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The state and vascularization of the bone marrow transplanted in the diffusion chamber to the rat neurovascular bundle

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ABSTRACT

Background. The diffusion chamber method helps solve the problem of delivering a biomaterial with minimal losses, while creating an isolated environment in the recipient's body. The issue of vascularization of diffusion chambers to preserve the functional capacity of the biomaterial remains relevant. A bioengineered diffusion chamber model, together with the vascular adventitia, promotes vascularization of the biomaterial placed in the chamber.

The **aim** of the study was to assess the state of the bone marrow placed in the diffusion chamber and transplanted to the femoral neurovascular bundle of a rat.

Materials and methods. The experimental part of the study was carried out on mature male Wistar rats. The animals were divided into two groups. Group 1 was experimental (n = 4), in which a polycaprolactone diffusion chamber filled with bone marrow was implanted in the femoral neurovascular bundle. Group 2 was control (n = 3), in which the diffusion chamber without bone marrow was implanted in a similar bundle.

Results. The histologic examination of the structure of the compact capsule in the bioengineered model in the experimental group revealed areas of woven bone tissue in 25% of the rats. An increase in the vascularization coefficient by 96% and a rise in the Kernohan index by 7% in the experimental group compared to the control group indicated that sufficient conditions were formed to develop the microvasculature while maintaining the bone marrow differentiation path.

Conclusion. The reliability of these results is confirmed by immunohistochemical markers of vascularization VEGF and CD34.

Keywords: diffusion chambers, biodegradable polymer, cell technologies, bone marrow transplant, microfluidic technologies, laboratory rats

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Состояние и васкуляризация костного мозга при имплантации в диффузионной камере на сосудисто-нервный пучок

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РЕЗЮМЕ

Введение. Метод диффузионных камер способствует решению задачи по доставке биологического трансплантата с минимальными потерями, создавая при этом условия изоляции в организме реципиента. Актуальным остается вопрос васкуляризации диффузионных камер для сохранения функциональности биоматериала. Модель биоинженерной конструкции диффузионной камеры в совокупности с адвентицией сосуда способствует васкуляризации биоматериала, помещенного в камеру.

Целью исследования явилась оценка состояния сингенного костного мозга, имплантированного в диффузионной камере на бедренный сосудисто-нервный пучок крысы.

Материалы и методы. Дизайн исследования включал в себя экспериментальную часть, проводимую на половозрелых самцах крыс линии Вистар. Животные были разделены на две группы: 1-я – экспериментальная (n = 4), имплантация диффузионной камеры из поликапролактона на бедренный сосудисто-нервный пучок с костным мозгом; 2-я – контрольная (n = 3), на аналогичный пучок имплантировалась камера без содержимого.

Результаты. При гистологическом исследовании в структуре компактной капсулы биоинженерной конструкции в экспериментальной группе выявлены участки грубоволокнистой костной ткани у 25% крыс. Повышение коэффициента васкуляризации на 96% и индекса Керногана на 7% в экспериментальной группе по сравнению с контрольной свидетельствует о формировании достаточных условий для развития микроциркуляторного русла при сохранении направления дифференцировки костного мозга.

Заключение. Достоверность приведенных результатов подтверждается иммуногистохимическими маркерами васкуляризации VEGF и CD34.

Ключевые слова: диффузионные камеры, биодеградируемый полимер, клеточные технологии, имплантация костного мозга, микрофлюидные технологии, лабораторные крысы

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

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INTRODUCTION

The use of the diffusion chamber (DC) is associated with a loss of the volume and biological properties of the cellular material as well as with the possibility of expanding the range of possible implantation. The search for methods for isolating cellular biomaterial is relevant due to high percentage of cellular biomaterial loss despite a high number of mesenchymal stem cells (MSC) in the bone marrow. The complexity of bone marrow transplant is associated with the necessity to imitate bone marrow hematopoietic niche (vascular, endosteal) as a physiological microenvironment [1]. That is why understanding the difficulties of the implantation process contributed to the progress in the development of 3D constructs with the possibility of immobilizing proliferation and differentiation factors [2] and programming hypoxic gradient [3].

Bone marrow vascularization associated with the expansion of the vascular niche is considered to

be the key problem in its functionality. Studies have noted the association between changes in bone marrow microcirculation and the progression of hematologic tumors as well as solid cancer [4]. The features of bone marrow vascularization present an opportunity for *in vitro* microfluidic technologies to generate separate compartments of cell and molecular signals in one biomaterial [5]. In addition, maintenance of bone marrow stem cells is regulated by different types of blood vessels with different permeability properties [6]

Researchers who attempted to consider the listed parameters in the *in vitro* system succeeded in creating the first vascularized bone-marrow-on-a-chip models [7]. It is known that new capillaries can grow not just in the process of neoangiogenesis, but also via the vascularization mechanism. The adventitia of major vessels is a depo for progenitor cells that participate in the microvasculature regeneration in response to a wide range of pathological stimuli. In addition, *vasa vasorum* has a range of molecular cell factors that initiate vasculogenesis [8]. The listed above allows to consider the bioengineered DC and vascular adventitia as an element of an experimental model of *in situ* vascularization of a syngeneic bone marrow transplant.

The **aim** of the study was to assess the state of the bone marrow placed in the 3D DC and transplanted to the femoral neurovascular bundle (FNB) of a rat.

MATERIALS AND METHODS

In this study, a bioengineered DC was designed in the form of a closed polymer capsule with a removable lid, latches, and the possibility to fill the cavity with cellular material (Fig. 1). The 3D-model of the DC (Fig. 1, *b*) was designed in the open source software environment Blender. The experimental samples (Fig. 1, *a*) were obtained by fused filament fabrication (FFF) on the CreatBot Duo 3D printer (CreatBot 3D Printer, China). End-walls of the DC have recesses to fix the construct to the vessel. The DC was made of polycaprolactone (Natural Works Ingeo 40–43d NatureWorks LLC) that is a biodegradable polyester approved for medical use with a low melting point (59–64 °) [9].

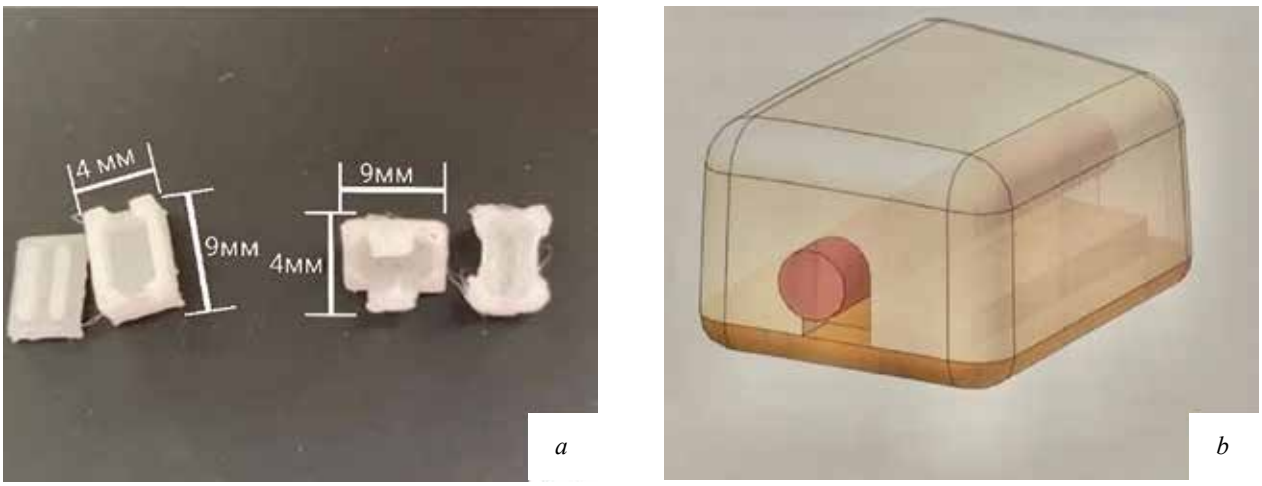

Fig. 1. 3D-printed construct (*a*) based on the 3D diffusion chamber model (*b*)

Sterilization of DC was done in 100% ethylene oxide vapor at 37 ˚C for 9 hours in the gas sterilizer 3M Steri-Vac Sterilizer/Aerator (3M, USA) according to the recommendations of GOST ISO 11135-2017 [10]. The study was conducted on adult male Wistar rats weighing 280–300 g. The animals were held in standard vivarium conditions in the Laboratory of Biological Models of Siberian State Medical University (Tomsk). The animals were divided into two groups. Group 1 was experimental (n = 4), in which a DC filled with bone marrow was implanted in FNB. Group 2 was control (n = 3) in which a DC without bone marrow was implanted in a similar bundle.

Two rats comparable in weight and age to the study groups became bone marrow donors. Bone marrow obtained under aseptic conditions in a laminar flow hood by washing the diaphysis of the femurs with a sterile culture medium, was placed *ex vivo* in an implantation chamber. Implantation of

DCs in experimental animals was carried out under isoflurane anesthesia. The volume of bone marrow placed in the DC was 100 μl. 15 minutes before surgery, atropine was administered intramuscularly at a dosage of 0.2 mg / kg to prevent hypersecretion of mucus in the bronchi. Surgical access was provided through a 2–3 cm incision deep in the inguinal fold, inward from the pulsation of the femoral artery [11]. All manipulations with the animals were carried out in accordance with the Directive of the European Parliament No. 2010/63eu of 22.09.2010 "On the protection of animals used for scientific purposes".

Six hours after the surgery, the rats were considered stable. On day 40 after the end of the implantation period, the animals were euthanized by CO_2 inhalation in compliance with the rules and norms of the European Council (86/609 EEC), the Declaration of Helsinki, and orders of the Ministry of Healthcare of the USSR (No. 742 of 13.11.1984 and No. 48 of 23.01.1985).

A macroscopic (visual) evaluation of the implantation site was carried out at 40 days of the experiment during necropsy. The implantation site was assessed by the following criteria: the degree of blood supply in the vessels, encapsulation and visual signs of an inflammatory reaction (the presence of hyperemia, edema, and infiltration) according to the scoring system, where 0 points was the absence of a sign, 1 point – weak degree, 2 points – moderate degree.

Histologic samples were prepared after necropsy according to standard methods [12]. Microscopy was performed on the Carl Zeiss Observer D1 light microscope (Germany). Rabbit polyclonal VEGF antibodies (Anti-VEGFA antibody, ab46154 antibodies from Dako (Mouse monoclonal [EIC] to VEGF receptor 2), CD34 (Anti-CD34 antibody, ab185732 antibodies from Dako to CD34 (Clone QBEnd 10)), and CD45 (CD45-APC-Cy7, Biolegend, USA)) were used for immunohistochemistry staining. Staining was carried out according to the manufacturer's instructions. To analyze the results of immunohistochemical reactions, a method for assessing dye expression on a point scale was used, where 3 points (+++) was strong staining, 2 points (++) – moderate staining, and 1 point (+) – weak staining [13]. Microscopy was performed on the Carl Zeiss Observer D1 light microscope (Germany). Morphometry with an assessment of the vascularization coefficient was carried out using images obtained by the Zeiss AxioCam ICc5 digital camera for light microscopy (Germany).

To quantify the degree of bone marrow vascularization in the DC implanted in rat FNB, the following coefficients were used: the vascularization coefficient (CV) was estimated using the formula: $CV = S_v/S_p \times 100\%$, where Sv was the area of all microvessels, Sp was the area of the photograph [14], excluding the femoral artery and vein, the Kernohan index (KI) was calculated as: $KI = (2 \times L_{\text{artery wall}}) / D_{\text{artery}}$, where L was the thickness of the tunica media of the artery), D was the diameter of the artery lumen [15].

Ten fields of view were assessed in each group to calculate the parameters. Statistical processing was carried out using the Statistica 10.0 program, IBM (USA). Statistical hypothesis testing to determine the nature of trait distribution was carried out using the Shapiro – Wilk test for small ($n < 30$) samples. Descriptive and nonparametric statistical methods were used to process the results obtained. The studied parameters were described as the median (Me) and the interquartile range (Q_1; Q_3). When comparing independent samples, the Kruskal – Wallis test with the median test was used; and the Wilcoxon test was used for paired comparisons. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Intraoperatively, camera fixation did not disrupt general blood circulation and innervation (Fig. 2). There were no visible postoperative inflammatory reactions at the implantation sites during the follow-up period (40 days) (Fig. 3).

Fig. 2. The diffusion chamber implantation bed

a *b* *A*

Fig. 3. The diffusion chamber implantation site after the surgery: *a* – experimental group, *b* – control group

The results of the macroscopic (visual) assessment of the implantation site according to the degree of blood supply in the vessels, encapsulation and visual signs of an inflammatory reaction (presence of hyperemia, edema, and infiltration) showed (Table 1) that in the experimental group, implantation was accompanied by mild (according to GOST ISO 10993-6-2021) changes in the form of hyperemia and formation of a connective tissue capsule.

Table 1

Macroscopic changes in the implantation bed of the diffusion chamber filled with bone marrow implanted in FNB on day 40 of the experiment, points, *Me* (Q_1; Q_3)

Group	Inflammation	Hyperemia	Chamber encapsulation
Experimental group, $n = 4$	0 (0; 0)	1 (1; 1.5)	1.5 (1; 1.5)
Control group, $n = 3$	0 (0; 0)	0 (0; 1)	–

Note. The number of animals in each group – *n*.

The histologic examination revealed that the implanted bioengineered structures were covered with a compact capsule more than 50 microns thick, made of mature connective tissue (Fig. 4). This tissue was classified as loose fibrous irregular connective tissue with developed microcirculation. These compact capsule structures could not be detected in the control group.

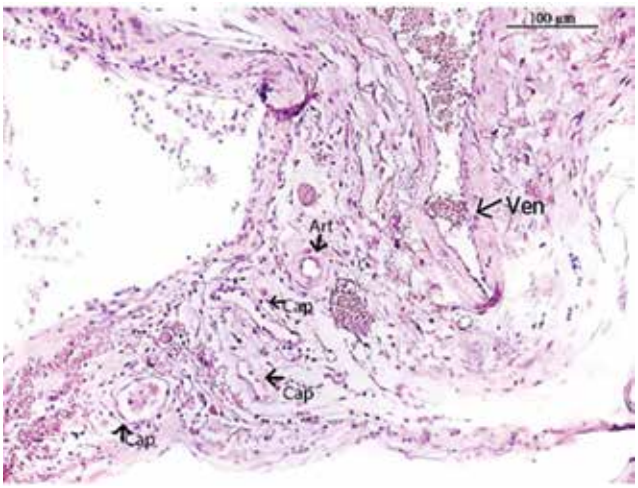

Fig. 4. Microscopic images of the distribution of microvasculature in the connective tissue capsule around the diffusion chamber on day 40 after the implantation. Light microscopy, × 40

Connective tissue was also found in the cavity of the chamber adjacent to the polymer wall (Fig. 5, *a*), similar in its structure and components to the connective tissue capsule of the DC (Fig. 5).

Microscopy of the chamber contents showed that all animals in the experimental and control groups had areas of loose fibrous connective tissue with a large number of microvessels (Fig. 5). Microscopy of a transverse section of the FNB, as an implantation site of DC filled with bone marrow, did not reveal a narrowing of the vessel lumen compared to a similar parameter in animals of the control group. The vessel walls showed homogeneity of endothelial cells and an increase in the layer of muscle cells. An increase in the number of small vessels in the muscle layer and adventitia (*vasa vasorum*) with an increase in the lumen of the latter was noted (Fig. 6).

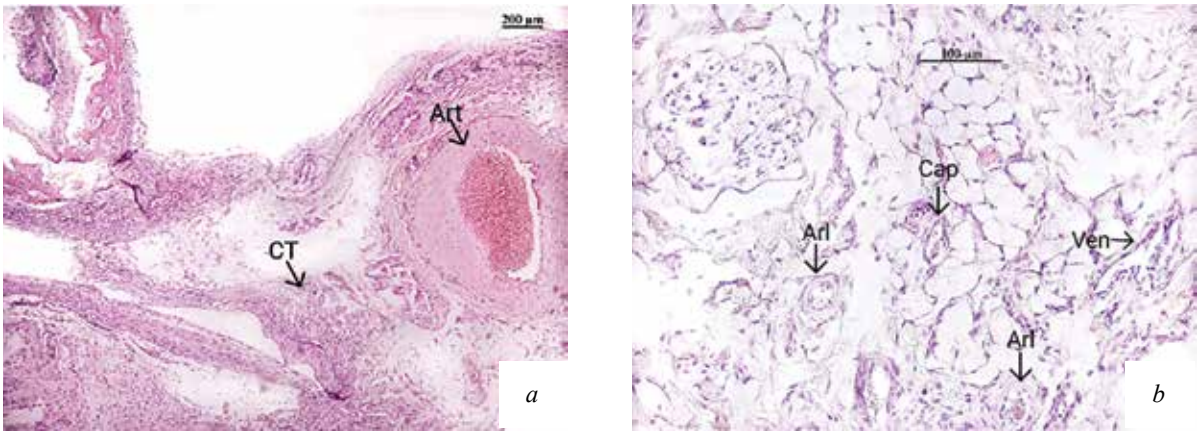

Fig. 5. Microscopic image of the connective tissue content of the diffusion chamber (*a*) and its microcirculation (*b*) on day 40 after implantation: CT – connective tissue, Cap – capillaries; Art – arterioles; Ven – venules. H&E stain. Light microscopy, ×10 (*a*); × 40 (*b*)

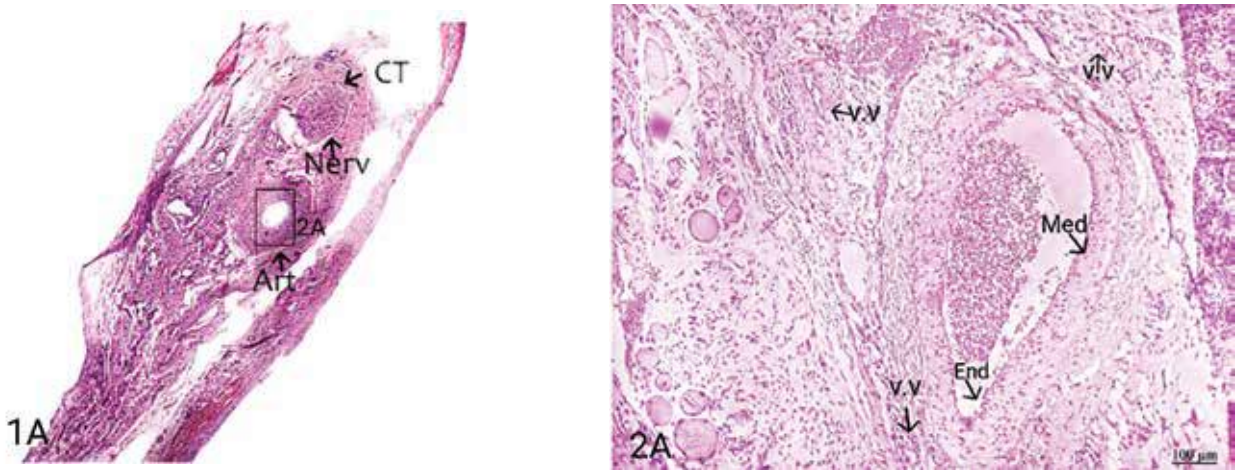

Fig. 6. 1A – microscopic image of the rat FNB with the DC: Art – artery, Nerv – nerve, CT – connective tissue content in the diffusion chamber. H&E stain. Light microscopy, × 10. *2A* – Microscopic image of an artery in the neurovascular bundle. Med – media, *v.v.*– *vasa vasorum*, End – endothelium. H&E stain. Light microscopy, × 40

The results of a quantitative assessment of the angiogenesis of DC with bone marrow implanted in rat FNB, according to the vascularization coefficient and the Kernohan index, are presented in Table 2. According to Table 2, during the DC implantation in FNB, sufficient conditions are formed along the femoral artery with a bioengineered construct that promote the growth of microvasculature in the zone of regenerative metaplasia of the bone marrow into connective tissue and its derivatives (adipose and bone tissue).

According to the immunohistochemical analysis, expression of VEGF, CD34, and CD45 was observed in the sections in all animals of the experimental and control groups, but in the experimental group with bone marrow metaplasia, it was more pronounced for CD34 (Fig. 7, *c, d*) and VEGF (Fig. 8, *a, b*). In both groups, the presence of the hematolymphoid marker CD45 was shown before day 40 of the study (Fig. 7, *e, f*).

Table 2

Parameters of vascularization of the diffusion chamber filled with bone marrow implanted in the FNB, *Me* (Q_1; Q_3)

Parameter	Group	
	Experimental group, $n = 4$	Control group, $n = 3$
Vascularization coefficient, %	1.28* (0.93; 1.60)	0.65 (0.37; 0.71)
Kernohan index, pxl	0.72* (0.69; 0.73)	0.67 (0.66; 0.68)

* – statistically significant differences in the parameters of the experimental group compared to the corresponding control value at $p < 0.05$.

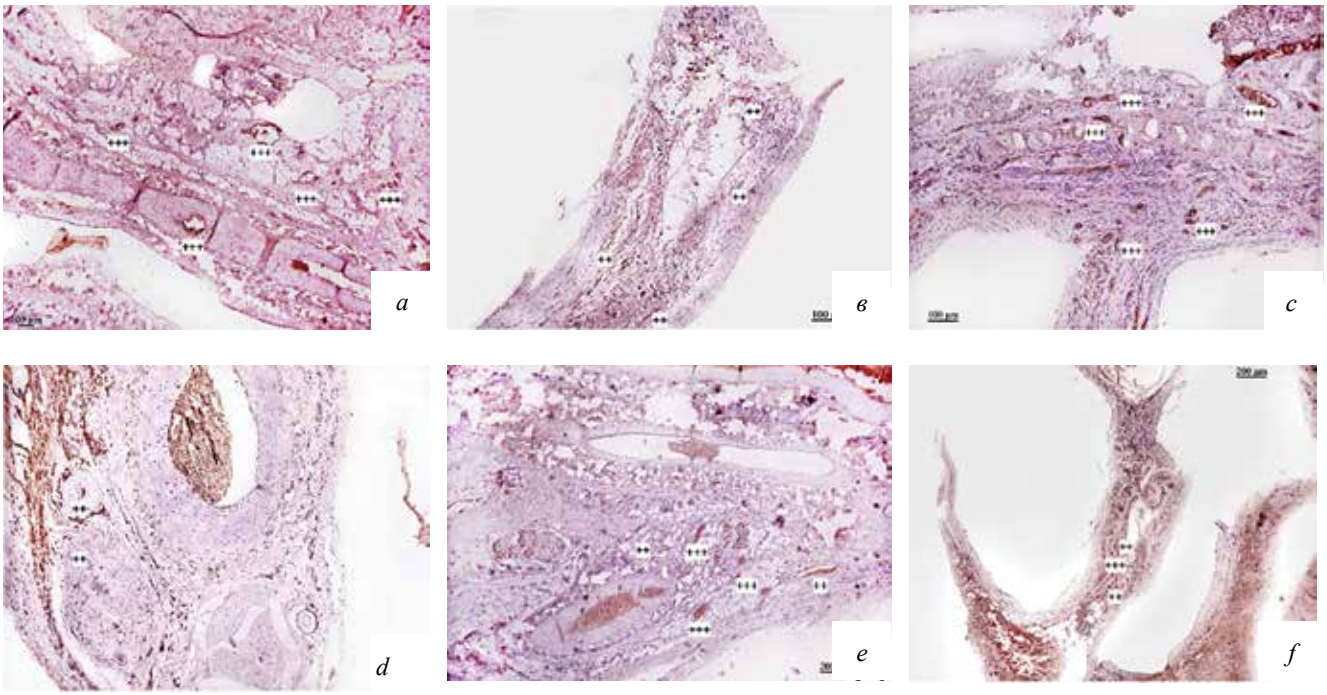

Fig. 7. Expression of VEGF, CD34, and CD45 in the experimental and control groups, × 10: +++ – strong staining, ++ – moderate staining. Expression of VEGF in DC contents in the experimental group. The expression degree is 3 points (+++) (*a*); VEGF expression in DC contents in the control group. The expression degree is 2 points (++) (*b*); CD34 expression in DC contents in the experimental group. The expression degree is 3 points (+) (*c*); CD34 expression in DC contents in the control group. The expression degree is 2 points (+) (*d*); CD45 expression in DC contents in the experimental group. The expression degree is 3 points (+) (*e*); CD45 expression in DC contents in the control group. The expression degree is 2 points (++) (*f*)

DISCUSSION

The study demonstrated the main morphological features of vascularization during bone marrow transplant to the FNB in DC. The material used for DC is polycaprolactone that is a biocompatible and biodegradable polymer. The degradation products of polycaprolactone are water, carbon dioxide, and caproic acid that are safe for animals [16].

The above literature data are confirmed by the conducted study which showed the absence of the damaging effect of polycaprolactone degradation products in the form of inflammatory reactions at the implantation site. The assessment of postoperative tissue state in the animals revealed no signs of inflammatory reactions. The revealed increase in the number of microvessels and the vascularization coefficient in the DC filled with bone marrow showed that bone marrow promotes the formation of vascularized stroma around major vessels. Immunohistochemical vascularization markers VEGF and CD34 confirmed the histology results. VEGF is a signaling protein produced for the induction of vasculogenesis and angiogenesis; it is responsible for restoring oxygen flow to tissues [17]. According to the manufacturer's instructions, CD34 expression in the present study was interpreted as a

marker of endothelial cells in blood and lymphatic vessels [18], and it increased during implantation of the DC filled with bone marrow. CD45 is a member of the tyrosine phosphatase family. The gene encoding this protein is specifically expressed in hematopoietic cells. The protein plays a role in signal transmission from cellular antigen receptors [19]. Expression of CD45 in histology sections at the implantation site of DC with bone marrow and without it (control) suggests migration of blood cells to the damaged area with subsequent active participation in regenerative processes [20].

Polycaprolactone has adsorption properties toward mesenchymal stem cells (MSCs) and low cellular toxicity [21]. Both local (vascular and BM) and circulating MSCs and pericytes can induce neoangiogenesis [22]. However, the presence of bone marrow in the DC significantly enhances the vascularization of the implant. At the same time, the literature also indicates the anti-inflammatory / regenerative effect of bone marrow MSCs at the implantation site.

The cytokine profile of MSCs may affect the absence of an inflammatory reaction at the implantation site [23]. Thus, vasculogenesis inducers (for example, VEGF, interleukin (IL)-10) secreted by MSCs are cytokines that also regulate tissue regeneration [24]. From the point of view of physiology, bone marrow MSCs can be differentiated based on the formation of well-vascularized loose irregular connective tissue during subcutaneous implantation [24], which was preserved under conditions of bone marrow implantation to the FNB in DC.

CONCLUSION

Diffusion chamber made of polycaprolactone and implanted in the femoral neurovascular bundle does not cause mechanical damage, inflammation, and post-implantation complications. Bone marrow placed in diffusion chambers undergoes regenerative metaplasia with differentiation of mesenchymal stem cells into fibroblasts and, possibly, endothelial cells. Increased vascularization in the zone of ectopically regenerating bone marrow creates conditions for *in situ* engineering of parenchymal organs that require preserved blood supply (liver, etc.). In general, the formation of a functional "DC – bone marrow – macrocirculation" system can be useful for the development of experimental tissue engineering.

REFERENCES

1. Zhang P., Zhang C., Li J., Han J., Liu X., Yang H. The physical microenvironment of hematopoietic stem cells and its emerging roles in engineering applications. *Stem Cell Res. Ther.* 2019;10(1):327. DOI: 10.1186/s13287-019-1422-7.
2. Mahadik B.P., Pedron Haba S., Skertich L.J., Harley B.A. The use of covalently immobilized stem cell factor to selectively affect hematopoietic stem cell activity within a gelatin hydrogel. *Biomaterials.* 2015;67:297–307. DOI: 10.1016/j.biomaterials.2015.07.042.
3. Sharma M.B., Limaye L.S., Kale V.P. Mimicking the functional hematopoietic stem cell niche in vitro: recapitulation of marrow physiology by hydrogel-based three-dimensional cultures of mesenchymal stromal cells. *Haematologica.* 2012;97(5):651–660. DOI: 10.3324/haematol.2011.050500.
4. Batsivari A., Haltalli M.L.R., Passaro D., Pospori C., Lo Celso C., Bonnet D. Dynamic responses of the haematopoietic stem cell niche to diverse stresses. *Nat. Cell Biol.* 2020;22(1):7–17. DOI: 10.1038/s41556-019-0444-9.
5. Cosson S., Lutolf M.P. microfluidic patterning of protein gradients on biomimetic hydrogel substrates. *Methods in Cell Biology.* 2014:121:91–102. DOI: 10.1016/B978-0-12-800281-0.00007-5.
6. Itkin T., Gur-Cohen S., Spencer J.A., Schajnovitz A., Ramasamy S.K., Kusumbe A.P. et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. *Nature.* 2016;532(7599):323–328. DOI: 10.1038/nature17624.
7. Jeon J.S. et al. Generation of 3D functional microvascular networks with mural cell-like human mesenchymal stem cells in microfluidic systems by vasculogenesis-like process. *Integr. Biol. (Camb.).* 2014;6(5):555–563. DOI: 10.1039/c3ib40267c.
8. Mulligan-Kehoe M.J. The vasa vasorum in diseased and nondiseased arteries. *Am. J. Physiol. Heart Circ. Physiol.* 2010;298(2):H295–305. DOI: 10.1152/ajpheart.00884.
9. Zhou X., Pan Y., Liu R. et al. Biocompatibility and biodegradation properties of polycaprolactone/polydioxanone composite scaffolds prepared by blend or co-electrospinning. *Journal of Bioactive and Compatible Polymers*. 2019;34(2):115–130. DOI: 10.1177/0883911519835569.
10. GOST (All-Union State Standard) ISO 11135-2017 Sterilization of medical products. Ethylene oxide. Requirements for the development, validation and routine control of a sterilization process for medical devices (in Russ.).
11. Kokozidou M., Katsargyris A., Verhoeven Eric L.G., Schulze-Tanzil G. Vascular access animal models used in research. *Ann. Anatomy.* 2019:225:65–75. DOI: 10.1016/j.aanat.2019.06.002.
12. Bogdanov L.A., Kutikhin A.G. Optimization of staining of elements of the circulatory system and the hepatolienal system with hematoxylin and eosin. *Fundamental and Clinical Medicine.* 2019;4(4):70–77 (in Russ.). DOI: 10.23946/2500-0764-2019-4-4-70-77.
13. Konyaeva A.D., Varakuta E.Yu., Leyman A.E., Rafiev D.O., Bolbasov E.N., Stankevich K.S. Morphological features of regeneration of the oral mucosa when using polymer piezoelectric membranes. *Bulletin of RSMU*. 2023;(3):61–68 (in Russ.). DOI: 10.24075/vrgmu.2023.020.
14. Nesterova E.S., Kravchenko S.K., Gemjian E.G., Osmanov E.A., Kovrigina A.M. Assessment of vascularization and microenvironment of tumor tissue in follicular lymphoma. *Therapeutic Archive.* 2013;85(7):57–64 (in Russ.).

15. Nikel V.V., Samotesov P.A., Efremova V.P., Batukhtina N.P., Vakhtina L.Yu., Bezoschetnov V.E. Morphofunctional state of arterial vessels of hollow parenchymal organs. *Journal of Siberian Medical Sciences.* 2015;(3):76 (in Russ.).
16. Kazantseva E.A. Designing and evaluating the effectiveness of controlled delivery systems for agricultural preparations of various actions: Master's thesis. Moscow, 2018 (in Russ.).
17. Stepanov I.V., Altybaev S.R., Starch N.V., Rachkovsky K.V., Sorokin D.A., Afanasyev S.G., et al. The relationship of the parameters of tumor neoangiogenesis with lymphogenic metastasis in rectal cancer. *Siberian Journal of Oncology.* 2017;16(3):46–51 (in Russ.). DOI: 10.21294/1814-4861-2017-16-3-46-51.
18. Vasuri F., Fittipaldi S., Giunchi F., Monica M., Ravaioli M., Degiovanni A. Facing the enigma of the vascular network in hepatocellular carcinomas in cirrhotic and non-cirrhotic livers. *J. Clin. Pathol.* 2016;69(2):102–108. DOI: 10.1136/jclinpath-2015-203028.
19. Park S., Kim J.-Y., Jang G.-B., Choi J.-H., Kim J.-H., Lee C.-L. Aberrant activation of the CD45-Wnt signaling axis promotes stemness and therapy resistance in colorectal cancer cells. *Theranostics.* 2021;11(18):8755–8770. DOI: 10.7150/thno.63446.
20. Yurova K.A., Khaziakhmatova O.G., Malashchenko V.V., Norkin I.K., Ivanov P.A., Khlusov I.A., et al. Cellular and molecular aspects of inflammation, angiogenesis and osteogenesis. A brief overview. *Cytology.* 2020;62(5):305–315 (in Russ.). DOI: 10.31857/S0041377120050090.
21. Woodruff M.A., Hutmacher D.W. The return of a forgotten polymer – polycaprolactone in the 21st century. *Prog. Polym. Sci.* 2010;35(10):1217–1256. DOI: 10.1016/j.progpolymsci.2010.04.002.
22. Murr L.E. Strategies for creating living, additively manufactured, open-cellular metal and alloy implants by promoting osseointegration, osteoinduction and vascularization: An overview. *J. Mater. Sci. Technol.* 2019;35(2):231–241. DOI: 10.1016/j.jmst.2018.09.003.
23. Dang J., Yang J., Yu Z., Chen L.,Zang Z., Wang K. et al. Bone marrow mesenchymal stem cells enhance angiogenesis and promote fat retention in fat grafting via polarized macrophages. *Stem Cell Res. Ther.* 2022;13(1):52. DOI: 10.1186/s13287-022-02709-2.
24. Khlusov I., Litvinova L., Shupletsova V., Khaziakhmatova O., Malashchenko V., Yurova K. et al. Costimulatory effect of rough calcium phosphate coating and blood mononuclear cells on adipose-derived mesenchymal stem cells in vitro as a model of *in vivo* tissue repair. *Materials.* 2020;13(19):4398. DOI: 10.3390/ma13194398.

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