 (CC), 44,8±11,0 (CT), 50,2±9,6 (TT), p=0,19.На 5-ый день лечения: 6,07±2,7 (CC), 6,7±2,3 (CT), 6,0±2,8 (TT), p=0,56. По шкале HADS в 1-ый день лечения были получены баллы: 25,5±4,4 (CC), 27,3±3,4 (CT), 27,58±3,6 (TT), p=0,22. На 5-ый день лечения: 3,6±22 (CC), 4,9±1,3 (CT), 4,0±1,39 (TT), p=0,11. По шкале UKU в 1-ый день лечения получены следующие баллы:1,28±0,46 (CC), 1,3±0,48 (CT), 1,17±0,39 (TT), p=0,53. На 5-ый день лечения: 8,7±0,6 (CC), 8,5±0,5 (CT), 8,5±0,5 (TT), p=0,59.

Выводы.По результатам исследования не было получено статически значимой разницы в показателях безопасности и эффективностибромдигидрохлорфенилбензодиазепина (Феназепама) у пациентов с тревожными расстройствами, коморбидными с алкоголизмом. Исходя из этого можно говорить об отсутствии влияния полиморфизма гена ABCB1 на профиль эффективности и безопасности применения Феназепама. В тоже время необходимо отметить, что, вероятно, отсутствие статистически значимой разницы в показателях не выявлено, ввиду недостаточного объема выборки. Необходимо продолжение исследования для решения данного вопроса.

ASSEMBLING CHEMOMES TO CREATE PHARMACEUTICAL MOLECULES AGAINST DRUG TARGETS

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This talk introduces a *de novo* chemotype (substructure) generation algorithm (DSGA) that derives frequent substructures in order to avoid the subjectivity of empirical method, and avoid the meaningless substructures generated from algorithmic approaches by statistical analyses. DSGA derives frequent chemical substructures (FCS) from a large compound library. In a FCS, substructures are not inter-included. When the library is big enough to represent the chemical diversity, such as ZINC database (27 million medicinal compounds), the resulting FCS is termed as the FCS dictionary (FCSD) for drug-like compounds. For a focused compound library (FL), DSGA can derive a focused FCS (*f*FCS) from FL. *f*FCS can be used as structural descriptors for focus library SAR studies.

Six focused libraries against targets PDE4D, mTOR, HDAC1, DPP4, BACE and ALR2 were tested with DSGA approach. Using the *f*FCSs as structural descriptor sets, six virtual screening models were generated to predict ligands against the targets, the prediction accuracies are greater than 90%.

Three methods were proposed to assembly drug-like molecules from substructures: (1) using the laws in the nature, such as isoprene rule; (2) organic synthesis rules, such as retro-synthon rules proposed by E. J. Corey; (3) pharmaceutical rules derived from a focused compound library against a specific target. We use DSGA to figure out rules that are used to compose privileged scaffolds by assembling FCS.

It can be chemically challenging to make the compounds proposed by these assembling approaches. By combining DSGA method, bioisoterism method and click chemistry, we generated privileged chemome (substructures/chemotypes) from Hsp90 inhibitor library, then find out available chemical fragments with bioisoterism rules. With SPR technology, we confirmed the fragments that interacted with Hsp90. Finally, we used click chemistry to assembly the substructures, and produced nanomolar selective Hsp90 inhibitors.

APPROACHES TO INCREASING THE DOCKING ACCURACY

Sulimov V.B., Kutov D.K., Sulimov A.V.

Molecular modeling, especially docking, is increasingly used in the development of medicines. With the help of docking, the molecule (ligand) of the test substance is positioned in the active center of the target protein and the protein-ligand binding energy is estimated: the more this energy, the more effective the drug. Reviews mention more than 60 docking programs in which a great variety of different simplifications are realized, because many decades docking programs are developed in the paradigm of «faster and faster» to dock many thousands of ligands on a laptop. But the calculation «on the lap» in Russian has a meaning: rough, slapdash, etc., and this reveals itself in the widespread use of docking programs. The positioning of ligands is performed satisfactorily by docking programs, but they tend to give large errors in binding energies.

The report discusses sources of docking errors, and approaches to their elimination, recently implemented in supercomputer programs FLM and SOL-P. The SOL-P program successfully docks flexible ligands to proteins with several dozen mobile atoms in the conformational space with an unprecedentedly large number of dimensions due to the application of a new method of global optimization - the tensor trains method. The conclusion is made that almost all existing simplifications that limit the accuracy of docking can be overcome, and a new generation of docking programs can be created, the use of which for the drug development should significantly increase its efficiency. The work was financially supported by the Russian Science Foundation, Agreement no. 15-11-00025.

A SCALABLE FABRICATION PROCESS OF POLYMERIC DISSOLVING MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY

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Microneedles are designed to create temporary channels on the skin without reaching to the dermis to enhance the drugs transport across the skin barrier. Dissolving microneedle array (DMNA) fabricated from biodegradable polymers can release the encapsulated drugs by dissolving in the skin without any biohazardous waste. In recent years, remarkable progress has been achieved in developing DMNA for successful transdermal delivery of biological small molecules, macromolecular drugs and vaccines.

High-temperature molding, UV photo-polymerization curing, and aqueous solution casting have been used for molding DMNA from dissolving materials. However, these methods are rather complicated involved with several processes, and the radiation source, polymerizing reagents or elevated temperatures may impair the stability of the biomacromolecular drugs and cause skin irritation. Although two-step molding has been widely utilized for the fabrication of DMNA, insufficient drug loading in the needle portion and lack of practicable industrial preparation method have severely impeded their further application.

A modified two-step method using different solvents for needle and base portions under mild conditions was developed by our group, and a series of DMNA containing various model drugs such as levonorgestrel, thymopentin, and salmon calcitonin were successfully prepared. Insertion assessment, stability test, and drug release study were conducted *in vitro*. The therapeutic efficiency was confirmed via *in vivo* pharmacodynamic study. In addition, a novel automatic microneedles array fabrication system (AMAFS 1.0) was initially developed by our group, which can continuously manufacture microneedles with a simple and reproducible operation to control mechanical parameters.

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РАЗРАБОТКА ПРЕПАРАТА ДЛЯ ИНДУЦИРОВАНИЯ ПРОДОЛЖИТЕЛЬНОГО, СТАБИЛЬНОГО И ОБРАТИМОГО ГИПОМЕТАБОЛИЧЕСКОГО И ГИПОТЕРМИЧЕСКОГО СОСТОЯНИЯ У КРЫС В ТЕРМОНЕЙТРАЛЬНЫХ УСЛОВИЯХ

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Как известно, разработка технологий введения человека в искусственное гипобиотическое состояние с контролируемым уровнем обмена веществ и температуры ядра тела является одной из наиболее важных задач современной биологии и медицины. Ее решение позволит обеспечить длительное выживание человека в замкнутом пространстве в условиях гипоксии, холода и дефицита ресурсов жизнеобеспечения, при проведении сложнейших многочасовых операций, а также продлить «золотой час» при оказании раненым неотложной медицинской помощи. Нами разработана эффективная технология получения стабильных насыщенных ксеноном жидких смесей с включением известных, используемых в клинике фармакологических препаратов. Полученная фармакологическая композиция после внутривенной инъекции крысам, вызывает быстрое снижение ЧСС, вслед за которым через 1,5-2 часа наблюдается стабильное снижение температуры тела животных в среднем на 7˚С-8˚С, которое может длиться до 8 часов при температуре окружения 21˚С-22˚С. По истечении времени действия препаратов происходит спонтанное повышение температуры тела до исходных величин. В период максимального падения температуры потребление кислорода может снижаться двукратно, при этом сатурация крови не нарушается.

Показано, что экспериментально обоснованная фармкомпозиция является уникальной, и отсутствие в составе одного из компонентов существенно снижает ее эффективность.

После выхода из состояния гипометаболии и гипотермии животные не обнаруживают отклонения в поведении и сохраняют ранее приобретенный навык.

Как показали предварительные данные, разработанная композиция многократно повышает устойчивость организма к внешнему охлаждению и гипоксии, переводя животное на новый уровень жизнеобеспечения, что позволяет временно понизить кислородные и энергетические запросы организма, с последующим полным восстановлением его жизнеобеспечивающих функций.

PLATELET GLUTATHIONE REDUCTASE AND GLUTATHIONE-S-TRANSFERASE ACTIVITY IN FIRST-EPISODE PATIENTS WITH SCHIZOPHRENIA AND SCHIZOAFFECTIVE DISORDER

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Background – First-episode psychosis is the most important time range for antipsychotic treatment and prevention of repeated psychoses, relapses, and further disease progression.

Aim – Comparative assessment of platelet glutathione reductase (GR) and glutathione-S-transferase (GST) activities in first-episode psychoses and in control group, and search for link between these activities and clinical assessments of the patients.

Methods – PANSS scores and platelet GR and GST enzymatic activities in the first-episode patients (men) with schizophrenia (SZ, n=21) or schizoaffective disorder (SZA, n=32) were assessed before and after the treatment course with antipsychotics and recorded in database. Control group consisted of 33 men volunteers. Non-parametric statistics was used for between-group comparisons (Mann-Whitney U-test), and search for correlations (Spearmen rank order correlations).

Results – significantly reduced GR activities were found in SZ or in SZA group, both before and after the treatment course, as compared with the control group. Significantly reduced GST activities were found in ShD group, both before and after the treatment course, as compared with the control group. Negative correlation was found between GST activity measured before the treatment course and PANSSneg, PANSSpsy, and PANSStotal assessed in SZ group after the treatment course ($R=-0.48$, $p<0.05$).

Conclusion – reduction of GR and GST activities may evidence for decreased glutathione antioxidant defence in first-episode psychoses, correlation between GST activity and PANSS may have a prognostic value for antipsychotic treatment efficacy.

PCA3 AND TMPRSS2:ERG GENES EXPRESSION ANALYSIS IN URINE SEDIMENT OF THE PATIENTS WITH ADENOCARCINOMA, BENIGN PROSTATE HYPERPLASIA AND OTHER PATHOLOGICAL CHANGES OF PROSTATE

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Prostate tumor cells and nucleic acids slip into the lumen of the urinary tract and are present in the urine sediment, in which mutations and changes of gene expression can be determined. The aim of the our study was to analyze *PCA3* and *TMPRSS2:ERG* expression in urine sediment with benign prostate hyperplasia (BPH) and prostate cancer (PCa) to determine the diagnostic significance of the combined expression level of these genes as PCa marker. The study included 51 patients with BPH and/or prostatitis (control), 59 patients with PCa. After prostate massage, RNA was extracted from the of urine sediment, reverse transcription was performed, then gene expression was analyzed in real-time PCR and the deltaCt value (Ct PCA3-KLK3) was calculated. Medians were 4.09 in the control and -0.20 in the PCa. Using ROC analysis the optimal deltaCt threshold was found to be 1.23. The accuracy of the *PCA3* overexpression was 82, sensitivity 76, specificity 88%. Expression of the chimeric oncogene *TMPRSS2:ERG* was absent in the control and was detected in 59% of the PCa. DeltaCt does not differ in patients with BPH, low and high grade PIN, prostatitis, while significantly increased in PCa with respect to any of the control subgroups listed above ($p<0.01$). Thus, the *PCA3* overexpression and the *TMPRSS2:ERG* are characteristic of PCa. Analysis of these genes expression with the proposed modification of RT-PCR in urine sediment allows to diagnose PCa with accuracy 82%.

REGENERATION OF RAT SKELETAL MUSCLE INDUCED BY MSC-POPULATED COLLAGENOUS SCAFFOLDS

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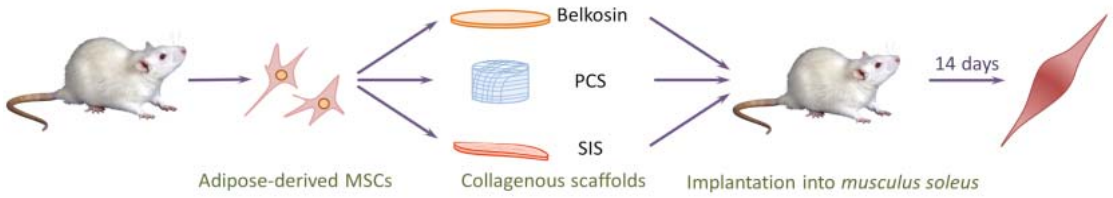
Introduction. Application of mesenchymal stem cells (MSCs) for tissue regeneration is currently a well-developed practice performed on many types of tissues, including the skeletal muscle. The best survival rates of MSCs in the implantation site are achieved by delivering MSCs on biocompatible scaffolds, as they provide adhesive surface and mechanical protection. A very popular material for such scaffolds is collagen, as it is non-immunogenic, biodegradable and can be used in various forms.

The aim of this study is to compare the influence of three collagen-based scaffolds on regeneration of rat skeletal muscle in the presence of adipose-derived MSCs.



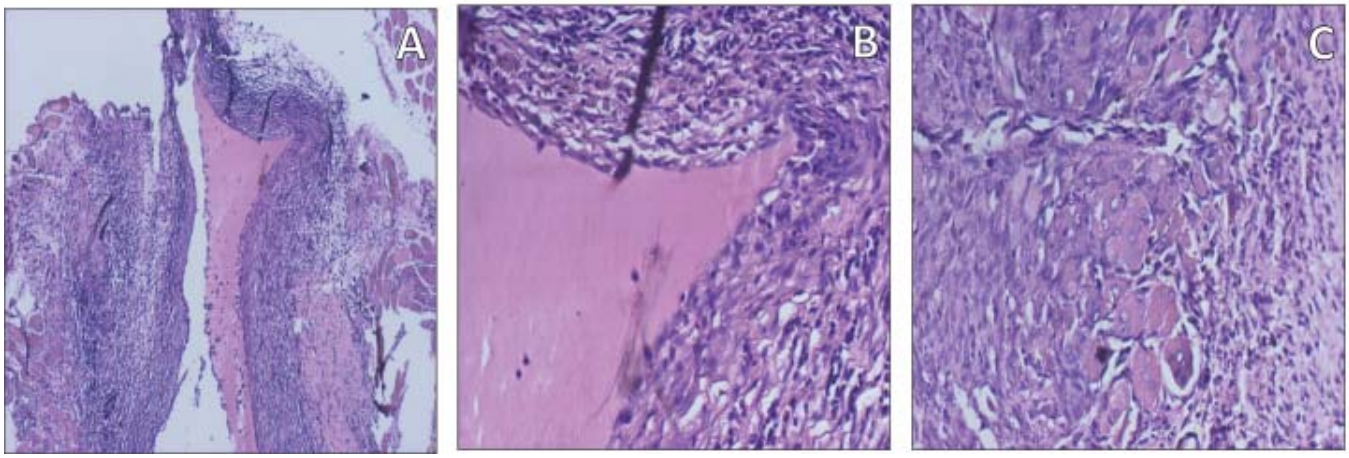
Materials and Methods. MSCs obtained from Wistar rat adipose tissue were cultivated on 3 different collagenous scaffolds for 14 days in 10^6 cells/ml concentration. Each scaffold was placed into musculus soleus laceration site immediately after the injury and sealed with sutures. Histological examination was performed 14 days after the operation.

Results. All the examined grafts caused inflammation at the implantation site, but presented different effect on muscle regeneration and vascularization. Effectiveness of muscle regeneration was estimated by the amount of muscle fibers with centralized nuclei.

Belkosin is a type of collagenous sausage casing used in meat industry. It consists of reorganized collagen fibers, which spawned the question, whether it can be used as a scaffold for stem cell delivery.

At the implantation site Belkosin showed almost no biodegradability and maintained its solid structure after 14 days. Very few cells were able to penetrate the scaffold and the rest remained mostly on its surface.

Groups of regenerating muscle fibers were found at the periphery of the inflammation zone. Single blood vessels were found among the inflammatory cells.

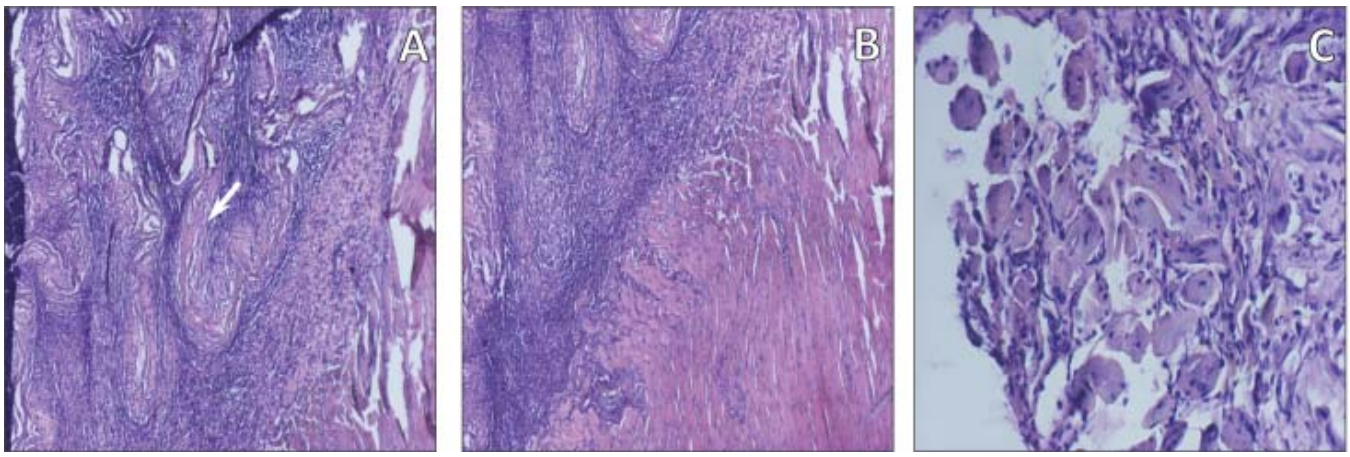


A. Site of Belkosin implantation. In many cross-sections surrounding tissue seems to be detached from the scaffold, hinting at its poor adhesive properties. A few cells impregnate the scaffold, but mot remain on its surface. **B**. Structure of Belkosin. **C**. A group of regenerating muscle fibers.

SIS (small intestinal submucosa) is a native fibrous layer derived from swine intestine wall. SIS is less dense than Belkosin, therefore more elastic and soft.

SIS exhibited good biodegradability at the implantation site, as it became thinner and slightly dispersed. Fibers of the scaffold were interlaced with connective tissue and inflamatory cells.

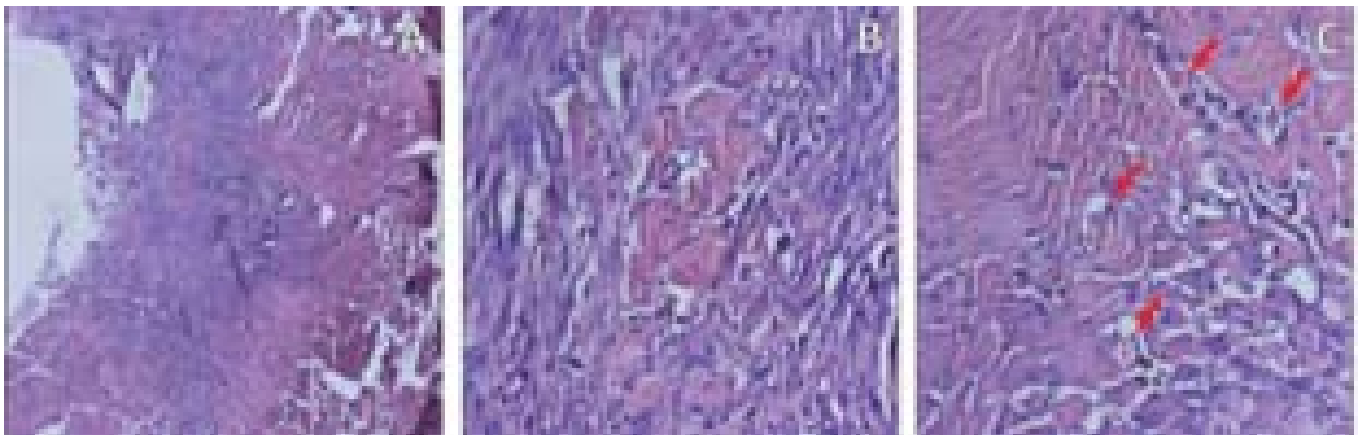
The area of inflammation was rather vast, similar to Belkosin, and regenerating muscle fibers and new blood vessels were only seen at its periphery. However, there were noticeably more groups of regenerating muscle fibers around SIS than around Belkosin.



A. Site of SIS implantation. White arrow marks the remains of the scaffold. The area is filled with numerous neutrophils. **B**. The verge of the inflamed area. Groups of regenerating muscle fibers are visible. **C**. A group of regenerating muscle fibers.

PCS (porous collagen scaffold) is a 3D structure of crosslinked collagen fibers. Due to loose porous structure it provides the best adhesive surface for the cells.

Of all scaffolds used, PCS caused the least inflammation, roughly confined to the area of the scaffold itself. 14 days after implantation PCS was almost fully degraded and well infiltrated with recipient connective tissue and cells. Unlike Belkosin and SIS, regeneration of muscle tissue wasn't limited to the verges of inflammation area, but also took place in its center, impregnating the scaffold's structure and its nearest surroundings. Moreover, PCS graft seemed to cause the most active vascularization at the implantation site.

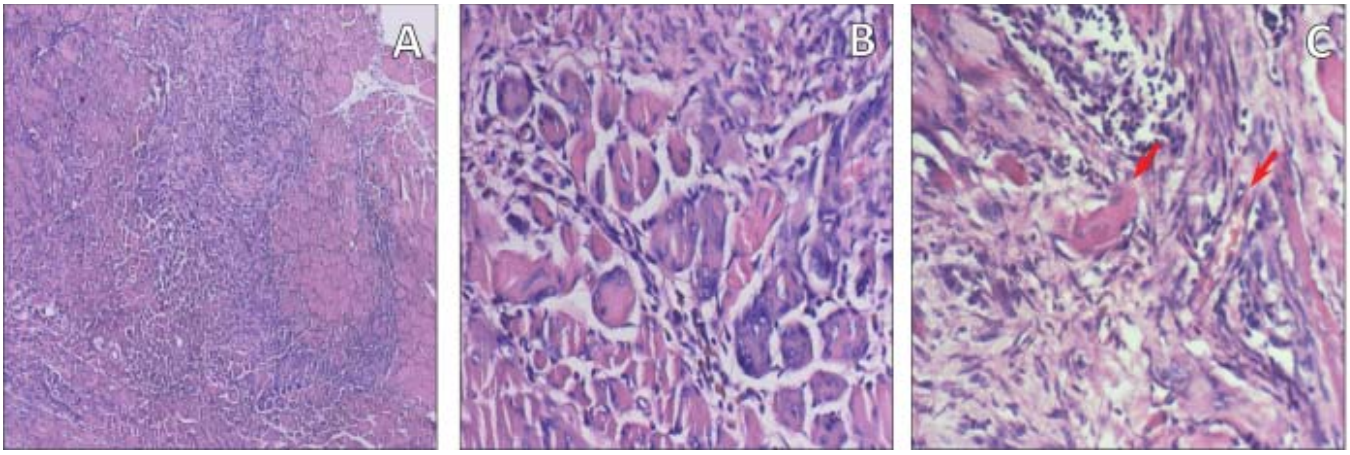


A. Site of PCS implantation. There are seemingly fewer neutrophils around the scaffold, and the area seems to contain more connective tissue, compared to Belkosin and SIS. **B**. Young muscle fibers appear near the middle of the inflamed area. **C**. Active vascularization around the implantation site. Red arrows mark blood vessels.

MSC suspension.

To compare MSC effectiveness with and without a scaffold, we also attempted to deliver MSCs into the laceration site by injection of the cell suspension. The same concentration of cells was used for injections, as for populating the scaffolds.

Inflammation caused by such cell delivery was minimal, as no foreign material was introduced. Some regenerating muscle fibers were found around and inside the inflamed area, however they appeared thinner than those growing in presence of scaffolds.

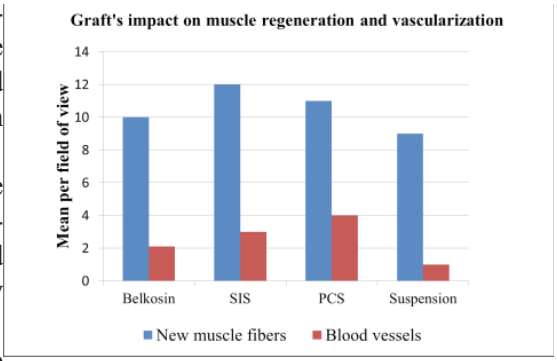


A. Site of MSC suspension injection. Quite few neutrophils are present at the site. **B**. Bundle of regenerating muscle fibers. **C**. Regenerating muscle fibers and a vessel in the middle of the inflamed area. A lot of connective tissue is present along with inflammatory cells.

Conclusion. Out of three examined collagenous scaffolds, Belkosin showed the least biocompatibility, as it barely disintegrated in the tissue. SIS and PCS allowed better permeation by recipient cells and tissue, but, evidently, PCS provides the best structural organization for tissue regeneration due to its soft porous structure.

SIS+MSC and PCS+MSC seemed to induce slightly more active muscle regeneration and vascularization than Belkosin and MSC suspension. Muscles regenerating with engrafted PCS+MSC exhibited the most positive result. The least active regeneration was induced by injection of MSC suspension.

Further experiments with the same scaffolds will be carried out to compare the effect of adipose- and bone marrow-derived MSCs on muscle regeneration.



REGENERATION OF RAT SKELETAL MUSCLE INDUCED BY MSC-POPULATED COLLAGENOUS SCAFFOLDS

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Application of mesenchymal stem cells (MSCs) for tissue regeneration is a well-developed practice in regenerative medicine. Delivering MSCs on biocompatible scaffolds provides the best survival rates of MSCs after implantation. Many scaffolds are made of collagen that is non-immunogenic and biodegradable. This study aims to compare the influence of three collagen-based scaffolds on skeletal muscle regeneration in the presence of adipose-derived MSCs.

MSCs obtained from rat adipose tissue were cultivated on 3 different collagenous scaffolds for 14 days in 10^6 cells/ml concentration. Each scaffold was placed into *musculus soleus* laceration site immediately after the injury and sealed with sutures. Histological examination was performed 14 days later. The scaffolds used were Belkosin (BLK), porous collagen scaffold (PCS) and small intestinal submucosa (SIS). For comparison MSCs were delivered by injection of the cell suspension into the injury site.

All the grafts caused inflammation at the implantation site. SIS+MSC and PCS+MSC seemed to induce slightly more active muscle regeneration and vascularization than Belkosin and MSC suspension. Muscles regenerating with engrafted PCS+MSC exhibited the most positive result. BLK showed the least biocompatibility, as it barely disintegrated in the tissue. SIS and PCS allowed better permeation by recipient cells. Evidently, PCS provides the best structural organization for tissue regeneration due to its porous structure.

MECHANISMS OF POSSIBLE ANTIBIOTICS ACTION POTENTIATING USING SECONDARY METABOLITES FROM PLANTS

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Background: Multidrug resistance is worldwide problem of 21st century. It occurs in microbial cell and results from activation of different mechanisms in cell structures. Secondary metabolites from plants are synthesized during the photosynthesis. Their release results from activation of intrinsic protective mechanisms.

Aim: To clarify possible mechanisms of antibiotic action potentiating using secondary metabolites from plants.

Materials and methods: We analyzed experimental papers and scientific reviews on the subject using NCBI MedLine, Scholar.Google, and Elsevier databases.

Results: Main antibiotic potentiating mechanisms of secondary metabolites from plants aimed on disturbing structures which are responsible for multidrug resistance forming. In bacterial cell secondary metabolites are able to inhibit β-glucane synthesis, depress efflux. Along with that in fungal cells much more mechanisms occur. For instance, secondary metabolites can inhibit ergosterol synthesis.

In the other hand, secondary metabolites can depress different life processes in pathogen cell: inhibition of nucleinic acid synthesis, DNA-gyrase and glycosidase activity, spindle apparatus forming and mitochondrial electron transport chain.

Conclusion: Wide biological activities of plant secondary metabolites makes them be considered as an effective aid for antibiotic action potentiating in the case of multidrug resistance. Our plan calls for further experimental research on optimal proportions of secondary metabolites for that.

APPLICATION OF METABOLOMICS TOOLS INTO MICRONUTRIENT RESEARCH: PAST PROGRESS, FUTURE CHALLENGES AND OPPORTUNITIES WITH SPECIAL FOCUS ON RUSSIA

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Introduction: Micronutrient deficiencies are widespread among infants, young children, women and the elderly in developing and developed countries. New methods in modern nutrition science, specifically “omics” tools represent an opportunity to improve diagnostics, assessment of nutritional status and biomarkers discovery, among others. Objective: The purpose of this presentation is to review the state of knowledge and to make recommendations on applications of metabolomics into micronutrient research, with special focus on Russia.

Methodology: 1. Evidence on the past and current prevalence of vitamin and mineral deficiencies in Russia will be identified. 2. A systematic search of scientific articles published in PubMed will be performed to identify studies that connect micronutrient research and metabolomics. 3. Recommendations will be proposed to fill the existing gaps on the magnitude of micronutrient deficiencies in Russia and the applications of metabolomics into micronutrient research.

Results: 1. Historical data and the current profile for vitamins and mineral deficiencies in Russia will be presented. 2. Studies connecting micronutrient and metabolomics research will be summarised (i.e. metabolomics profiles of vitamin B-12 and vitamin B-6 deficiencies). 3. Recommendations will be conceptualized to improve the current state of knowledge and application of metabolomics into micronutrient research.

Conclusions: The available knowledge on the magnitude of vitamin and mineral deficiencies in Russia, and the existing progress on integration of metabolomics into micronutrient deficiencies will be presented. Future challenges and opportunities for science, public health and industry will be discussed.

VEGFA PARTICIPATES TO THE MECHANISMS SUSTAINING THE ONSEDT OF ACQUIRED RESISTANCE TO BRAF INHIBITORS IN MELANOMA

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The introduction of BRAF inhibitors (BRAFi) has improved response rate and overall survival of metastatic melanoma patients compared to standard chemotherapy. However, acquired drug resistance occurs in nearly all patients. The comprehension of cellular and molecular mechanisms underlying BRAF inhibitor resistance could help to identify novel actionable pathways in the treatment of BRAF dependent tumors.

VEGFA is an attractive target for combinatorial cancer therapy and besides regulating angiogenesis has an immune-suppressive effect in tumors. In my presentation, I'll present published and unpublished data indicating that VEGFA removal antagonizes the acquired resistance to BRAF inhibitor by a mechanism involving myeloid cells and a remodeling of extracellular matrix. This positive effect synergizes with immune-checkpoint inhibitor targeting PD-1. These preclinical results offer the rationale for novel combinatorial approaches including co-targeting of signaling molecules, tumor angiogenesis and immune system.

SUPERCRITICAL FLUID TREATMENT OF THREE-DIMENSIONAL SCAFFOLDS FORMED USING LASER STEREOLITHOGRAPHY

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There are a variety of different techniques to fabricate and functionalize scaffolds, most of which depend on the material in question. In the present work, three-dimensional scaffolds based on photosensitive chitosan hydrogels were formed by laser stereolithography. The developed photosensitive composition allows to form macro-designed scaffolds and might therefore be a suitable candidate for laser-based 3D printing. Supercritical fluid treatment have been applied to hydrogel scaffolds in order to improve mechanical, chemical, and physical properties – such as biocompatibility, roughness, Young's modulus and surface wettability. The hydrogel scaffolds treated in the supercritical carbon dioxide have no cytotoxic effect, their mechanical properties increases from 10 to 80000 Pa.

This work was supported by Russian Foundation for Basic Research (Project № 18-32-00222) in part of formation of hydrogel scaffolds and their treatment in the supercritical carbon dioxide and by the Federal Agency of Scientific Organizations (Agreement No 007-Г3/Ч3363/26) in part of development of new systems for 3d printer technologies.

FUNCTIONAL TISSUE ENGINEERING OF TENDON: BIOMECHANICAL CHARACTERISTICS OF THE RESTORED ACHILLES TENDON OF THE RABBIT USING MSC

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Surgical repair of a damaged tendon is a standard practice, but it is not very effective. In most cases, the restoration occurs by the formation of scar tissue instead of native tissue. The tissues recovered in this way have significant biomechanical dysfunction, and in some cases, it can lead to a disability.

The aim of our work is to develop a method for restoration of the Achilles tendon, which will return it into a morphological and biomechanical state similar to the native one. To do this, we cut out a part of the tendon's middle beam in a rabbit model and filled it with a tissue-engineered construct from a Vicryl mesh folded into a tube and filled with mesenchymal stem cells. After the follow-up period, the regenerated tendons were taken from the operated rabbits and compared with intact and control samples (scaffold without MSC). The obtained data indicates a complete restoration of the tissue in the regenerated tendon with the structure similar to the intact one. Moreover, the regenerated tendon significantly strengthened while maintaining elasticity.

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PCA3 AND *TMPRSS2:ERG* GENES EXPRESSION ANALYSIS IN URINE SEDIMENT OF THE PATIENTS WITH ADENOCARCINOMA, BENIGN PROSTATE HYPERPLASIA AND OTHER PATHOLOGICAL CHANGES OF PROSTATE

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Prostate tumor cells and nucleic acids slip into the lumen of the urinary tract and are present in the urine sediment, in which mutations and changes of gene expression can be determined. The aim of the our study was to analyze *PCA3* and *TMPRSS2:ERG* expression in urine sediment with benign prostate hyperplasia (BPH) and prostate cancer (PCa) to determine the diagnostic significance of the combined expression level of these genes as PCa marker. The study included 51 patients with BPH and/or prostatitis (control), 59 patients with PCa. After prostate massage, RNA was extracted from the of urine sediment, reverse transcription was performed, then gene expression was analyzed in real-time PCR and the deltaCt value (Ct PCA3-KLK3) was calculated. Medians were 4.09 in the control and -0.20 in the PCa. Using ROC analysis the optimal deltaCt threshold was established 1.23. The accuracy of the *PCA3* overexpression was 82, sensitivity 76, specificity 88%. Expression of the chimeric oncogene *TMPRSS2:ERG* was absent in the control and was detected in 59% of the PCa. DeltaCt does not differ in patients with BPH, low and high grade PIN, prostatitis, while significantly increased in PCa with respect to any of the control subgroups listed above ($p<0.01$). Thus, the *PCA3* overexpression and the *TMPRSS2:ERG* are characteristic of PCa. Analysis of these genes expression with the proposed modification of RT-PCR in urine sediment allows to detect PCa with accuracy 82%.

MOLECULAR GENETIC AND MORPHOLOGICAL DIAGNOSTIC OF GRAVITY ENDOMETRIUM AND CHORIAL TISSUE IN EARLY PREGNANSY LOSS

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Summary: The results of clinical, molecular-genetic and morphological diagnostics of gravity endometrium and chorionic tissue in three types of non-developing pregnancy, identified by using ultrasound criteria, are presented. The obtained data can be improved the efficiency of the treatment and pre-conception training after suffering early pregnancy loss. Non-developing pregnancies account for 45-80% of the total number of early reproductive losses. Aim: to increase the effectiveness of treatment after suffering a NB in the early stages taking into account ultrasound data and results of molecular - genetic and morphological studies gravidarum endometrium and chorionic tissue. Patients and methods: We examined and treated 70 women with the diagnosis of non-developing pregnancy (less than 12 weeks). The patients ages was from 18 to 40 years old admitted to the Gynaecology Department of University Clinical Hospitals No. 4 of I.M. Sechenov First Moscow State Medical University (Sechenov University). The average obstetrical gestational period in EM and ES were diagnosed made 9 ± 2.0 and 8 ± 2.0 weeks, respectively. Upon admission, 45 patients (64%) noted scarce bleeding, 25 patients (36%) did not have any bleeding at all. For surgical treatment of NP, manual vacuum extraction aided by video hysteroscopy was used. After gestational sac extraction, all patients had their diagnosis confirmed morphologically. A molecular - genetic research of chorial tissues was carried out in 42 women with NP for the search for aneuploidy on the chromosomes X, Y, 13, 14, 16, 18, 21 and 22. Test method: multilocus quantitative fluorescent PCR (Amel, DXS6809, DXS6803, DXS8377, SBMA, D13S258, D13S634, D13S742, D18S535, D18S386, D18S391, D21S11, D21S1411, IFNAR, D14S122, D14S127, D14S128, D16S534, D16S476, D16S690, D22S683, D22S691, D22S873) followed by fragment analysis on the genetic analyzer ABI 3100. **Results:** embryonic miscarriage (EM) (no heart beat of the embryo) had 30 (43%) of patients, with Empty sac (ES) (no embryo and yolk sac) were 31(44%) of cases. Yolk sac (YS) (no embryo with yolk sac present) had 9 (13%) patients (Kolte A.M., et all., 2015). During gravity endometrium examination, attention was paid to the nature and rate of inflammatory changes. Chromosomal abnormalities were detected in 40% cases on chromosomes X, Y, 13, 14, 16, 18, 21 and 22. Structure of the revealed pathology: triploidy – 6 (14.2%) of cases, trisomy 16, 21, 22 – 2 (4.7%) each cases, respectively, but trisomy 13, 14, 18, monosomy X, monosomy 21 were detected in 1 (2.3%) each, respectively. Morphological examination revealed that 7 (10%) of patients with ***ES*** *were* without any bleeding. Endometrium was edematous, with focal haemorrhage, small areas of necrosis, slight leukocytic infiltration in 29% and lack of lymphoid cell infiltration. In **ES,** 63% of women with bleeding had moderate and marked infiltration with polymorphonuclear leukocytes in their endometrium, even with foul foci formation; 17% of women had focal lymphoid cell infiltration. In **YS,** 3 (33.3%) patients out of 9 had no bleeding, but their endometrium demonstrated slight leukocytic infiltration; lymphoid cell infiltration had 67% of patients. Patients with **EM** had lymphoid cells and did not have any bleeding (33%) or had bleeding (40%); more pronounced the phenomenon was in women with blood type A (II)Rh+. Where bleeding was present, patients with **EM** had moderate and marked infiltration with polymorphonuclear leukocytes, even with foul foci formation, 1.5 times **rarer** than patients with **ES.** When chorial tissue was assessed it was found out that 47 (67%) women out of 70 patients had dystrophic and necrobiotic changes in their chorionic villi. In **EM**, dystrophic, necrotic and sclerotic changes in villi were more frequent than in patients with ES (33% and 26%, respectively). In general, most marked morphological signs of an acute inflammation process in endometrium (heavy leukocytic infiltration, foul foci formation) were more common for women with bleeding typical of miscarriage. Therefore, in ES where patients *did not complain of scarce bleeding, inflammatory changes in endometrium were less pronounced* compared to those having bleeding. In **YS** and **EM**, most common is *lymphocitic infiltration both with and without bleeding*. Lymphocitic infiltration which is more typical of patients with **EM** was accompanied with inactive viral inflammation in endometrium. **Conclusion:** Multilocus quantitative fluorescent PCR method is used for research of numerical chromosomal abnormalities of the first trimester and provides a frequency of chromosomal abnormalities in 40% patients with non-developing pregnancy. If numerical chromosomal abnormalities are detected, it is recommended to refer patients to a clinical genetics consultation for examination and planning of subsequent pregnancy. Morphological examination are shown the need for ultrasound screening at week 8-9 in order to timely identify non-developing pregnancy. Early hospitalisation into an inpatient unit and timely minimally invasive extraction of dying gestational sac facilitates prevention of complications, e.g. acute and chronic endometritis. Analysis of morphological examinations (taking into account the non-developing pregnancy type) requires personalised approach to antibacterial (fluoroquinolones, macrolides) and antiviral therapy (viferon 1,000,000 IU rectal suppositories, viferon gel 40,000 IU for local therapy) after minimally invasive therapeutic and diagnostic extraction of a pathological gestational sac.

PHOTO-CROSSLINKED POLYLACTIDE PARTICLES AS A PROMISING CARRIER FOR THE DEVELOPMENT OF SUSTAINED RELEASE DRUGS

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Nowadays, one of the key issues in pharmaceutical industry is to develop new effective technologies of sustained-release drugs and find new materials as a drug carrier. Polylactides are promising, because they are biocompatible and biodegradable. Moreover, their degradation rate can be easily modified by varying their chemical structure and cross-linking conditions. Here, we sought to assess FITC release from photo-crosslinked polylactide particles. To prepare carrier material, we used photosensitive poly(D,L)-lactide modified with methacryloyl chloride and mixed it with 4,4′-bis(diethylamino) benzophenone in dichloromethane. To assess FITC release kinetics, we dipped the particles in 0.1% FITC for 12 h and washed with PBS. Then they were placed into PBS with constant mixing at 36-37°C for 12 h. We chose the following time points: 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h. The FITC concentration was measured via a spectrophotometer using a calibration curve. We revealed that 67% of loaded FITC released during first 30 min; and then the substance released linearly from 2h till 8h. In 8h, the FITC release was insignificant. Thus, we showed that photo-crosslinked polylactide particles might be used as a drug carrier and provide sustained release. This work was supported by the Russian Foundation for Basic Research, grant 17-34-80151.

PHOTO-CROSSLINKED HYDROGEL SCAFFOLDS BASED ON HYALURONIC ACID DERIVATIVE FOR TISSUE ENGINEERING

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3D hydrogel-based scaffolds with predesigned structures and functionality has gained a lot of interest in tissue engineering. Scaffolds should imitate physical-chemical properties of replaced tissue for the best implant survival rate. This fact requires prerequisite scaffold material properties, such as biocompatibility, biodegradability, adhesion properties of surface, high porosity. Hyaluronic acid is considered to be one of the most advantageous material. However, scaffolds based on non-modified hyaluronic acid possess relatively limited mechanical properties and can be vulnerable to rapid degradation and contraction.

Here, we demonstrate a method of scaffold production based on hyaluronic acid modified with glycidyl methacrylate (HAGM). UV or visible light irradiation of solution, containing HAGM and photoinitiator, induced photo-crosslinking, resulting in production of tough and insoluble hydrogel. Photoinduced gelation is possible due to radical reaction of double bond moieties of glycidyl methacrylate in HAGM. The role of photoinitiator played endogenous compound: riboflavin mononucleotide (vitamin B2). Scaffolds were fabricated via 3D printing by using home-built 3D printer.

HAGM-based scaffolds were produced for *in vitro* and *in vivo* applications. The lack of cytotoxicity and good cell adhesion were demonstrated in MTT assay and in proliferation of immortalized human fibroblasts BJ-5ta on hydrogel surface. Biocompatibility was confirmed by the lack of significant inflammation processes at subcutaneous positioning of scaffold in mice.

The reported study was funded by RSCF according to the research project № 17-19-01416

AORTIC ROOT DECELLULARIZATION IN SUPERCRITICAL CARBON DIOXIDE MEDIUM: A PILOT STUDy

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Development of prosthetic heart valves is the one of the greatest challenges in tissue engineering. Among them, decellularized transplants are the most promising due to appropriate hemodynamic behavior and biocompatibility. Decellularization is aimed to decrease immunological response of the recipient's body while preserving the composition and structure of extracellular matrix. One of the disadvantages of currently used decellularization protocols is the long duration of treatment (about 14 days). Our project aims to develop the aortic root decellularization protocol utilizing supercritical carbon dioxide ($scCO_2$) medium as non-toxic agent, which has ability to eliminate lipids and other cell compounds in a short period (several hours).

In order to investigate impact of $scCO_2$ to decellularization process ovine aortic roots were divided into 2 groups. Group 1: samples underwent alkaline pre-treatment with further $scCO_2$ processing. Group 2: samples were treated with $scCO_2$ supplemented with ethanol. Efficiency of decellularization was examined via hematoxylin-eosin staining.

The comparative analysis of the samples provided new insights into decellularization of complex 3D-structures and paved the way for further investigations.

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CARBON NANOPARTICLES AS NOVEL CARRIERS FOR RADIOPHARMACEUTICALS

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At present, nuclear medicine is becoming more and more relevant in the developed countries of the world and finds new practical applications for the diagnosis and treatment of socially significant diseases. One of the main requirements for radiopharmaceuticals (RFs) is accumulation in the target organ. Directed transport of the radiopharmaceutical is provided by the interaction of two components: transport nanocarriers delivering a radioactive isotope to the target organ; and the isotope. Due to the variety of radionuclides and so much of potential nanocarriers capable of delivering the isotope to the target organ, today we can create the RFs for the diagnosis or treatment of any system of the body. Basic requirements of nanocarriers in RFs are biocompatibility, non-toxicity, selective accumulation in targeted organs, radiation resistance and a significant ability to complexation with radionuclides.

In this work, to solve the problem of delivering a radioactive isotope to a specific organ, it is promising to use new carriers – carbon nanomaterials: nanodiamond, multiwall carbon nanotube and graphene oxide. They possesses suitable physicochemical properties: nanoscale, developed free surface (which is likely to determine high sorption capacity), chemical and radiation resistance, biocompatibility, non-toxicity and the possibility of surface modification by creating certain functional groups by gas and liquid-phase chemical reactions, as well as grafting of various substances to hold radionuclides of different nature.

Sorption experiments of radioactive ions ^{99}Tc and ^{211}Pb on the surface of carbon nanomaterials were carried out under strictly controlled conditions, in which the time to achieve the mobile equilibrium, the dependence of sorption on pH, the total sorbate concentration, etc. were investigated. Sorption parameters and capacity of carbon nanocarriers were determinated.

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CARBON NANOPARTICLES AS NOVEL CARRIERS FOR RADIOPHARMACEUTICALS

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This work is supported by the Russian Science Foundation (project № 18-13-00413).

IMMUNOLOGICAL MARKERS OF REMODELING OF NERVOUS TISSUE AT FOCAL TRAUMATIC INJURIES OF THE BRAIN

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Objective: To study of dynamics of the content of markers remodeling of neural tissue in patients with focal traumatic brain injury.

Material and Methods: Research object was 40 patients both males and females aged 43±7,5 years with traumatic injuries of the brain and 40 conditionally healthy persons correlated according to sex and age. Quantitative content of the protein S-100, CNTF in blood serum were determined by ELISA test. Statistical analysis of the findings was carried out with the help of the software packages IBM SPSS 20 Statistics.

Results. Protein S-100 level on the first day from the moment of obtaining an injury increased by 27,05 times in comparison with the control ($p<0,001$). In the rest observation periods index concentration gradually decreased ($p_{1-4}<0,001$), but was authentically higher than the control ($p<0,001$).

CNTF level on the first day from the moment of obtaining an injury increased by 64,67 times in comparison with the control ($p<0,001$) and remained high in the rest examination period.

Conclusion: Dynamics of the content of neurospecific proteins in patients with focal traumatic brain injury allows to estimate separate mechanisms of neural tissue remodeling process selectively.

CLONAL-DIRECTED PERSONALIZED CHEMOTHERAPY FOR BREAST CANCER

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Abstract. In previous studies, it has been shown that during neoadjuvant chemotherapy (NAC) under the influence of chemotherapeutic agents a clonal evolution of the tumor takes place, at which the tumor clones change (TC) - complete / partial disappearance or appearance of new clones. New clones that were formed under the influence of NAC contain amplifications in the following loci: **5p, 6p, 7q, 8q, 13q, 9p, 9q, 10p, 10q21.1, 16p, 19p, 18chr**. At the same time, it was revealed that the appearance of any two or more amplifications of these regions in the residual tumor during NAC leads to 100% hematogenous metastasis. All other patients with elimination of TC, lack of action of chemotherapy on the clones or patients with the appearance of deletion clones did not have hematogenous metastases in the 5-year follow-up period (according to the Kaplan-Mayer method, p = 0.00001 Log-rank test). **The aim of this work** was to evaluate the efficacy of NAC and the 2-year metastasis-free survival of patients with breast cancer (BC) on the basis of studying the presence of amplification clones in the primary tumor of patients and evaluating the expression of somatic-stem cells transition genes.

Material and methods. The material for the study was samples of biopsy material before treatment and tumor material after NAC for each patient. The presence of amplifications was determined in the above regions using the CytoScan HD Array microarray (Affymetrix, USA). Three groups of patients were formed. 1-st: patients, whose tumors were determined by the presence of any 2 amplifications. They received a personalized NAC (in accordance with patent RU 2594251). The second group included patients who initially had a 0-1 amplification clone in the tumor and started treatment from the operative stage. Patients of the 3rd group also initially had a 0-1 clone, the treatment started with a personalized NAC. The remaining stages of treatment were standard. According to the main clinical and morphological parameters, the groups did not differ.

Results. 86% of patients in 1st group (n = 29) achieved complete or partial regressions after NAC, the 2-year survival rate in the group was 97% (28/29). Survival in the second group was 93% (13/14). In the third group, the efficacy of NAC was 84% (16/19), but the survival was statistically significantly lower than in the 1st group - 68% (13/19) (p = 0.011). Proceeding from the proof of the origin of the tumor from tumor stem cells, we proposed the existence of a somatic-stem cells transition program in the occurrence of TC. The next stage of the work was the study of expression of somatic-stem cells transition genes(TERT; OCT3; SMO; MYC; SNAI2; MOB3B; TGFBR1; KLF4; BMI1; VIM; FLT3; LAT; SMAD2; LMNB2; KLF1; TGFb). It was shown that in patients without metastases before treatment, 5 genes are overexpressed - OCT3; BMI1; LMNB2; TGFb1 и FLT3; in patients with metastases before treatment, 7 genes are overexpressed - OCT3; BMI1; LMNB2; TGFb1; TERT; SNAI2; TGFbR1. In the residual tumor of patients without hematogenous metastases, the frequency of overexpressed genes after NAC does not change. In patients with metastases after NAC in the residual tumor, 14 of 16 studied genes are overexpressed - except for KLF1 and SMAD2. At the same time, it was shown that, in overexpression in the residual tumor of the three OCT3, LAT and LMNB2 genes, 69% of patients (11/16) had hematogenous metastases. With the hypoexpression of at least one of these genes, the 5-year metastatic-free survival rate is 94% (34/36).

Conclusion. 1. The first results are indicative of the predictive and prognostic significance of the presence of amplification clones in the primary tumor in breast cancer patients. The appointment of NAC is most appropriate in patients with 2 or more clones in the primary tumor, while at 0-1 clone chemotherapy in preoperative mode is not appropriate. 2. A model for predicting the onset of hematogenous metastasis is obtained on the basis of an analysis of the expression of 16 somatic-stem cells transition genes. The sensitivity of the metastatic forecast is 69%, specificity 94%, accuracy of diagnostic 82%.

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