УДК 602.6/.9
https://doi.org: 10.20538/1682-0363-2018-3-217–228
For citation: Khlusov I.A., Litvinova L.S., Yurova K.A., Melashchenko E.S., Khaziakhmatova O.G., Shupletsova V.V., Khlusova M.Yu. Modeling of the mesenchymal stem cell microenvironment as a prospective approach to tissue bioengineering and regenerative medicine (a short review). *Bulletin of Siberian Medicine*. 2018; 17 (3): 217–228.

Modeling of the mesenchymal stem cell microenvironment as a prospective approach to tissue bioengineering and regenerative medicine (a short review)

Khlusov I. A.[1,2,3], Litvinova L. S.[3], Yurova K. A.[3], Melashchenko E. S.[3], Khaziakhmatova O. G.[3], Shupletsova V. V.[3], Khlusova M. Yu.[1]

[1] *Siberian State Medical University, 2, Moskow Tract, Tomsk, 634050, Russian Federation;*

[2] *National Research Tomsk State University, 36, Lenina Av., Tomsk, 634050, Russian Federation;*

[3] *Immanuel Kant Baltic Federal University, 14, Nevskogo str., Kaliningrad, 236041, Russian Federation*

ABSTRACT

One of the promising areas is the design and modification of materials for control of the fate of multipotent mesenchymal stromal cells (MMSCs) that will allow stroma of various human and animal organs and tissues to be constructed. However, the discussion of the existence and functioning of microenvironment for the MMSCs is just beginning to develop. The design of artificial materials that are able biomimetically to reproduce the cellular and tissue microenvironment are based on ideas and main elements borrowed from wildlife is the current direction of the development of medical materials technology and tissue bioengineering. Scaffold technology is a promising experimental approach to simulate the properties of natural microenvironment of stem cells. Our aim is a short review of key elements of MMSC microterritories, its advanced investigations and the attempts of modeling in application to tissue bioengineering and regenerative medicine.

Key words: stem cells, microenvironment, extracellular matrix, biomaterials, bone, bioengineering.

INTRODUCTION

The nature of the microenvironment and its interaction with stem cells is one of the fundamental questions in biology and medicine [1]. Grand aspirations exist in the field of cell technologies and tissue bioengineering for correction of diseases in cardiology, pulmonology, traumatology and orthopedics, and other biomedical segments. There are analyses of global market trends for stem cell applications, with data from 2016 and 2017, and projections of compound annual growth rates (CAGRs) through 2022 according to the reports of BCC Research LLC (USA, www.bccresearch.com) [2] and other marketing companies. Nevertheless, cell usage in a regime of cell therapy did not lead to proposed results, and a development of foreign market of regenerative medicine was previously delayed [3].

✉ *Khlusov Igor A.*, e-mail: khlusov63@mail.ru.

The first singular steps in tissue-engineer reconstruction were done with blood vessels, heart valves, esophagus, trachea, and bladder. In turn, reconstructive surgical interventions because of the lack of biocompatibility and longevity of endoprosthesis, are accompanied by the severe complications (thrombosis, infarct, stroke, etc.), that lead to disability and even death [4]. As a consequence, one of the anticipated trends is tool convergence of pharmacology, cell technologies, medical material science, and bioengineering to design *in vitro* models that push closer to the physiological conditions [5].

Due to a classification of [6] biomaterials evolution for tissue engineering is presented as follows: 1) First generation of implants on the basis of bio-inertness principle (a minimal interaction with an organism) began to develop in the 50s of the XX century; 2) Second generation on the basis of bioactivity (resorbable materials with the controlled interaction with physiological microenvironment) firstly appeared and have been developed in the 80s of the XX century; 3) Third generation began to develop in the XXI century for structural-functional tissues regeneration on the basis of stimulation of the specific cell response at the molecular level. In the given segment the implants with the functionalized surface and biomimetic structure/surface imitating natural extracellular matrix imitation (ECM) occur [7]. Currently, calcium phosphate (CP) materials and coatings imitating the mineral part of the bone are supposed to be more real prototypes of the ECM for MSCs [8].

Current trends lead to the following question [9]: What does the concept of the stem cell and MSC microenvironments truly mean today? It is very important, because MSCs form the stroma of any organ and tissue, and affect the activity of other stem cell types. Here, we summarized our short analysis concerning recent concepts and controversies related to the MSC microenvironment in mammals and its studies and prospects for tissue bioengineering and regenerative medicine.

MESENCHYMAL STEM CELLS AND THEIR MICROENVIRONMENT

A subset of colony-forming units of fibroblast-like cells (CFU-Fs), stromal stem cells [10] or MSCs [11] were first identified in the bone marrow, where they are present at approximately ten-fold higher concentrations than in the circulation, and can be obtained from all tissues with varying frequencies [12, 13]. These cells have been recently named skeletal stem cells [14].

The International Society for Cellular Therapy (ISCT) issued a nomenclature clarification that restricted the use of the term MSCs to cells that meet the stem cell criteria and recommended the term “multipotent mesenchymal stromal cells” (MMSCs) for fibroblast-like plastic adherent cells regardless of the tissue of origin [15]. The minimal criteria for defining MMSCs that are required by the ISCT are as follows: 1) adherence to a plastic surface; 2) the expression of the CD73, CD90, and CD105 antigens; 3) the lack of expression of CD45, CD34, CD14 or CD11b, and CD79a or CD19; 4) HLA-DR surface molecules; and 5) the ability to differentiate *in vitro* into the osteogenic, chondrogenic, and adipogenic lineages [16].

There is heterogeneity within the MSC population [15, 17, 18]. MSCs are widely distributed *in vivo*. There are central (bone marrow) and regional tissue-resident (amnion, placenta, adipose tissue, periosteum, synovial membrane, skeletal muscle, dermis, pericytes, blood, trabecular bone, umbilical cord, and lung) pools of MSCs [15, 19, 20] that respond to injury *via* cell homing and paracrine regulation. The MSC pool is a rare population (approximately 0.001%–0.01%) of the adult human bone marrow [21].

The primary role of MSCs as a hematopoietic inductive microenvironment (HIM) component is to support hematopoietic stem cell (HSC) survival, the maintenance of quiescence and differentiation, and the reparation of tissue injury [22] by providing growth factors, cell-cell interactions, and matrix proteins. This cell type has potential utility in regenerative

medicine. Mesenchymal cells have putative roles in maintaining tissue homeostasis and are increasingly recognized as components of the stem cell niche. Overall historical background and current understanding of MSCs and the HIM have been presented in detail [15]. A long list of biologically important paracrine and autocrine molecules produced by MSCs is permanently renewed [13, 23].

In addition, these cells seem to reside in their own bone marrow microterritories, where stromal stem cells are able to self-renew and generate mature conjunctive/stromal cell types [23]. A thorough discussion of the existence and function of an microterritory (niche) for MSC vital functions has started recently in the scientific literature. What are the microenvironment and the microterritory for MSCs? Do HSCs and MSCs share the same niche and exchange signals to drive proliferation and differentiation [23]? At present, these are unanswered questions.

There are difficulties with localizing the MSC anatomical sites (microterritoris) in the bone marrow cavity [13]. In this connection, MSCs were found lining blood vessels in the human bone marrow and dental pulp with the use of the markers Stro-1 and CD146 [24]. These cells also expressed α-smooth muscle actin (α-SMA), and some cells even expressed 3G5, a pericyte-associated cell-surface marker, as noted previously [25]. Doherty et al. (1999) hypothesized that pericytes are in fact MSCs because they can differentiate into osteoblasts, chondrocytes, and adipocytes [26]. Correspondingly, certain studies proposed a perivascular nature of the MSC niche on the basis of the expression of α-SMA in MSCs isolated from all tissue types tested [12].

The proliferative capacity of human MMSCs has been demonstrated to be better maintained *in vitro* under hypoxic versus normoxic conditions (2% and 20% oxygen, respectively) [27]. Kolf et al. (2007) suggested the hypoxia-induced enhancement of the proliferative capacity ("stemness") and plasticity of MMSCs [25]. In that case, the MMSC microterritories are thought to be located elsewhere, near the endosteum, with a hypoxic milieu. Indeed, our recent experiment revealed the *in vitro* osteogenic differentiation and maturation of human lung prenatal stromal cells (regional pool of MSCs) after brief contact with a rough CP scaffold simulating bone inorganic matrix [28].

Herein, the existence of a structural-functional hierarchy of MMSC niches may be speculated by analogy with HSC niches.

EXTRACELLULAR MATRIX

Key structural-functional components of the cell microenvironment include soluble factors, cell-cell contacts, and cell-matrix adhesions.

The well-known microenvironment structure of the bone marrow can be subdivided into mobile components (T lymphocytes, hormones, neurotransmitters, etc.) and resident stromal elements, which are fixed in certain regions to form their 'meshwork'. The morphological components of the microenvironment were defined previously [29], as follows: 1) blood vessels; 2) a tissue component consisting of resident and migrating cells that penetrate the ECM with fibers and soluble molecules; and 3) nerve terminals. Microenvironment components control stem cell fate via direct (cell-cell and cell-matrix) and indirect (soluble molecules) mechanisms. ECM tightly regulates growth factor distribution in space and time by binding and limiting their diffusion, modulating the effects of matrix and intercellular contacts [30]. ECM components include, in general, the following factors:

1) Fibrillar proteins that contain collagen, reticulin fibers, fibronectin, laminin, tenascin, hemonectin, and some other components of the filamentous network. Collagen is considered to play a key role among the proteins in the ECM by providing mechanical stability [31]. Fibrous ECM (e.g., fibronectin and laminin) binds cell transmembrane integrins through arginine-glycine-aspartic acid (RGD) sequences. This event promotes the interaction of integrins with the cytoskeleton at focal adhesion complexes (protein aggregates that include vinculin, α-actinin, and talin). In turn, this interaction can initiate

the production of intracellular messengers or directly mediate nuclear signals. Collectively, changes in cytoskeletal tension and cytosol components lead to shape transformation, locomotion, cell growth, and differentiation [7, 32]. The adhesive, anchorage, binding and epigenetic nuances of ECM glycoproteins and their integrin receptors have been presented in detail previously [7, 19, 33, 34].

2) Glycosaminoglycans (GAGs), primarily hyaluronic acid (HA), are a key component of the ECM. HA (40% of all GAGs in the bone marrow) [31] is a prominent ECM polymer in supporting human embryonic stem cell (ESC) growth in undifferentiated masses (i.e., embryoid bodies) [35]. HA plays a role as a modulator of the supportive function of the bone marrow niche via its receptor, CD44 (a multifunctional transmembrane protein), which is expressed by a wide variety of cells, including MSCs [13] and HSCs [36]. Chung et al. (2008) demonstrated the ability of HA hydrogels to promote the chondrogenic differentiation of encapsulated human MSCs [37].

3) Multiple soluble factors (e.g., cytokines, growth factors, and ions) in the ECM induce intracellular signal pathways by means of their membrane receptors or ion channels.

At the same time, no specific matrix components have been identified to date that help to maintain MSCs in their naпve state [25]. However, MSCs constitute a specific niche composed of ECM proteins with unique features. For instance, Djouad et al. (2007) hypothesized that the induction of MSC differentiation towards chondrocytes in the articular cartilage might be induced and/or influenced by molecules from the microenvironment. Therefore, a crosstalk between ECM components of the microenvironment and MSCs within the cartilage is responsible for the chondrogenic differentiation of MSCs [38]. ECM left by osteoblasts on titanium scaffolds after its decellularization increased osteogenesis markers, such as ALP and calcium deposition, in MSCs seeded [39]. ECM deposited by microvascular endothelial cells enhances MSC endotheliogenesis [25].

Bone-like ECM structure alone can regulate MSC differentiation into osteoblasts (see below). On this basis, ECM properties underlie MSC microterritories development, hierarchy and function, with potential applications for tissue engineering and regenerative medicine. More quantitative and molecular information about the ECM–MSC interaction is currently needed.

BONE AS A SPECIALIZED EXTRACELLULAR MATRIX FOR MSCS

Current estimates of the bone significance for MSCs are incomplete. There is evidence that bone ECM alone can regulate MSC differentiation, with potential applications for tissue engineering. The mineralization of synthetic scaffolds is known to be an effective technique for promoting cell adhesion, stimulating the osteogenic differentiation of MSCs and improving the osteointegration of implant materials [40].

An increasing ionic calcium concentration immediately arises around the region where osteoblasts and osteoclasts actively remodel bone. Bone cells, particularly osteoblasts, chondrocytes and osteoclasts, exhibit functional responses to ionic calcium (Ca^{2+}). Scadden (2007) hypothesized that stem cells may be able to recognize extracellular calcium content [41]. The CaR was recently found to facilitate the retention of HSCs on the endosteal bone surface. CaR [42] is a member of family of protein-coupled membrane receptors and does not serve as a channel for calcium transport but recognizes extracellular calcium. CaR responds to multiple extracellular cations (gadolinium and aluminum), with calcium as its major ligand [43].

Liu et al. (2009) established *in vitro* the significant effects of both extracellular Ca^{2+} and inorganic phosphate (Pi) levels on the growth and osteogenic differentiation of rabbit bone marrow-derived MSCs. Their results showed that the optimal extracellular Ca^{2+} and Pi concentrations for cell proliferation and differentiation were 1.8 mM and 0.09 mM, respective-

ly, which are the concentrations supplied in many commonly used culture media, such as DMEM and alpha-MEM. Greater Ca^{2+} concentrations did not change cell proliferation but significantly inhibited cell differentiation and enhanced cell mineralization. In turn, MSC proliferation and differentiation decreased significantly with higher or lower concentrations of the Pi supplement. Higher Pi concentrations also led to significant cell apoptosis [44].

In that way, the bone tissue undergoes throughout the life span a process of remodeling via a tight coupling between bone formation by osteoblasts (derived from MSCs) and bone resorption by osteoclasts (which are hematopoietic in origin) [45]. This process provides Ca^{2+} for the CaR on stem cells near the endosteal bone surface, as proposed by [41], and Pi, which has its own bioactivity.

In fact, not only ionic calcium but also phosphate, as products of the metabolism of the mineral bone matrix, could affect stem cells chemically. Ions with a positive or negative charge influence the value of the cellular zeta-potential that is connected to the cell (trans) membrane voltage. In turn, membrane hyperpolarization promotes the osteogenic (alkaline phosphatase gene expression and intracellular calcium levels) differentiation of human MSCs, unlike the depolarized state [46, 47].

Further, the bone topography changes permanently. There is a fatal disruption of preexisting osteoblastic niches at bone remodeling sites. Each site of trabecular or cortical bone is exposed to physiological remodeling every 3-10 years, on average [48]. Thus, MSC microenvironment cannot be stable structures, and their microterritories could appear *de novo*. Bone is a native substrate for marrow MSCs, and the surface topography of the mineralized bone surface critically affects cell fate [49].

Thus, we hypothesized distinct physical-chemical effects of smooth or rough bone (predominantly, its mineral matrix) surfaces on the quiescent or active status of the stem cells, respectively [50]. Blood mononuclear leukocytes (T cells, B cells, and monocytes) modulate bone turnover during health, stress and disease (for instance, osteogenesis imperfecta) [51, 52].

MSC MICROENVIRONMENT MODELING

Biomaterials are rapidly being developed to display and deliver stem cell-regulatory signals in a precise and near-physiological manner and to serve as powerful artificial microenvironments in which to study and instruct stem cell fate both in culture and *in vivo*. A synergism of cell biological and biomaterials technologies promises to have a profound impact on stem cell biology and provide insights that will advance stem cell-based clinical approaches to tissue bioengineering and regeneration [53]. The ability to better engineer an artificial ECM that can control cell behavior, through physical and molecular interactions, may further extend our capabilities in engineering tissue substitutes from adults or ESCs [7, 54].

The ECM from different tissues (bone, marrow, brain, cardiac muscle, etc.) is physically and chemically diverse. Herein, Wolff's law of bone growth and remodeling articulated in 1892 that changes in force applied to the bone result in changes in its structure, mass, and strength. This law can be applied to the interconnection between ECM structure and stem cell structure, function and commitment. Today, a set of key physicochemical parameters of artificial ECM that affect cells have already been identified, namely, architecture and mechanical properties [55], mechanical integrity, the rate of scaffold degradation, fluid transport [56], cell-recognizable surface chemical properties, the ability to induce signal transduction [57], surface free energy and wettability [58], surface electrical charge [59, 60], chemical composition [61], surface geometry, topography, and stiffness [62].

A wide variation in matrix stiffness is known to influence the focal adhesion structure and the cytoskeleton [63, 64]. Mechanotransduction is one recent direction for the study of stromal mechanocytes (fibroblasts and reticular cells) [65] and lineage progenitors [66].

Solid and soft tissues exhibit a range of stiffness values, as measured by the elastic modulus (E). Stem cells may possess more than the typical ensemble of force-coupled signaling pathways as a means of sensitizing themselves to three-dimensional (3D) microenvironments that range – physically – from flowing fluids and strained tissues to solid tissues of varied elasticity.

The neurogenic markers of MSCs have been determined to be highest on polyacrylamide gels with E = 0.1–1 kPa. When MSCs are grown on firm gels that mimic the elasticity of muscle (E = 8-17 kPa) and that are coated with collagen-I, myogenic markers are upregulated. When naпve MSCs are grown on rigid gels (E = 34 kPa) that mimic precalcified bone, the cells demonstrate an osteogenic origin [66]. The authors showed that soluble inductive factors tend to be less selective than matrix stiffness in driving specification and cannot reprogram MSCs that are precommitted for weeks on a given matrix.

Thus, carefully made biomimetic materials with well-defined quantitative parameters can prime the control of specific progenitors mediated by ECM epigenetic signals. Size control is important for minimizing gradients of oxygen and other physical or chemical factors that regulate stem cell fate [67]. For example, to control *in vitro* the area of human MSC growth with the help of micropatterned substrates, one group [68] printed two-dimensional (2D) fibronectin "islands" and cultured a single MSC on each island for 1 week. In proliferation-arrested MSCs, the authors found that adipogenesis was induced on small fibronectin islands (1024 μm^2), and osteogenesis was induced on large islands (10,000 μm^2, which maximize contractile anchorage). Cell shape alone was concluded to drive the commitment of human MSCs to the adipocyte or osteoblast fate by means of cytoskeletal tension.

Bone tissue growth in 3D porous CP ceramics has been observed *in vivo* at a pore diameter of 100-800 μm [69, 70], which corresponds to approximate section areas of 8000-500000 μm^2. Adhesive circular microdomains (100–400 μm diameter) on glass cover slips may be manufactured by a maskless photolithography system. Using this system, 200 μm circular domains were found to have the optimal diameter for the cardiac differentiation of murine stem cells in uniform-sized ESC aggregates [71].

Peerani et al. (2007) developed methods to culture human ESCs in defined microenvironments by micropatterning colonies on an extracellular matrix substrate (Matrigel™) printed with distinct features. Poly(dimethylsiloxane) stamps with a diameter (D) of circular features from 200 to 800 μm and a distance between circular features from 500 to 1000 μm were fabricated using standard soft lithography techniques. ESCs were seeded as single cells onto the patterned substrates and cultured without any exogenous growth factors. Decreased pluripotency (enhanced differentiation) of human ESCs was observed at *D* = 200 μm. Colony size has been speculated to change the ratio of perimeter to internal cells as well as the levels and distribution of mechanical stress within a colony to influence gene expression and paracrine signaling feedback [72]. The authors concluded that the results presented in this work demonstrated a role of niche size in human ESC self-renewal control.

De Barros et al. (2010) developed *in vitro* a complex multicellular 3D spheroid model with human bone MSCs, which were undifferentiated (noninduced) or induced to differentiate into osteoblasts for 1 week. After 4 days, the MSCs formed spheroid structures of an approximately 400 μm diameter (420 ± 23.7 μm; size correlated with 6 to 50 thousand plated cells) with a complex 3D network similar to that of reticular cells in the marrow cavity. Mixed spheroids had osteoinduced MSCs at the center and noninduced MSCs around the spheroid-simulated microenvironment near subendosteal region in the bone marrow. By surrounding osteoinduced spheroids with MSCs, the authors sought to mimic the trabecular bone that is also surrounded by reticular

cells of bone marrow. Hematopoietic $CD34^+$ progenitors with restrained proliferation and migration were located in close proximity to osteoinduced MSCs [73].

Since 1964, Curtis and Varde have provided important details about the effect of surface topography on cell behavior [74]. A wide range of cells (fibroblasts, osteoblasts, osteoclasts, nerve cells, MSCs, ESCs, etc.) respond to the surface micro- [74, 75] and nanotopography; the main influencing factor *in vitro* is the nanotopography rather than the microtopography [61, 76]. However, biomechanical forces led to another result in an *in vivo* subcutaneous test in mice. Only macroscale grooves of a 1 mm width and depth on a sub-microrough (average Ra < 500 nm) CP surface promoted donor bone marrow survival and bone/marrow system remodeling. No smooth nanorough CP scaffolds affected ectopic MSC growth [50]. Therefore, the pronounced 3D structure (texture) of the ECM has its own *in vivo* potential to direct stem cell fate [77].

Thus, material architecture can be used to model the ECM for MSCs. The key elements of a microenvironment are known, but the "cocktail" of biophysical mechanisms is not understood. The achieved results are not unequivocal, and different conditions for fabricating scaffoldings were recognized as possible factors for varying outcomes [78]. Nevertheless, designing artificial matrices that can mimic the quantitative parameters of the tissue microenvironment and regulate the appropriate differentiation of stem cells is a promising approach to therapeutic applications [25].

CONCLUSION

The ability to sustain stem cells and tissues inside and outside the body requires substantial improvement to advance clinical applications. Once isolated from tissues, stem cells rapidly lose their status, function and viability. The failure of stem cell function happens because the support network of the microenvironment (cell-cell contacts, contacts with matrices and captured insoluble adhesion proteins and support cells) no longer exists. It is better to support cells inside privileged microenvironments, where they do not lose their special characteristics and where their specialization can be programmed, maintained, and regulated *in vitro* and *in vivo* for a long time.

The key concept is that a biomaterial surface and volume can contain specific chemical and structural information that controls tissue formation, in a manner analogous to cell-cell communication and patterning during embryological development [7]. The successful application of stem cells in the bioengineering of new tissues and the recovery of damaged tissues is dependent on the use of an appropriate scaffold to maintain native 3D cell distribution and on the use of specific molecules to drive tissue-specific ECM development [79].

Individualized artificial microenvironment may help to overcome the problems of cell-based tissue bioengineering and regenerative medicine. A prolific strategy has been to use the ECM directly, with its cells removed. Alternatively, copies of the ECM structure and function are made with biomaterials. The most promising substrates that increasingly match native ECMs are protein polymers and synthetic polymers with oligopeptide additions, adhesion receptors, and soluble and insoluble ligands that increase cell interactions and stimulate natural tissue remodeling [80, 81].

A promising strategy is to define the size of native structural-functional microterritories to model step by step the physicochemical and epigenetic features that influence the stem cell developmental program.

CONFLICT OF INTEREST

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

SOURCE OF FINANCING

This investigation was supported by the Federal Target Program of the Ministry of Education and Science of the Russian Federation (agreement 14.575.21.0164, ID number RFMEFI57517X0164) "Research and innovations in priority areas of development of Russian scientific and technological complex for 2014-2020".

REFERENCES

1. Terskikh V.V., Vasiliev A.V., Vorotelyak E.A. Stem Cell Niches. *Biology Bull*. 2007; 34 (3): 211–220. DOI: 10.1134/S1062359007030016.
2. The global market for stem cells (Report ID: 2274672, March 2018): 162 p. Available from: https://www.reportbuyer.com/product/2274672/the-global-market-for-stem-cells.html; last visit 12.05.2018.
3. Zorin V.L., Zorina A.I., Cherkasov V.R. Analysis of the overseas market of regenerative medicine. *Kletochnaya transplantologiya i tkanevaya inzheneriya*. 2009; 4: 68–78 (in Russ.).
4. Holmes D.R. Jr. State of the art in coronary intervention. *Am J Cardiol*. 2003; 91: 50A–53A.
5. Schrattenholz A., Klemm M. How human embryonic cell research can impact in-vitro drug screening technologies of the future. Drug Testing In Vitro: Breakthroughs and Trends in Cell Culture Technology. Weinheim: WILEY-VCH Verlag Gmbh Co., 2007: 205–228.
6. Rabkin E., Schoen F.J. Cardiovascular tissue engineering. *Cardiovasc. Pathol*. 2002; 11 (6): 305–317. https://doi.org/10.1016/S1054-8807(02)00130-8.
7. Ratner B.D., Hoffman A.S., Schoen F.J., Lemons J.E. Biomaterials Science: an introduction to Materials in Medicine. 2nd ed. San Diego: Elsevier Academic Press, 2004: 864.
8. Khlusov I.A., Shevtsova N.M., Khlusova M.Y. Detection in vitro and quantitative estimation of artificial microterritories which promote osteogenic differentiation and maturation of stromal stem cells. *Methods Mol. Biol*. 2013; 1035: 103–119. DOI: 10.1007/978-1-62703-508-8_9.
9. Lander A.D., Kimble J., Clevers H., Fuchs E., Montarras D., Buckingham M., Calof A.L., Trumpp A., Oskarsson T. What does the concept of the stem cell niche really mean today? *BMC Biology*. 2012; 10: 19. DOI: 10.1186/1741-7007-10-19.
10. Owen M., Friedenstein A.J. Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found. Symp.* 1988; 136: 42–60.
11. Caplan A.I. Mesenchymal stem cells. *J. Orthop. Res.* 1991; 9: 641–650.
12. Da Silva Meirelles L., Chagastelles P.C., Nardi N.B. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J. Cell Sci.* 2006; 119: 2204–2213. DOI: 10.1242/jcs.02932
13. Aerts F., Wagemaker G. Mesenchymal stem cell engineering and transplantation. In: Nolta J.A., Ed. Genetic engineering of mesenchymal stem cells. The Netherlands: Springer, 2006: 1–44. DOI: 10.1007/1-4020-3959-X_1.
14. Bianco P., Robey P.G. Skeletal stem cells. In: Lanza R., Ed. Handbook of Stem Cells. V.2. New York: Academic Press. 2004: 415–424. DOI:10.1016/B978-012436643-5/50129-2.
15. Kfoury Y., David T., Scadden D.T. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell*. 2015; 16: 239–253. http://dx.doi.org/10.1016/j.stem.2015.02.019.
16. Dominici M., Le Blanc K., Mueller I., Slaper-Cortenbach I., Marini F., Krause D., Deans R., Keating A., Prockop Dj., Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8: 315–317. DOI: 10.1080/14653240600855905.
17. Han W., Yu Y., Liu X.Y. Local signals in stem cell-based bone marrow regeneration. *Cell Research*. 2006; 16: 189–195. DOI:10.1038/sj.cr.7310026.
18. Ho A.D., Wagner W., Franke W. Heterogeneity of mesenchymal stromal cell preparations. *Cytotherapy*. 2008; 10: 320–330. DOI: 10.1080/14653240802217011.
19. Docheva D., Popov C., Mutschler W., Schieker M. Human mesenchymal stem cells in contact with their environment: surface characteristics and the integrin system. *J. Cell Mol. Med*. 2007; 11 (1): 21–38. DOI: 10.1111/j.1582-4934.2007.00001.x.
20. Armstrong L., Lako M., Buckley N., Lappin T.R., Murphy M.J., Nolta J.A., Pittenger M., Stojkovic M. Editorial: Our Top 10 Developments in Stem Cell Biology over the Last 30 Years. *Stem sells*. 2012; 30: 2–9. DOI: 10.1002/stem.1007.
21. Pittenger M.F., Mackay A.M., Beck S.C., Marshak D.R. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284: 143–147. DOI: 10.1126/science.284.5411.143.
22. Tuljapurkar S.R., Jackson J.D., Brusnahan S.K., O'Kane B.J., Sharp J.G. Characterization of a mesenchymal stem cell line that differentiates to bone and provides niches supporting mouse and human hematopoietic stem cells. *Stem Cell Discovery*. 2012; 2(1): 5–14. DOI:10.4236/scd.2012.21002.
23. Nardi N.B., da Silva Meirelles L. Mesenchymal stem cells: isolation, in vitro expansion and characterization. *Handb. Exp. Pharmacol*. 2006; 174: 249–282.
24. Shi S., Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J. Bone Miner. Res. 2003; 18: 696–704. DOI: 10.1359/jbmr.2003.18.4.696
25. Kolf C.M., Cho E., Tuan R.S. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. *Arthritis Res. Ther*. 2007; 9 (1): 204–219. DOI: 10.1186/ar2116.
26. Doherty M.J., Canfield A.E. Gene expression during vascular pericyte differentiation. *Crit. Rev. Eukaryot Gene Expr*. 1999; 9: 1–17.
27. Grayson W.L., Zhao F., Izadpanah R., Bunnell B., Ma T. Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs. *J. Cell Physiol*. 2006; 207: 331–339. DOI: 10.1002/jcp.20571.
28. Khlusov IA, Khlusova MYu, Zaitsev K.V., Kolokol'tsova T.D., Sharkeev Yu.P., Pichugin V.F., Legostaeva E.V., Trofimova I.E., Klimov A. S., Zhdanova A. I. Pilot *in vitro* study of the parameters of artificial niche for osteogenic differentiation of human stromal stem cell pool. *Bull. Exp. Biol. Med*. 2011; 150 (4): 535–542. DOI: 10.1007/s10517-011-1184-4.

29. Tavassoli M. Studies on hemopoietic microenvironments. *Exp Hematol*. 1975; 3: 213-26.
30. Discher D.E., Mooney D.J., Zandstra P.W. Growth factors, matrices, and forces combine and control stem cells. *Scici*. 2009; 324 (5935): 1673–1677. DOI: 10.1126/science.1171643.
31. Dygai A.M., Zhdanov V.V. Theory of hematopoiesis control. In: SpringerBriefs in Cell Biology. V.5. Switzerland: Springer International Publishing, 2014: 1–93.
32. Ingber D.E. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ. Res*. 2002; 91: 877–887.
33. Taichman R.S. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. *Blood*. 2005; 105 (7): 2631–2639. DOI: 10.1182/blood-2004-06-2480.
34. Stabenfeldt S.E., Brown A.C., Barker T.H. Engineering ECM complexity into biomaterials for directing cell fate. In: Gefen Amit, Ed. Studies in Mechanobiology, Tissue Engineering and Biomaterials. V.2. Roy K., Ed. Biomaterials as Stem Cell Niche. Berlin Heidelberg: Springer-Verlag, 2010: 1-18. DOI: 10,1007/8415_2010_32.
35. Gerecht S., Burdick J.A., Ferreira L.S., Townsend S.A., Langer R., Vunjak-Novakovic G. Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. *Proc. Natl. Acad. Sci. USA*. 2007; 104 (27): 11298–11303. DOI: 10.1073/pnas.0703723104.
36. Iwasaki H., Suda T. Hematopoietic stem cells and their niche. In: Kondo M., Ed. Hematopoietic Stem Cell Biology, Stem Cell Biology and Regenerative Medicine. New York: Humana Press, 2010: 37–55. DOI: 10.1007/978-1-60327-347-3.
37. Chung C., Burdick J.A. Influence of three-dimensional hyaluronic acid microenvironments on mesenchymal stem cell chondrogenesis. *Tissue Eng. Part A*. 2009; 15 (2): 243–254. DOI: 10.1089/ten.tea.2008.0067.
38. Djouad F., Delorme B., Maurice M., Bony C., Apparailly F., Louis-Plence P., Canovas F., Charbord P., Noλl D., Jorgensen C. Microenvironmental changes during differentiation of mesenchymal stem cells towards chondrocytes. *Arthritis Res. Ther*. 2007; 9 (2): R33. DOI: 10.1186/ar2153.
39. Datta N., Holtorf H.L., Sikavitsas V.I., Jansen J.A., Mikos A.G. Effect of bone extracellular matrix synthesized in vitro on the osteoblastic differentiation of marrow stromal cells. *Biomaterials*. 2005; **26 (9)**: 971–977. DOI: 10.1016/j.biomaterials.2004.04.001.
40. Phadke A., Chang C.-W., Varghese S. Functional biomaterials for controlling stem cell differentiation. In: Gefen Amit, Ed. Studies in Mechanobiology, Tissue Engineering and Biomaterials. V.2. Roy K., Ed. Biomaterials as Stem Cell Niche. Berlin Heidelberg: Springer-Verlag, 2010: 19–44.
41. Scadden D.T. The stem cell niche in health and leukemic disease. *Best Pract. Res. Clin. Haematol*. 2007; 20 (1): 19–27. DOI: 10.1016/j.beha.2006.11.001.
42. McNeil S.E., Hobson S.A., Nipper V., Rodland K.D. Functional calcium-sensing receptors in rat fibroblasts are required for activation of SRC kinase and mitogen-activated protein kinase in response to extracellular calcium. *J. Biol. Chem*. 1998; 273: 1114–20.
43. Theman T.A., Collins M.T. The Role of the calcium-sensing receptor in bone biology and pathophysiology. *Curr. Pharm. Biotechnol*. 2009; 10 (3): 289–301.
44. Liu Y.K., Lu Q.Z., Pei R., Ji H.J., Zhou G.S., Zhao X.L., Tang R.K., Zhang M. The effect of extracellular calcium and inorganic phosphate on the growth and osteogenic differentiation of mesenchymal stem cells in vitro: implication for bone tissue engineering. *Biomed. Mater*. 2009; 4 (2): 025004. DOI: 10.1088/1748-6041/4/2/025004.
45. Purton L.E., Scadden D.T. The hematopoietic stem cell niche. In: The Stem Cell Research Community ed. StemBook. Cambridge (MA): Harvard Stem Cell Institute, 2008. Available from: doi/10.3824/stembook.1.28.1.
46. Sundelacruz S., Levin M., Kaplan D.L. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. *PLoS One*. 2008; 3 (11): e3737. DOI: 10.1371/journal.pone.0003737.
47. Sundelacruz S., Levin M., Kaplan D.L. Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Rev*. 2009; 5: 231–246. DOI: 10.1007/s12015-009-9080-2.
48. Riggs B.L., Melton III L.J. Osteoporosis: Etiology, diagnosis, and management. 2nd ed. Philadelphia. New York: Lippincott-Raven Publ, 1995: 524.
49. Dellatore S.M., Garsia A.S., Miller W.M. Mimicking stem cell niches to increase stem cell expansion. *Curr. Opin. Biotechnol*. 2008; 19 (5): 534–540. DOI: 10.1016/j.copbio.2008.07.010.
50. Khlusov IA, Shevtsova NM, Khlusova MYu, Zaytsev K.V., Sharkeev Y.P., Pichugin V.F., Legodtaeva E.V. Niche-relief conception for stem cells as a basis of biomimetic approach to bone and hemopoietic tissues engineering. *Cellular Transplantation and Tissue Engineering*. 2011; 6 (2): 55–64.
51. Pacifici R. The immune system and bone. *Arch. Biochem. Biophys*. 2010; 503 (1): 41–53. DOI: 10.1016/j.abb.2010.05.027.
52. Saprina T., Khlusov I., Borodulina A. Change of properties of mononuclear leukocytes at patients with an osteogenesis imperfecta after operative treatment with using nanosized hydroxylapatite coatings. *Bone*. 2010; 46 (Suppl.1): S69–70.
53. Lutolf M.P., Gilbert P.M., Blau H.M. Designing materials to direct stem-cell fate. *Nature*. 2009; 462: 433–441. DOI: 10.1038/nature08602.
54. Metallo C.M., Mohr J.C., Detzel C.J., de Pablo J.J., Van Wie B.J., Palecek S.P. Engineering the stem cell microenvironment. *Biotechnol. Prog*. 2007; 23: 18–23. DOI: 10.1021/bp060350a.
55. Saha K., Pollock J.F., Schaffer D.V., Healy K.E. Designing synthetic materials to control stem cell phenotype. *Curr. Opin. Chem. Biol*. 2007; 11: 381–387. DOI: 10.1016/j.cbpa.2007.05.030.

56. Kobel S., Lutolf M.P. Biomaterials meet microfluidics: building the next generation of artificial niches. Curr. Opin. Biotechnol. 2011; 22(5): 690–697. DOI: 10.1016/j.copbio.2011.07.001.
57. Dawson E., Mapili G., Erickson K., Taqvi S., Roy K. Biomaterials for stem cell differentiation. *Adv. Drug Deliv. Rev.* 2008; 60: 215–228. DOI: 10.1016/j.addr.2007.08.037.
58. Ponsonnet L., Reybier K., Jaffrezic N., Comte V., Lagneau C., Lissac M., Martelet C. Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behavior. *Mater. Sci. Eng. C.* 2003; 23(4): 551–560. DOI: 10.1016/S0928-4931(03)00033-X.
59. Ueshima M., Tanaka S., Nakamura S., Yamashita K. Manipulation of bacterial adhesion and proliferation by surface charges of electrically polarized Hydroxyapatite. *J. Biomed. Mater. Res.* 2002; 60: 578–584. DOI: 10.1002/jbm.10113.
60. Dekhtyar Yu., Dvornichenko M.V., Karlov A.V., Khlusov I.A., Polyaka N., Sammons R., Zaytsev K.V. Electrically functionalized hydroxyapatite and calcium phosphate surfaces to enhance immobilization and proliferation of osteoblasts in vitro and modulate osteogenesis *in vivo*. IFMBE Proc. 2009; 25/10: 245–248. https://doi.org/10.1007/978-3-642-03900-3_70.
61. Martinez E., Engel E., Planell J.A., Samitier J. Effects of artificial micro- and nano-structured surfaces on cell behaviour. *Ann. Anat.* 2009; 191: 126–135. DOI: 10.1016/j.aanat.2008.05.006.
62. Yu L.M.Y., Leipzig N.D., Shoichet M.S. Promoting neuron adhesion and growth. *Materials Today.* 2008; 11: 36–43. DOI: 10.1016/S1369-7021(08)70088-9.
63. Bershadsky A.D., Balaban N.Q., Geiger B. Adhesion-dependent cell mechanosensitivity. *Annu. Rev. Cell Dev. Biol.* 2003; 19: 677–695. DOI: 10.1146/annurev.cellbio.19.111301.153011.
64. Discher D.E., Janmey P.A., Wang Y.L. Tissue cells feel and respond to the stiffness of their substrate. *Science.* 2005; 310: 1139–1143. DOI: 10.1126/science.1116995.
65. Tomasek J.J., Gabbiani G., Hinz B., Chaponnier C., Brown R.A. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* 2002; 3: 349–363. DOI: 10.1038/nrm809.
66. Engler A.J., Sen S., Sweeney H.L., Discher D.E. Matrix elasticity directs stem cell lineage specification. *Cell.* 2006; 126 (4): 677–689. DOI: 10.1016/j.cell.2006.06.044.
67. Parmar K., Mauch P., Vergilio J.A., Sackstein R., Down J.D. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc. Natl. Acad. Sci. USA.* 2007; 104 (13): 5431–5436. DOI: 10.1073/pnas.0701152104.
68. McBeath R., Pirone D.M., Nelson C.M., Bhadriraju K., Chen C.S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell.* 2004; 6 (4): 483–495.
69. Gauthier O., Bouler J.-M., Aguado E., Pilet P., Daculsi G. Macroporous biphasic calcium phosphate ceramics: influence macropore diameter and macroporosity percentage on bone ingrowth. *Biomaterials.* 1998; 19 (1-3): 133–139. DOI: 10.1016/S0142-9612(97)00180-4.
70. Sous M., Bareille R., Rouais F., Clement D., Amedee J., Dupuy B. Cellular biocompatibility and resistance to compression of macroporous beta-tricalcium phosphate ceramics. *Biomaterials.* 1998; 19: 2147–2153. DOI: 10.1016/S0142-9612(98)00118-5.
71. Sasaki D., Shimizu T., Masuda S., Kobayashi J., Itoga K., Tsuda Y., Yamashita J.K., Yamato M., Okano T. Mass preparation of size-controlled mouse embryonic stem cell aggregates and induction of cardiac differentiation by cell patterning method. *Biomaterials.* 2009; 30 (26): 4384–4389. DOI: 10.1016/j.biomaterials.2009.05.003.
72. Peerani R., Rao B.M., Bauwens C., Yin T., Wood G.A., Nagy A., Kumacheva E., Zandstra P.W. Niche-mediated control of human embryonic stem cell self-renewal and differentiation. *EMBO J.* 2007; 26 (22): 4744–4755. DOI: 10.1038/sj.emboj.7601896.
73. de Barros A.P.D.N., Takiya C.M., Garzoni L.R., Leal-Ferreira M.L., Dutra H.S., Chiarini L.B., Meirelles M.N., Borojevic R., Rossi M.I.D. Osteoblasts and bone marrow mesenchemal stromal cells control hematopoietic stem cell migration and proliferation in 3D in vitro model. *PLoS One.* 2010; 5 (2): e9093-9111. DOI: 10.1371/journal.pone.0009093.
74. Curtis A.S.G., Wilkinson C. Topographical control of cells. *Biomaterials.* 1998; 18: 1573–1583.
75. Meyer U., Buchter A., Wiesmann H.P., Joos U., Jones D.B. Basic reactions of osteoblasts on structured material surface. *Eur. Cells Mat.* 2005; 9: 39–49.
76. Yim E.K., Pang S.W., Leong K.W. Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Exp. Cell Res.* 2007; 313 (9): 1820–1829. DOI: 10.1016/j.yexcr.2007.02.031.
77. Jing D., Fonseca A.-V., Alakel N., Fierro F.A., Muller K., Bornhauser M., Ehninger G., Corbeil D., Ordemann R. Hematopoietic stem cells in co-culture with mesenchymal stromal cells – modeling the niche compartments in vitro. *Haematologica.* 2010; 95: 542–550. DOI: 10.3324/haematol.2009.010736.
78. Klein C., de Groot K., Chen W., Li Y., Zhang X. Osseous substance formation induced in porous calcium phosphate ceramics in soft tissues. *Biomaterials.* 1994; 15: 31–34. DOI: 10.1016/0142-9612(94)90193-7.
79. Huang J.I., Yoo J.U., Goldberg V.M. Orthopaedic applications of stem cells. In: Blau H, Melton D, Moore M. Eds. Handbook of stem cells. V.2. New York: Elsevier Inc., 2004: 773–784. DOI: 10.1016/B978-012436643-5/50160-7.
80. Gattazzo F., Urciuolo A., Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim. Biophys. Acta.* 2014; 1840 (8): 2506–2519. DOI: 10.1016/j.bbagen.2014.01.010
81. Asada N., Takeishi S., Frenette P.S. Complexity of bone marrow hematopoietic stem cell niche. *Int J Hematol.* 2017; 106 (1): 45–54. DOI: 10.1007/s12185-017-2262-

Received 18.04.2018
Accepted 15.05.2018

Khlusov Igor A., DM, Professor, Department of Morphology and General Pathology, SSMU; NR TSU, Tomsk; Immanuel Kant BFU, Kaliningrad, Russian Federation. ORCID: http://orcid.org/0000-0003-3465-8452.
Litvinova Larisa S., DM, Head of Base Laboratory Immunology and Cell Biotechnology, Immanuel Kant BFU, Kaliningrad, Russian Federation. ORCID: https://orcid.org/0000-0001-5231-6910.
Yurova Kristina A., PhD, Researcher, Base Laboratory Immunology and Cell Biotechnology, Immanuel Kant BFU, Kaliningrad, Russian Federation. ORCID: http://orcid.org/0000-0001-6146-3330.
Melashchenko Elena S., Laboratory Assistant, Base Laboratory Immunology and Cell Biotechnology, Immanuel Kant BFU, Kaliningrad, Russian Federation. ORCID: http://orcid.org/0000-0002-8987-0664.
Khaziakhmatova Olga G., PhD, Researcher, Base Laboratory Immunology and Cell Biotechnology, Immanuel Kant BFU, Kaliningrad, Russian Federation. ORCID: http://orcid.org/0000-0002-5525-3529.
Shupletsova Valeria V., PhD, Researcher, Laboratory Immunology and Cell Biotechnology, Immanuel Kant BFU, Kaliningrad, Russian Federation.
Khlusova Marina Yu., PhD, Assistant Professor, Department of Pathophysiology, SSMU, Tomsk, Russian Federation. ORCID: http://orcid.org/0000-0001-9583-5358.

(✉) **Khlusov Igor A.**, e-mail: khlusov63@mail.ru.

УДК 602.6/.9
https://doi.org: 10.20538/1682-0363-2018-3-217–228
Для цитирования: Хлусов И.А., Литвинова Л.С., Юрова К.А., Мелащенко Е.С., Хазиахматова О.Г., Шуплецова В.В., Хлусова М.Ю. Моделирование микроокружения мезенхимных стволовых клеток как перспективный подход к тканевой инженерии и регенеративной медицине (краткий обзор). *Бюллетень сибирской медицины*. 2018; 17 (3): 217–228.

Моделирование микроокружения мезенхимных стволовых клеток как перспективный подход к тканевой инженерии и регенеративной медицине (краткий обзор)

Хлусов И.А.[1, 2, 3], Литвинова Л.С.[3], Юрова К.А.[3], Мелащенко Е.С.[3], Хазиахматова О.Г.[3], Шуплецова В.В.[3], Хлусова М.Ю.[1]

[1] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

[2] *Национальный исследовательский Томский государственный университет (НИ ТГУ)*
Россия, 634050, г. Томск, пр. Ленина, 36

[3] *Балтийский федеральный университет имени (БФУ) И. Канта*
Россия, 236041, г. Калининград, ул. А. Невского, 14

РЕЗЮМЕ

Одним из перспективных направлений являются разработка и модификация материалов для контроля жизнедеятельности мультипотентных мезенхимных стромальных клеток (ММСК), которые (ре)конструируют строму различных органов и тканей человека и животных. Тем не менее обсуждение вопроса о существовании и функционировании микроокружения ММСК только начинается. Это тормозит дальнейшее развитие клеточной биологии и тканевой инженерии. Дизайн искусственных материалов, способных к биомиметическому воспроизведению клеточного и тканевого микроокружения, основанный на идеях и элементах, заимствованных у природы, является современным направлением в развитии медицинского материаловедения и тканевой инженерии. Скеффолд-технологии – многообещающий экспериментальный подход к моделированию свойств природного микроокружения для стволовых клеток.

Цель – краткий обзор ключевых элементов микротерриторий ММСК, его перспективных исследований и попыток моделирования в приложении к тканевой инженерии и регенеративной медицине.

Ключевые слова: стволовые клетки, микроокружение, экстрацеллюлярный матрикс, биоматериалы, кость, биоинженерия.

КОНФЛИКТ ИНТЕРЕСОВ

Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

ИСТОЧНИК ФИНАНСИРОВАНИЯ

Исследование выполнено при финансовой поддержке Минобрнауки России, соглашение № 14.575.21.0164 от 26.09.17 (уникальный идентификатор RFMEFI57517X0164) в рамках Федеральной целевой программы «Исследования и разработки по приоритетным направлениям развития научно-технологического комплекса России на 2014–2020 годы».

Поступила в редакцию 18.04.2018
Подписана в печать 15.05.2018

Хлусов Игорь Альбертович, д-р мед. наук, профессор, кафедра морфологии и общей патологии, СибГМУ; ст. науч. сотрудник, НИ ТГУ, г. Томск; профессор-исследователь, БФУ им. И. Канта, г. Калининград. ORCID: http://orcid.org/0000-0003-3465-8452.
Литвинова Лариса Сергеевна, д-р мед. наук, зав. базовой лабораторией иммунологии и клеточных биотехнологий, БФУ им. И. Канта, г. Калининград. ORCID: https://orcid.org/0000-0001-5231-6910.
Юрова Кристина Алексеевна, канд. мед. наук, науч. сотрудник, базовая лаборатория иммунологии и клеточных биотехнологий, БФУ им. И. Канта, г. Калининград. ORCID: http://orcid.org/0000-0001-6146-3330.
Мелащенко Елена Сергеевна, аспирант, лаборант, базовая лаборатория иммунологии и клеточных биотехнологий, БФУ им. И. Канта, г. Калининград. ORCID: http://orcid.org/0000-0002-8987-0664.
Хазиахматова Ольга Геннадьевна, канд. биол. наук, науч. сотрудник, базовая лаборатория иммунологии и клеточных биотехнологий, БФУ им. И. Канта, г. Калининград. ORCID: http://orcid.org/0000-0002-5525-3529.
Шуплецова Валерия Владимировна, канд. биол. наук, науч. сотрудник, базовая лаборатория иммунологии и клеточных биотехнологий, БФУ им. И. Канта, г. Калининград.
Хлусова Марина Юрьевна, канд. мед. наук, доцент, кафедра патофизиологии, СибГМУ, г. Томск. ORCID: http://orcid.org/0000-0001-9583-5358.

(✉) **Хлусов Игорь Альбертович**, e-mail: khlusov63@mail.ru.