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Changes in Rat Bone Tissue at the Site of the Defect *In Vivo* under the Effect of a Cryogenically Structured Albumin Sponge Containing a Bioregulator

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We performed a morphological study of the bone tissue after implantation of a cryogenically structured albumin sponge containing a bioregulator isolated from blood serum into an extensive experimental defect of the femur. By day 90, no complete reparation of the bone tissue was achieved in the control group (without implantation of 3D carrier), a loose spongy bone is formed at the site of the defect. After implantation of the 3D carrier without serum bioregulator, the defect was closed, but the formed bone was loose and contained no inflammation foci. After the defect was filed with the albumin sponge with the bioregulator, the repair pattern corresponded to the processes of epimorphic tissue regeneration. The results suggest that cryogenically structured protein material in combination with a serum bioregulator ensured complete restoration of the bone tissue.

Key Words: *reparation of bone; 3D technologies; cryogels; bioregulators*

3D technologies are now actively used in surgery, especially in orthopedics and traumatology, for the regeneration of extensive and difficult to repair bone defects [2,3,8]. In these cases, biocompatible and resorbable materials containing various functionally active additives and/or living cells are often used to fill these defects [4,12,14].

Of particular interest are so-called cryogels and cryostructurates [9], because their 3D macroporous morphology is similar to the structure of cancellous bone [10,15]. The use of cryogenically structured spongy 3D carriers makes it possible, on the one hand, to introduce bioactive substances that stimulate osteogenesis, and, on the other hand, to populate them with cells, for example, mesenchymal or fibroblasts, which also promote regeneration of the bone tissue [11]. Various inorganic and organic substances are used as materials for implantation into the defect area. Since the process of bone restoration is long-lasting and takes about 2-3 months, it is important to have the information about the morphology of the bone tissue filling the extensive defect at later stages of repair.

We have previously shown [1] that the use of cryogenically structured spongy 3D carriers containing a new group bioregulator, a protein-peptide complex (PPC) isolated from the bovine blood serum [7], ac-

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tivates reparative osteogenesis at the early stages (14 days) of *in vivo* wound healing in rats. In these experiments, different 3D matrices were used, of which the macroporous cryogel based on BSA turned out to be the most promising.

Here we studied the features of bone defect repair at delayed terms (90 days) of healing induced by PPC introduced into cryogenically structured spongy 3D carriers based on BSA.

MATERIALS AND METHODS

Cryogenically structured spongy carrier (a cylinder with a diameter of 5 mm and a height of 5 mm) was prepared from BSA as described previously [13]. Then the obtained spongy samples were incubated in an aqueous solution of a bioregulator, frozen, and lyophilized. The specific density of this albumin 3D carrier in dry form is ~0.11 g/cm^3, the total porosity of the biopolymer material is more than 85%, and the size of large communicating pores in the sponge equilibrium-swollen in water varies from 50 to 150 μm [13]. The same carriers without the bioregulator, also freeze-dried, served as the control.

The bioregulator was obtained from bovine blood serum (a commercial preparation used as a nutritional supplement for culture media, BioloT) by a method including proteins precipitation with ammonium sulfate, dialysis, concentration, and isoelectric focusing in a sucrose density gradient at pH 3-10. High degree of purification of the bioregulator was shown by PAAG electrophoresis and reverse phase HPLC [7].

In vivo experiments were carried out on male Wistar rats weighing 180-220 g. The animals were kept under standard vivarium conditions. Standard 2×4 mm defects in the femur diaphysis at a distance of 0.5 cm from the hip joint was created with a drill under ether anesthesia. The defects were filled with the test materials, 3D carriers with and without PPC, and the soft tissues and the skin were sutured.

The following experimental groups were formed (6 rats each): control (group 1; bone defect was not filled), implantation of the spongy albumin 3D carrier (group 2), and implantation of the spongy albumin 3D carrier containing PPC in a final concentration of 10^{-10} mg/ml.

The state of bone defects was studied on day 90, because delayed effects of the studied substances on restoration of the bone tissue can be evaluated during this period. On day 90 after surgery, the animals were sacrificed, the bone material from the defect area was isolated, fixed in formalin, decalcified, and embedded in paraffin; histological sections (10 μm) were prepared, stained with hematoxylin and eosin, and examined under a light microscope. For microscopy, 50 slides were prepared from each animal in the group (8 sections per slide).

RESULTS

Cryogenically structured material, macroporous cryogel based on a non-toxic and biocompatible polymeric precursor BSA, was used as a 3D carrier for the osteoinductive bioregulator. This matrix was chosen because it is bioresorbable (is cleaved by proteinases) and more effective than alginate and chitosan sponges at the early stages of bone defect healing [1]. Cryogels loaded with PPC were tested as possible osteoinducers and osteoconductors in a model of an experimental bone defect in rats *in vivo* on day 14 after surgery [1].

At delayed terms (90 days) of healing, signs of bone-forming activity of different intensity were observed (Fig. 1). It was most pronounced after implantation of albumin 3D carrier containing PPC (group 3).

Thus, in the control group, complete restoration of bone tissue was not achieved (the defect was not completely closed) and dense bone tissue was not formed; the defect contained a newly formed loose spongy bone tissue with numerous blood vessels (Fig. 1, *a*, *b*).

In the case of using albumin 3-D carrier without PPC (group 2), the defect was completely closed. The newly formed bone was loose spongy, without foci of inflammation and blood vessels. Osteon lacunae were large, which indicates the formation of incompletely formed dense bone (Fig. 1, *c*, *d*), less dense than native bone tissue.

After implantation of the spongy albumin carrier containing PPC (group 3), the defect was completely closed with the formation of the bone tissue of different degree of maturity: dense bone tissue, as well as cancellous tissue with elements of cartilage tissue in 50% rats (formation of fragments of immature bone tissue with elements of cartilage tissue; Fig. 1, *d*, *e*). In other rats of this group, dense bone tissue was completely restored without intermediate stages of new bone formation. The presence of bone marrow cells in the newly formed bone is worthy of note. Fragments of dense bone tissue with small lacunae in the osteons were also seen.

The results suggest that implantation of the 3D carrier based on BSA and containing PPC isolated from blood serum ensured complete restoration of a large defect in the bone. The pattern of this repair corresponds to the processes of epimorphic tissue regeneration. It should be noted that the formation of bone tissue in the area of the experimental defect occurred by the mechanism of embryonic bone development, because the formation of cartilaginous tissue and coarse fibrous tissue is noted at certain stages of recovery (Fig. 1, *e*). A similar mechanism of the

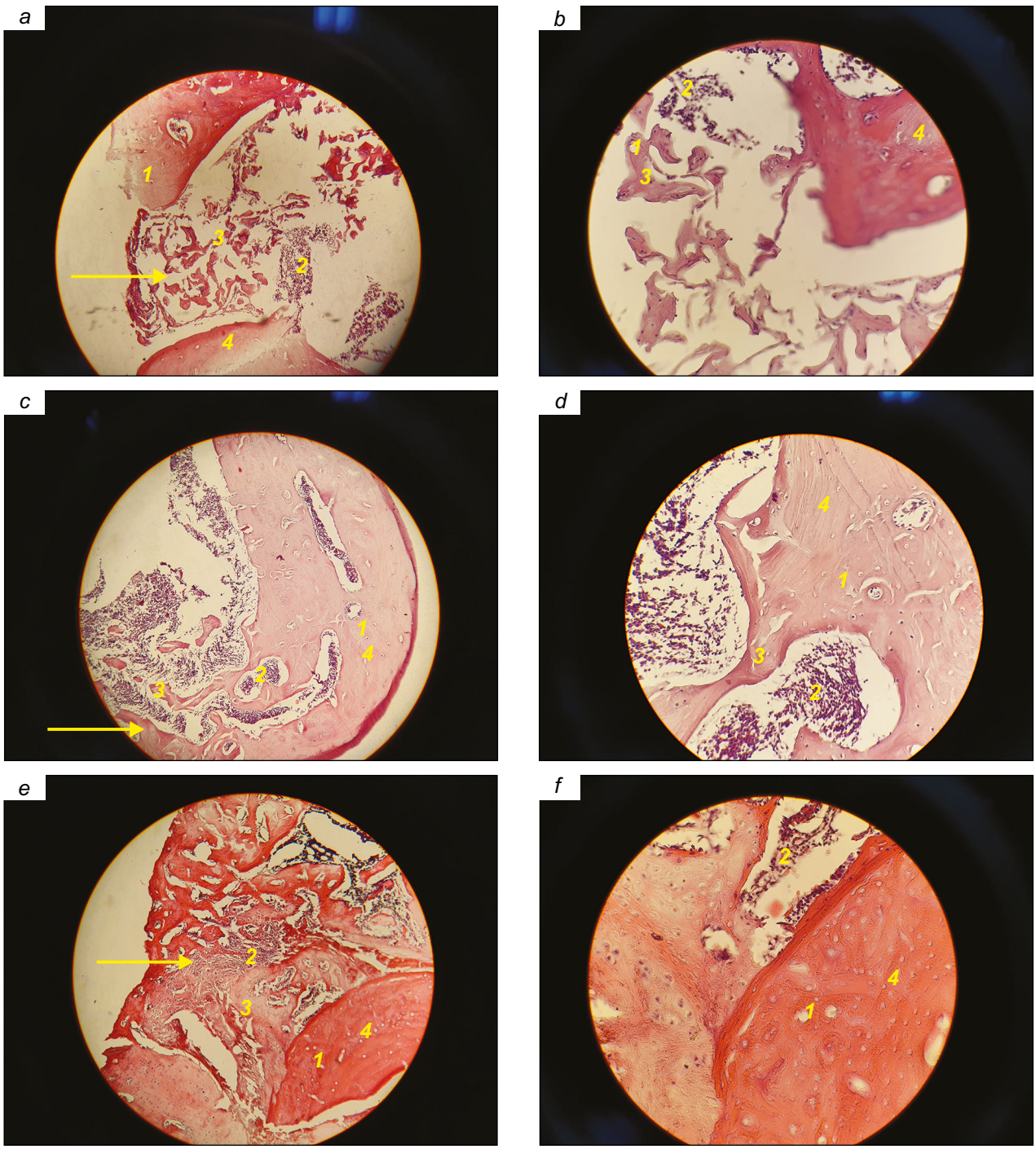

Fig. 1. Histological picture of a cross section of rat femur in the area of the defect 90 days after surgery. Staining with hematoxylin and eosin. ×100 (*a*, *c*, *e*), ×400 (*b*, *d*, *f*). *a*, *b*) Group 1 (control), *c*, *d*) group 2 (implantation of albumin 3D carrier without bioregulator), *e*, *f*) group 3 (implantation of albumin 3D carrier containing PPC). *1*) Osteons, *2*) bone marrow, *3*) cancellous bone tissue, *4*) dense bone tissue. Arrows show the defect area.

action of this bioregulator was also observed during skin regeneration in a model of wound healing in rats and mice [5]. This can be due to activation of cellular sources of connective tissue regeneration by this bioactive PPC [6]. Thus, in cases of extensive damage to various types of connective tissues (skin, bone, cartilage, *etc.*), when their regeneration and restoration are difficult and are accompanied by the formation of a scar or callus, the use of 3D carriers containing a bioregulator isolated from blood serum leads to activation of reparation pathways with restoration of all structures typical of normal tissue.

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REFERENCES

1. Krasnov MS, Shaikhaliev AI, Korshakov EV, Efimenko MV, Soloshenkov PP, Davidova TR, Zvukova ND, Sinitskaya ES, Yamskova VP, Yamskov IA, Lozinsky VI. Induction of Osteogenesis in Rat Bone Tissue Using Cryogenically Structured Porous 3D Materials Containing a Bioregulator. Bull. Exp. Biol. Med. 2019;168(1):99-103. doi: 10.1007/s10517-019-04657-z
2. Muraev AA, Gazhva YV, Ivashkevich SG, Riabova VM, Korotkova NL, Semyonova YA, Metsuku IN, Faizullin RL, Ivanov SY. A novel approach to alveolar bone complex defects 3D reconstruction. Sovremen. Tekhnol. Med. 2017;9(2):37-45. Russian.
3. Muraev AA, Ivanov SYu, Ivashkevich SG, Gorshenev VN, Teleshev AT, Kibardin AV, Kobets KK, Dubrovin VK. Orthotopic bone implants for bone regeneration. Stomatologiya. 2017;96(3):36-39. Russian.
4. Muraev AA, Ivanov SYu, Ryabova VM, Artifexova AA, Volodina EV, Polyakova IN. Toxicity and Biological Activity of a New Bone Substituting Material Based on Nondemineralised Collagen Containing Vascular Endothelial Growth Factor. Sovremen. Tekhnol. Med. 2012;(3):19-25. Russian.
5. Stretskii GM, Krasnov MS, Rybakova EY, Avdeenko OE, Tikhonov VE, Shaikhaliev AI, Yamskova VP, Yamskov IA. Effect of a composition containing chitosan gel and a bioregulator from blood serum on healing of purulent wounds in mice. Bull. Exp. Biol. Med. 2012;152(4):524-7. doi: 10.1007/s10517-012-1568-0
6. Yamskova VP, Krasnov MS, Yamskov IA. On mechanisms underlying regeneration and reparation processes in tissues. Bull. Exp. Biol. Med. 2010;149(1):140-143. doi: 10.1007/s10517-010-0893-4
7. Yamskova VP, Krasnov MS, Yamskov IA. New Experimental and Theoretical Aspects in Bioregulation. The Mechanism of Action of Membranotropic Homeostatic Tissue-Specific Bioregulators. Saarbrücken, 2012. Russian.
8. Liu X, Ma PX. Polymeric scaffolds for bone tissue engineering. Ann. Biomed. Eng. 2004;32(3):477-486.
9. Lozinsky VI. Cryostructuring of polymer systems. 50. Cryogels and cryotropic gel-formation: terms and definitions. Gels. 2018;4(3):77. doi: 10.3390/gels4030077
10. Mahumane GD, Kumar P, du Toit LC, Choonara YE, Pillay V. 3D scaffolds for tissue regeneration: architectural challenges. Biomater. Sci. 2018;6(11):2812-2837.
11. Petrenko YA, Ivanov RV, Petrenko AY, Lozinsky VI. Coupling of gelatin to inner surfaces of pore walls in spongy alginate-based scaffolds facilitates the adhesion, growth and differentiation of human bone marrow mesenchymal stromal cells. J. Mater. Sci. Mater. Med. 2011;22(6):1529-1540.
12. Qi C, Yan X, Huang C, Melerzanov A, Du Y. Biomaterials as carrier, barrier and reactor for cell-based regenerative medicine. Protein Cell. 2015;6(9):638-653.
13. Rodionov IA, Grinberg NV, Burova TV, Grinberg VY, Lozinsky VI. Cryostructuring of polymer systems. Proteinaceous wide-pore cryogels generated by the action of denaturant/reductant mixtures on bovine serum albumin in moderately frozen aqueous media. Soft Matter. 2015;11(24):4921-4931.
14. Shakya AK, Kandalam U. Three-dimensional macroporous materials for tissue engineering of craniofacial bone. Br. J. Oral Maxillofac. Surg. 2017;55(9):875-891.
15. Wubneh A, Tsekoura EK, Ayranci C, Uludağ H. Current state of fabrication technologies and materials for bone tissue engineering. Acta Biomater. 2018;80(1):1-30.