https://doi.org/10.23873/2074-0506-2023-15-2-177-187

Optimization of the technique for manufacturing the osteoplastic material based on type 1 human collagen and allogeneic bone chips

A.A. Ofitserov[1], M.S. Makarov✉[1], M.V. Storozheva[1],
N.V. Borovkova[1,2], I.N. Ponomarev[1]

[1]N.V. Sklifosovsky Research Institute for Emergency Medicine,
3 Bolshaya Sukharevskaya Sq., Moscow 129090 Russia;
[2] Department of Transplantology and Artificial Organs, N.I. Pirogov Russian National Research Medical University,
1 Ostrovityanov St., Moscow 117997 Russia

✉Corresponding author: M.S. Makarov, Cand. Sci. (Biol.), Senior Researcher of the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine, MakarovMS@sklif.mos.ru

Abstract

Introduction. *Biological grafts, including bone chips and collagen, are supposed to be promising in the treatment of bone tissue treatment. Nevertheless, manufacturing of these grafts still needs to be standardized.*

Aim*. To optimize methodology of osteoplastic material production, based on allogenic 1 type collagen and bone chips.*

Material and methods. *Osteoplastic material grafts were produced, using with allogeneic bone chips 180-800 μm and type 1 collagen solution in acidic acid. We studied total integrity of graft, collagen quality, morphofunctional properties of line M-22human cells interacting with different type of osteoplastic material grafts.*

Results. *Procedures for manufacturing the osteoplastic material did not significantly affect the quality of collagen in its composition, while*

lyophilized grafts had pronounced acidogenic and toxic effects in cell culture. Soaking osteoplastic material in isotonic solution for 30 min or longer increased its biocompatibility in vitro. Adhesion properties of osteoplastic material widely varied depending on collagen concentration and bone chips size.

Conclusion. *Osteoplastic material prepared with allogeneic bone chips of 180-800 μm and collagen solution in acidic acid had pronounced acidogenic and toxic effects in vitro that could be considerably reduced by soaking in isotonic solution. Varying the collagen concentration and the size of bone chips one could produce biocompatible osteoplastic material grafts with high and low adhesion properties.*

Keywords: osteoplastic material, collagen, biocompatibility, cells, proliferation activity

Conflict of interests Authors declare no conflict of interest

Financing The study was performed without external funding

For citation: Ofitserov AA, Makarov MS, Storozheva MV, Borovkova NV, Ponomarev IN. Optimization of the technique for manufacturing the osteoplastic material based on type 1 human collagen and allogeneic bone chips. *Transplantologiya. The Russian Journal of Transplantation.* 2023;15(2):177–187. (In Russ.). https://doi.org/10.23873/2074-0506-2023-15-2-177-187

OPM osteoplastic material

DMEM, Dulbecco's modified Eagle's medium

λ, wavelength

Introduction

Currently, there is an active development of implants and biological structures to stimulate osteogenesis and bone tissue regeneration. The term “osteoplastic material” (OPM) is often used to designate such products [1]. According to their chemical composition,

OPMs are classified into bioorganic, ceramic, synthetic, and composite ones [2, 3]. OPMs can include diploid cells, as well as growth factors, differentiation factors, and cytokines [4–6]. In specialized medical departments involved in the production of tissue grafts, there is the possibility of producing OPM using allogeneic bone and a solution of human type 1 collagen. Collagen has been shown to significantly increase the bioconductive properties of grafts, to stimulate the migration of the patient's native cells, and therefore can it be used as a substrate for the formation of native bone for them [3, 7, 8]. When using bone material in the form of dispersed structures (bone chips), it becomes possible to obtain plastic grafts of a given size and shape. The collagen solution promotes the incorporation of bone granules, reducing the risk of their displacement or extrusion under mechanical stress [9, 10]. Bone chips have osteogenic and osteoinductive properties and is widely used in clinical practice [2–4]. Products based on bone chips and collagen are considered very promising in the treatment of defects in cancellous and cortical bone [11, 12]. Meanwhile, well-known products contain xenogenic materials in their composition [1, 5, 8–10]. In this regard, it seems relevant to develop a method for obtaining OPM based on allogeneic materials, such as collagen and bone chips.

The aim was to optimize the technique for obtaining osteoplastic material based on allogeneic type 1 collagen and bone chips.

Material and methods

The study was conducted at the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine in 2022.

For manufacturing the OPM, solutions of human type 1 collagen isolated from the tendons of tissue donors by acid extraction in 0.01 M

acetic acid [13] and bone chips obtained from the bone of tissue donors [14] were used. The size of bone chip granules varied from 180 μm to 800 μm. In all experiments, the amount of collagen solutions used was 100 ml. To determine the optimal ratio, 3 to 10 grams of bone chips were added to 100 ml of collagen solution and mixed, trying to achieve its uniform distribution and the absence of conglomerates. For experiments in vitro, a mixture with an optimal ratio of bone chips and collagen was used. The mixture of collagen sol and bone chips was stirred until it became homogeneous, then it was placed on Petri dishes with an area of 10x10 cm, frozen, and then lyophilized in a chamber at rare atmosphere. The finished osteoplastic material was sterilized using ultraviolet rays.

At the first stage, we studied the effect of an osteoplastic material made from 0.6–0.8% human type 1 collagen sol, used to potentiate the osteoconductive properties of bone tissue grafts [12], and bone chips with a size of 315–430 μm on the human fibroblast culture cells. OPM was placed into the fibroblast culture without additional treatment. A total of 5 samples were investigated.

At the second stage, the method efficacy for reducing the OPM toxicity was assessed. In the first series of experiments, grafts were prepared according to the above method, then the samples were soaked in isotonic sodium chloride solution for 10, 20, 30, 40, 50, or 60 minutes, after which they were placed in cell culture. In the second series of experiments, the mixture of collagen sol and bone chips was washed in distilled water before freezing. One cycle included: centrifugation of a mixture of bone chips and collagen in a Falcon type tube with an acceleration of 3000 g for 20 minutes, a complete replacement of the supernatant with an equivalent volume of sterile distilled water, and resuspension of the contents in the tube. The number of washing cycles varied from 1 to 4. After washing, the mixture of collagen and bone chips

was frozen, and lyophilized; soaking in isotonic sodium chloride solution was not performed before being placed in cell culture. A total of 20 samples were studied.

At the third stage, the OPM adhesive properties were assessed as achieved by using bone chips of different diameters and a collagen solution in 2 concentrations. In parallel, collagen sponges obtained from a solution with the same collagen concentration, but without using bone chips, were studied. We used 8 types of OPM obtained from 0.8% or 1.1% human type 1 collagen sol and bone chips of one of the sizes: 180 μm, 315 μm, 630 μm, 800 μm. After combining the components, the nature of the bone chip distribution in collagen (homogeneity) was evaluated; after lyophilization, the stability of the finished product was evaluated. Next, in the cell culture, the toxicity of the OPM samples and the feasibility of its reduction were evaluated by their washing in physiological sodium chloride solution for 30 minutes. A total of 22 samples were studied.

All studies in vitro were conducted on a culture of human M-22 line fibroblasts in 20–22 passages. Ten thousand cells in Dulbecco's modified Eagles medium (DMEM) containing 10% fetal bovine serum were placed in each well having a growth surface area of 1.9 cm^2 (a 4-well plate). Cell suspension without OPM was added to the control wells; samples of OPM or collagen sponge were placed into the experimental wells, then the cell suspension was added. Cells were incubated at 37°C and 5% carbon dioxide concentration in the atmosphere for 3 days. The number of cells at the bottom of the wells and on the surface of the grafts, the overall structural integrity of the cells, and their morphology were assessed. For that, the cells were stained with a vital (lifetime) fluorochrome dye based on tripaflavin-acridine orange or tripaflavin-rhodamine C and examined using a Nikon 80i confocal luminescent

microscope (Nikon, Japan) [15]. In addition, the autofluorescence of collagen fibers in the OPM was evaluated. Registration of collagen autofluorescence in non-fixed unstained OPM samples was performed with a blue light filter (excitation λ of 380–420 nm, emission λ from 450 nm, exposure of 1 sec). Collagen autofluorescence was quantified using the mean luminescence intensity parameter (in foot candles, FC). For comparison, the intensity of the luminescence of collagen fibers was evaluated in the composition of collagen dressings prepared using the same collagen solution according to a well-known method [13].

Statistical processing of the obtained data was made by the methods of variational statistics using "Microsoft Excel 2000" software package. Arithmetic mean values (M) and standard deviations (σ) were calculated. Differences were assessed using Student's t-test. The distribution was tested for normality using the Kolmogorov–Smirnov test. Differences in values were considered statistically significant at a significance level over 95% ($p<0.05$).

Results and discussion

In the development of the OPM, it was necessary to obtain a whole graft with a homogeneous distribution of components. It was experimentally shown that in 100 ml of collagen solution it was possible to homogeneously distribute up to 6 g of bone chips. If the mass of bone chips exceeded 6 g, the resulting mixture lost its homogeneity, conglomerates formed, and after lyophilization, the graft was fragmented. Thus, in manufacturing OPM, the optimal ratio of bone chips to collagen solution was 6 g per 100 ml.

At the first stage of in vitro research, we used the OPM samples made on the basis of a 0.8% solution of type 1 collagen and bone chips of 315–430 μm in size. What drew the attention was the change in the color

of the DMEM nutrient medium indicator, suggesting a shift in pH to the acidic part (acidogenicity), which manifested itself as soon as within 1 hour after placing the OPM in the experimental well, and it was not associated with contamination of the samples with pathogenic flora. After 1 hour of the OPM incubation in the wells, the pH value averaged 4.5 at a rate of 7.0–7.2 for the DMEM. After 3 days of culturing, the number of cells was very low at the bottom of these wells, and did not exceed a mean of 1 thousand per cm^2. Meantime, the number of cells in the control wells was 14000–15000 cells per 1 cm^2. Cells were not found in the OPM content, thus, a noticed decrease in the number of cells at the bottom of the wells was not associated with their migration into the graft. Meantime, in the culture medium of experimental wells, a large number of stunned cells were noted. Such cells have a characteristic round shape, do not contact with each other, have pronounced defects in the nucleus and vacuolar system, which is clearly detected when stained with vital fluorochrome dyes. Such changes are characteristic of severely deformed or apoptotic cells. Thus, the finished OPM samples had a pronounced acidogenicity and toxicity, which led to massive cell death.

To reduce the OPM toxicity, 2 methods were used: soaking the finished OPM in an isotonic sodium chloride solution or washing the OPM in distilled water at the manufacturing stage. Control samples in both studies were comparable. In the control wells (without OPM), the number of cells at the bottom of the wells after 3 days of culturing was 23–24 thousand/cm^2, while in experiments with the initial OPM, this parameter did not exceed 0.9–1.0 thousand/cm^2, and there were absolutely no cells on the OPM surface (Table 1). In the wells where the OPM samples had been pre-soaked in isotonic sodium chloride solution, the number of cells at the bottom ranged from 3.0±0.5 to 17.5±0.8 thousand/cm^2 , increasing with the product soaking duration from 10 to 50

minutes. In parallel, the number of cells on the graft increased: after soaking for 20 minutes, their number was 1.0±0.2 thousand/cm^2; and after 50 minute soaking, it reached 4.5±0.7 thousand/cm^2. It should be emphasized that in the experiments where the OPM soaking lasted for 10–20 minutes, many cells at the bottom of the wells and on the graft surface was altered in appearance, had poor contact with the plastic, and had defects in the nucleus and cytoplasm. Vital staining of cells showed a noticeable decrease in the outer membrane luminescence, an abrupt decrease in the number of secretory vesicles in the cells, indicating cell deformation, destruction of their vacuolar structures, disruption of contact interactions, which together can be regarded as a toxic effect. In experiments where soaking was 30–50 minutes, there were no deformed cells; accordingly, the cell morphology was normal or close to normal. In the case of OPM soaking for 60 minutes, the number of cells and their morphology did not significantly differ from the results of the experiments where soaking was 50 minutes.

Table 1. Biocompatibility assessment of osteoplastic material grafts prepared by different methods

Soaking the finished lyophilized graft in isotonic sodium chloride solution		
Osteoplastic material exposure time in isotonic sodium chloride solution	The density of line M-22 cells after 3 days of culturing, (10^3)/cm^2	
	Well bottom	Graft surface
Control 1. Wells with cells without graft	24.1±1.2	—
Control 2. 0 min (graft without soaking)	1.0±0.2	0
10 min	3.0±0.5	0
20 min	5.3±0.4	1.0±0.2
30 min	11.1±1.0*	2.6±0.5*
40 min	13.0±1.0*	2.8±0.6*
50 min	17.5±0.8*	4.5±0.7*
60 min	17.0±1.0*	4.0±0.6*

Distilled water washing the mixture of liquid collagen and bone chips from the acidic medium, followed by lyophilization		
Number of washes	The density of line M-22 cells after 3 days of culturing, $(10^3)/cm^2$	
	Well bottom	Graft surface
Control 1 (wells with cells without graft	23.5±1.0	—
Control 2 (without washing)	0.9±0.2	0
1 time	6.5±1.2	1.0±0.3
2 times	7.5±1.6	1.0±0.2
3 times	7.5±1.9	1.6±0.2
4 times	5.0±0.9	1.8±0.2

*statistically significant at $p < 0.05$ relative to control 2 and experiments with 10- and 20-minute soaking

In a series of experiments where a mixture of collagen and bone chips was washed before lyophilization, a gradual change in its pH from 3.3 to 6.1 was noted as the number of treatment cycles increased to 4.

In an in vitro study, the number of cells at the bottom of OPM-containing wells having been subjected to different numbers of additional treatment cycles, ranged from 5.0 to 7.5 thousand/cm^2 ($p>0.05$). At the same time, the cell density on the graft surface after 3–4 collagen washings was higher than after 1–2 washings. However, the absolute figures of cell density were very low in all cases, not exceeding 2000/cm^2 and were lower than in the experiments with the grafts soaked for 30–60 minutes (Table 1). Thus, soaking the finished lyophilized OPM grafts in isotonic sodium chloride solution is more effective in reducing their toxicity, and the soaking time should be at least 30 minutes.

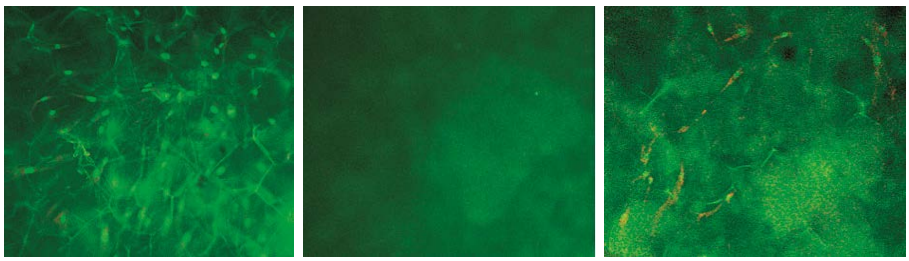
When assessing the OPM obtained using chips of different sizes and 2 different collagen concentrations, we found that when using 0.8% collagen sol, the most homogeneous mixture can be obtained in case of mixing relatively large bone chips (600–800 μm). In the experiments with 1.1% collagen solution, on the contrary, the most homogeneous mixture was formed while using fine chips (180 μm) (Table 2). After

lyophilization, all samples were stable and did not break in fragments. In parallel, the OPM samples and collagen-based dressings obtained from a solution with similar collagen concentrations (0.8 and 1.1%) were assessed in experiments in vitro. In the presence of collagen dressings, no cell deformations were seen either at the bottom of the wells, or in the grafts; the cells adhered to the surface of the dressings (Fig. 1A). All OPM samples without pre-soaking were toxic and prevented cell growth (Fig. 1B). In experiments where OPM was pre-soaked in an isotonic sodium chloride solution for 30–50 minutes, no marked cell deformations were observed. In OPM obtained from a 0.8% collagen solution, its samples with bone chips of 180–600 μm in diameter after 3 days of culturing had a similar cell density on their surface (Fig. 1C) and did not differ statistically significantly from collagen grafts without bone chips, while in the OPM with bone chips 800 μm in diameter, the number of cells was 3–4 times lower (Fig. 2A). The number of cells at the bottom of the wells was the largest in experiments where the diameter of the bone chips was 180 μm. In OPM obtained from 1.1% collagen solution, the cell adhesion on the OPM surface was much less pronounced than in control collagen dressing (without bone chips) and in the OPM prepared by using 0.8% collagen solution and the bone chips of 180–600 μm in diameter. We should note that at the bottom of the wells, the highest cell density was noted in those cases where the diameter of the bone chips was 180 and 800 μm (Fig. 2b). In general, it can be concluded that a 0.8% collagen solution is preferable for manufacturing OPM than a 1.1% solution. The OPM prepared using 0.8% collagen solution and bone chips with a diameter of 180–600 μm after pre-soaking are adhesive and non-toxic to cells.

Table 2. Homogeneity assessment in the mixture of bone chips and collagen solution

Options	Concentration of collagen in solution	
Size of bone chip granules, μm	0.8%	1.1%
180	Low	High
315	Medium	Low
630	High	Medium
800	High	Low

Notes. Low homogeneity is defined as the formation of chip and collagen clots occurring in the entire bulk of mixture; it is not possible to completely stir the clots
Medium homogeneity denotes the formation of certain clots while stirring; with more intensive mixing, clots disappear
High homogeneity denotes no clot formation while stirring



A **B** **C**

Fig. 1. Growth of M-22 cells on collagen matrices obtained from 0.8% collagen solution after 3 days of culturing. Magnification 100x. Tripaflavin-acridine orange stain. A: collagen dressing (without bone chips); B: osteoplastic material of 0.8% collagen and bone chips without soaking; C: osteoplastic material of 0.8% collagen and bone chips, pre-soaked in isotonic sodium chloride solution within 40 minutes

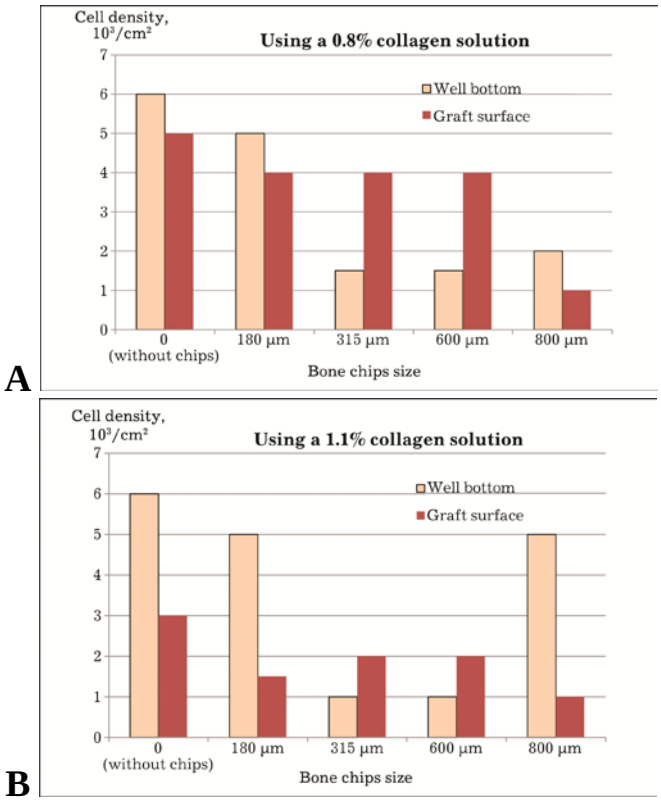
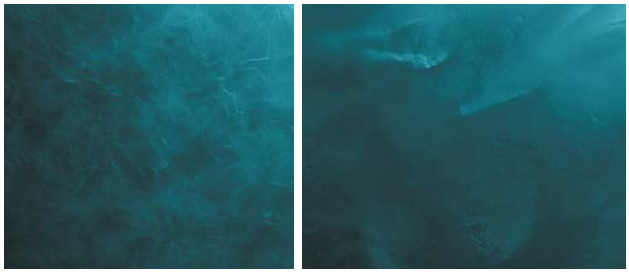


Fig. 2. Number of cells, cultivated in the presence of previously soaked osteoplastic material. To obtain osteoplastic material we used 0.8% collagen solution (A) or 1.1% collagen (B)

The collagen quality was assessed by studying the autofluorescence of collagen fibers in OPM and in collagen-based dressings obtained by using the collagen concentrations (0.8 and 1.1%) similar to those used for OPM manufacturing. The analysis of collagen autofluorescence in collagen-based dressings clearly revealed the areas with a diffuse collagen arrangement and the areas with collagen fibers (Fig. 3A). In OPM samples, collagen fibers were detected much less frequently, the main area was filled with a diffuse collagen layer (Fig. 3B). The mean intensity of collagen autofluorescence in dressings and OPM did not show statistically significant difference and averaged 28±1 FC. Comparison of OPM samples with different sizes of bone chips did not reveal any difference in the intensity of collagen luminescence.

According to our observations, the chemical degradation of collagen is manifested by an abrupt increase in fiber autofluorescence and exceeds 60 FC [11]. When examining the OPM samples, only individual insignificant fibers with such a level of luminescence were detected. In general, the collagen in the OPM had normal structural integrity.



A B

Fig. 3. Collagen autofluorescence in the collagen dressings (A) and in osteoplastic material (B) obtained from a 0.8% collagen solution. Magnification 40x

Conclusion

Currently, in manufacturing the osteoplastic material based on collagen and bone chips, no clear grounds have been given that would show the optimality of selected collagen and bone chips parameters [1, 2, 8–10]. It is known that the adhesive properties of collagen matrices largely depend on the collagen concentration in the baseline solution. Matrices with a diffuse distribution of collagen stimulate active cell migration, as well as their proliferation, while such matrices rapidly degrade in the wound [7]. Matrices with high collagen compaction are non-adhesive for human cells, but, at the same time, they have high mechanical strength and resorb much more slowly [6, 8]. Our study showed that, with regard to the conditions of preparing, the osteoplastic material samples can be either adhesive for cells or practically non-adhesive without toxicity. Thus, depending on the goals set, we can

obtain osteoplastic materials with different biological properties. One should take into account that the combination of bone chips and collagen sol in acetic acid causes acidogenicity of bone grafts after lyophilization. In many ways, this may be due to the formation of dihydrogen phosphate as a result of the acetic acid contact with calcium phosphate in the bone chip composition. Our study showed that preliminary soaking of the osteoplastic material in an isotonic sodium chloride solution significantly reduces the acidogenicity and toxicity of the osteoplastic material in cell culture. On the other hand, under in vivo conditions, the toxicity of non-soaked osteoplastic materials might be less pronounced due to various forms of biological activity that are absent in vitro. For a more detailed assessment of the osteoplastic material biocompatibility, as well as its adhesive and reparative properties, it is necessary to conduct further studies on experimental animals.

Conclusions

1. Osteoplastic material based on bone chips and collagen sol isolated by acid extraction method is acidogenic and toxic. The original samples of osteoplastic materials reduce the pH of the Dulbecco's modified Eagles culture medium from the normal value of 7.0–7.2 to 3.3–4.5, thereby preventing the adhesion and proliferation of M-22 cells, and cause severe disturbances in the structure of cell membranes; this toxic effect leads to mass death of M-22 cells in vitro.

2. To effectively reduce acidogenicity and toxicity, samples of osteoplastic material should be kept in an isotonic sodium chloride solution (0.9% NaCl) for at least 30 minutes before use. This method is more efficient than washing the mixture of bone chips and collagen before lyophilization. When ready-made osteoplastic materials were soaked for 30–60 min in an isotonic sodium chloride solution, the cell

density at the culture well bottom and on the surface of the matrices was 1.5–3 times statistically significant higher than the density figures in the experiments where the mixture of bone chips and collagen had preliminarily been washed with distilled water.

3. The use of large bone chips (of 800 μm in diameter) reduces the adhesive properties of the finished osteoplastic materials. Samples of bone graft material obtained using 0.8% collagen solution are 2-2.5 times statistically significant more adhesive to human cells compared to bone graft materials obtained of 1.1% collagen solution.

References

1. Kirilova IA, Baitov VS, Podorozhnaya VT, Pochueva NYu. Morphological presentation of osteogenesis in experimental application of KOSTMA osteoplastic material. *Hir Pozvonoc.* 2007;(4):58–61. (In Russ.).

2. Kirilova IA, Podorozhnaya VT, Ardashev IP, Chernitsov SV. Diffe-rent kinds of osteoplastic materials for bone structure restoration. *Polytrauma.* 2008;(4):60–64. (In Russ.).

3. Walsh W, Oliver RA, Christou C, Lovric V, Walsh ER, Prado GR, Hai-der T. Critical size bone defect healin-g using collagen–calcium phosphate bone graft materials. *PLoS ONE.* 2017;12(1):e0168883. https://doi.org/10.1371/journal.pone.0168883

4. Yoshida K, Sumita Y, Marukawa E, Harashima M, Asahina I. Effect of platelet-rich plasma on bone engineering with an alloplastic substitute containing BMP2. *Biomed Mater Eng.* 2013;23(3):163–172. https://doi.org/10.3233/BME-130741

5. Tanaka H, Toyoshima T, Atsuta I, Ayukawa Y, Sasaki M, Matsushita Y, et al. Additional effects of platelet-rich fibrin on bone regeneration in sinus augmentation with deproteinized bovine bone

mineral: preliminary results. *Implant Dent.* 2015;24(6):669–674. https://doi.org/10.1097/ID.0000000000000306

6. Civinini R, Macera A, Nistri L, Redl B, Innocenti M. The use of autologous blood-derived growth factors in bone regeneration. *Clin Cases Miner Bone Metab.* 2011;8(1):25–31.

7. Zhang L, Zhou Y, Su D, Wu S, Zhou J, Chen J. Injectable, self-healing and pH responsive stem cell factor loaded collagen hydrogel as a dynamic bioadhesive dressing for diabetic wound repair. *J Mater Chem B.* 2021;9(29):5887–5897. https://doi.org/10.1039/d1tb01163d

8. Kane RJ, Weiss-Bilka HE, Meag-her MJ, Liu Y, Gargac JA, Niebur G, et al. Hydroxyapatite reinforced collagen scaffolds with improved architecture and mechanical properties. *Acta Biomater.* 2015;17:16–25. https://doi.org/10.1016/j.actbio.2015.01.031

9. Jain G, Blaauw D, Chang SJ. A comparative study of two bone graft Substitutes-InterOss® collagen and OCS-B collagen®. *Funct Biomater.* 2022;13(1):28. https://doi.org/10.3390/jfb13010028

10. Fan Q, Zeng H, Fan W, Wu T, Sun J, Yan Q, et al. Ridge preservation of a novel extraction socket applying Bio-Oss® collagen: an experimental study in dogs. *J Dent Sci.* 2021;16(3):831–839. https://doi.org/10.1016/j.jds.2021.03.005

11. Vaza AYu, Fain AM, Borovkova NV, Galankina IE, Makarov MS, Zabavskaya OA, et al. The first experience of using the developed modified allogenic bone grafts in the surgical treatment of patients with severe fractures of the surgical neck of the humerus. *Russian Sklifosovsky Journal "Emergency Medical Care".* 2021;10(1):83–90. (In Russ.). https://doi.org/10.23934/2223-9022-2021-10-1-83-90

12. Vaza AYu, Fain AM, Ivanov PA, Klyukvin IYu, Slastinin VV, Borovkova NV, et al. Analysis of the application of different bone grafting procedures in patients with intra-articular fractures.

Transplantologiya. The Russian Journal of Transplantation. 2015;(4):6-12. (In Russ.).

13. Ermolov AS, Smirnov SV, Khvatov VB, Istranov LP, Koniushko OI, Kolokolchikova EG, et al. The use of bioactive wound dressing, stimulating epithelial regeneration of IIIa-degree burn wounds. *Bull Exp Biol Med.* 2008;146(1):153–157. (In Russ.). https://doi.org/10.1007/s10517-008-0225-0

14. Khvatov VB, Svishchev AV, Vaza AYu, Borovkova NV, Mironov AS, Pokhitonov DYu, et al. Method of manufacturing a lyophilized allograft bone. *Transplantologiya. The Russian Journal of Transplantation.* 2016;(1):13–18. (In Russ.).

15. Makarov MS, Storozheva MV, Borov-kova NV, Ponomarev IN. Growth-stimulating effect of platelet preparations obtained by different methods on human fibroblast culture. *Bull Exp Biol Med.* 2022;174(1):159–163. (In Russ.). https://doi.org/10.1007/s10517-022-05666-1

Information about the authors

Andrey A. Ofitserov, Researcher of the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine, https://orcid.org/0000-0003-2170-0009, OfitserovAA@sklif.mos.ru

25%, study design development, obtaining data for analysis, analysis of the obtained data, writing the text of the manuscript

Maksim S. Makarov, Cand. Sci. (Biol.), Senior Researcher of the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine, https://orcid.org/0000-0002-2184-2982, MakarovMS@sklif.mos.ru

25%, study design development, obtaining data for analysis, analysis of the obtained data; writing the text of the manuscript; review of publications on the topic of the article

Natalya V. Borovkova, Dr. Sci. (Med.), Head of the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine; associate Professor of the Department of Transplantology and Artificial Organs, N.I. Pirogov Russian National Research Medical University, https://orcid.org/0000-0002-8897-7523, BorovkovaNV@sklif.mos.ru

25%, study design development, analysis of the obtained data, writing the text of the manuscript

Mayya V. Storozheva, Researcher of the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine, https://orcid.org/0000-0003-1927-2404, StorozhevaMV@sklif.mos.ru

15%, obtaining data for analysis, analysis of the obtained data

Ivan N. Ponomarev, Cand. Sci. (Med.), Senior Researcher of the Scientific Department of Biotechnologies and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine, https://orcid.org/0000-0002-2523-6939, PonomarevIN@sklif.mos.ru

10%, obtaining data for analysis, analysis of the obtained data

The article was received on February 9, 2023;
approved after reviewing February 15, 2023;
accepted for publication March 29, 2023