Research Article

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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

A.Y. Vaza*✉*, A.M. Fain, N.V. Borovkova, I.E. Galankina, M.S. Makarov, O.A. Zabavskaya, R.S. Titov, L.N. Zimina, G.A. Nefedova, V.B. Bondarev, A.S. Mironov, I.N. Ponomarev

Trauma and Orthopedics Department
N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department
3 B. Sukharevskaya Sq., Moscow, 129090, Russian Federation

✉ **Contacts:** Aleksandr Y. Vaza, Leading Researcher, Department of Emergency Trauma of the Musculoskeletal System, N.V. Sklifosovsky Research Institute for Emergency Medicine.
Email: vazal@inbox.ru

BACKGROUND Fractures of the proximal humerus are more common in older adults. Two options for surgical treatment of the same fractures are routinely performed — primary arthroplasty and osteosynthesis with metal fixators. Both methods are most effective in elderly patients for augmentation of the proximal humerus. For this purpose, methyl methacrylate cement or bone allo-/autografts are used. However, the incidence of unsatisfactory functional results after surgery remains high.

AIM OF STUDY To develop allogeneic graft to strengthen the proximal humerus in the osteosynthesis of a comminuted fracture with a lack of bone tissue which has osteoconductive properties and capable of being a carrier of biologically active substances.

MATERIAL AND METHODS The head, neck and portion of diaphysis of a fibula of a cadaver were used for a graft. The graft 6–8 cm long was formed and saturated with a collagen solution. Ten patients with 4-fragment fractures of the proximal humerus underwent surgery. X-rays and computed tomography of the operated joint were performed the day after surgery, and then, 6 weeks, 6 months, and one year after the surgery. The follow-up period ranged from 6 to 18 months.

RESULTS When evaluating osteoconductive effect in cell culture during saturation with collagen, the content cells on the surface of bone was 5–8 thousand/cm^2 and 16–18 thousand/cm^2 on the sample, saturated with plasma and growth factors. The studied bone grafts were biocompatible and non-toxic for fibroblast culture. On the surface of the bone graft without collagen adhesion of minimum number of cells occurred. After saturation of the graft with collagen, the adhesive activity of cells on the bone matrix increased, which indicated the presence of an osteoconductive effect. The addition of plasma saturated with growth factors promoted an increase in the number of adhered cells; therefore, the graft can be used as a carrier of biologically active substances.

Postoperative X-ray didn't reveal secondary varus displacement of the head in any patient (except for ununiformed fracture case).

CONCLUSION The developed graft is non-toxic, does not possess immunogenicity, has more pronounced osteoconductive properties in comparison with native bone allografts, which contributes to its colonization with cells. The use of such a graft in clinical practice makes it possible to achieve healing of the fracture and prevent secondary displacement of fragments.

Keywords: shoulder joint, proximal humerus fractures, allograft

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Affiliations

Aleksandr Y. Vaza	Leading Researcher, Department of Emergency Trauma of the Musculoskeletal System, N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department; https://orcid.org/0000-0003-4581-449X, vazal@inbox.ru; 40%, text writing, selecting material, conducting operations, analyzing the data obtained
Aleksey M. Fain	Head of the Department of Emergency Trauma of the Musculoskeletal System, N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department; https://orcid.org/0000-0001-8616-920X, fainam@sklif.mos.ru; 10%, general supervision of scientific research, revision of the text of the article
Natalia V. Borovkova	Head of the Scientific Department of Biotechnology and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine; https://orcid.org/0000-0002-8897-7523, borovkovanv@sklif.mos.ru; 5%, supervision of the experimental part of scientific research, revision of the part of the text devoted to the description of experimental research
Irina Y. Galankina	Head of the Scientific Department of Pathological Anatomy, N.V. Sklifosovsky Research Institute for Emergency Medicine; galankinaie@sklif.mos.ru; 5%, supervision of histological examination, revision of the text on histological examination

Maksim S. Makarov	Senior Researcher, Department of Biotechnology and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine; makarovms@sklif.mos.ru; 5%, conducting an experiment with fibroblasts, making illustrations, writing a text about the experiment
Olga A. Zabavskaya	Senior Lecturer, Training Center, N.V. Sklifosovsky Research Institute for Emergency Medicine; https://orcid.org/0000-0001-68 93-7973, zabavskayaoa@sklif.mos.ru; 5%, analysis, description of CT scans, writing a text on computed tomography
Roman S. Titov	Traumatologist-orthopedist, Traumatology Department No. 1, N.V. Sklifosovsky Research Institute for Emergency Medicine; https://orcid.org/0000-0002-2960-8736, titovrs@sklif.mos.ru; 5%, operations, collection and analysis of clinical material
Larisa N. Zimina	Leading Researcher, Laboratory of Pathomorphology with Histochemistry, N.V. Sklifosovsky Research Institute for Emergency Medicine; ziminaln@sklif.mos.ru; 5%, production, study, analysis of histological material and preparations, illustrations
Galina A. Nefedova	Senior Researcher, Laboratory of Pathomorphology with Histochemistry, N.V. Sklifosovsky Research Institute for Emergency Medicine; https://orcid.org/0000-0002-8452-8499, nefedovaga@sklif.mos.ru; 5%, production, study, analysis of histological material and preparations, writing a text dedicated to histological studies
Vasily B. Bondarev	Traumatologist-orthopedist, Traumatology Department No. 1, N.V. Sklifosovsky Research Institute for Emergency Medicine; https://orcid.org/0000-0002-1183-3644, bondarevvb@sklif.mos.ru; 5%, selection and processing of literature used in the article, preparation of an article for printing procedure
Aleksandr S. Mironov	Surgeon, Head of the Department of Tissue Preservation and Graft Production with an Operating Unit, N.V. Sklifosovsky Research Institute for Emergency Medicine; mironovas@sklif.mos.ru; 5%, transplant production, writing a transplant production test
Ivan N. Ponomarev	Researcher of the Department of Biotechnology and Transfusiology, N.V. Sklifosovsky Research Institute for Emergency Medicine; https://orcid.org/0000-0002-2325-6939, ponomarevin@sklif.mos.ru ; 5%, participation in the experiment, illustrations for the experiment

INTRODUCTION

The most severe, multi-fragment fractures of the proximal humerus usually occur in the elderly. This is due to a number of factors, one of which is a change in bone tissue, which additionally complicates the choice of an effective and safe method of surgical treatment [1, 2].

Currently, there are two widespread options for the surgical treatment of such fractures - primary arthroplasty and osteosynthesis with metal fixators. The main disadvantage of primary arthroplasty is a large number of unsatisfactory functional results [3]. The most popular and effective type of osteosynthesis for multifragmental fractures of the proximal humerus is osteosynthesis with plates with angular stability [4]. Poor bone quality in elderly patients and bone deficiency after a fracture, when the head of the humerus looks like an eggshell, complicates both reduction and fixation of the fragments. Disruption of blood supply to the head does not improve the situation either [5]. The share of secondary varus displacement of fragments associated with unsatisfactory fixation and eruption of screws, associated with head collapse due to aseptic necrosis, can reach 49% [6].

In order to strengthen the cancellous bone, improve fixation, augmentation of the proximal humerus is used. According to some authors, functional results after osteosynthesis with augmentation are superior to functional results after primary arthroplasty in severe fractures of the proximal humerus [3].

The first option is augmentation with methyl methacrylate cement [7]. The disadvantages of this method include: heating the cement to high temperatures during polymerization, which increases the risk of bone tissue necrosis, toxicity, and lack of bioresorption.

The second most popular method of augmentation today is the use of a graft from the diaphyseal part of the fibula, which also has a number of disadvantages [8]. When using an autologous graft, the operation

time increases, and the patient receives additional trauma [9]. This graft consists of tubular bone, which does not have an osteoconductive effect and degrades poorly. Even 2 years after the operation, it is visible on X-ray as clearly as immediately after the operation. Tubular bone cannot serve as a carrier of biologically active substances. Some publications say that the use of such grafts does not lead to an improvement in functional and radiological results [10].

Purpose: to develop an allogeneic graft for strengthening the proximal humerus during osteosynthesis of comminuted fractures accompanied by a deficiency of bone tissue, possessing osteoconductive properties and capable of being a carrier of biologically active substances.

MATERIAL AND METHODS

The head, neck and part of the diaphysis of the fibula of the cadaver were used to make the graft. The fibula was taken from a tissue donor no later than 24 hours after death in accordance with the RF law of December 22, 1992 No. 4180-1 "On transplantation of human organs and (or) tissues" and stored during quarantine at a temperature of minus 40 ° C. After confirming the safety of the biomaterial, a fragment was cut from the fibula, including the head, neck, and part of the diaphysis, with a total length of 6–8 cm. The cortical layer of the graft, in our opinion, strengthens the fracture zone, and the cancellous substance serves as a conductor for the restoration of the bone tissue of the shoulder head. To accelerate the process of remodeling of the bone allograft while maintaining the strength characteristics, its weight was reduced by forming through holes with a 2 mm drill at a distance of 3–5 mm from each other. This approach made it possible to create additional niches for filling with type I collagen solution. Saturation with collagen solution was used to increase the osteoconductive properties of the graft. The bone graft blank was impregnated with a 0.7–0.8% solution of type I allogeneic collagen by repeated centrifugation. The graft soaked in collagen solution was lyophilized and sterilized with gamma rays. After lyophilization, the collagen solution turned into a sponge that filled both the formed holes and the intertrabecular spaces of the fibular head.

In order to assess the osteoconductive effect and biocompatibility of the graft, fragments of a bone graft without collagen (control), a bone graft with a collagen sponge (experiment 1), and a bone graft with a collagen sponge impregnated with plasma saturated with growth factors (experiment 2). To these fragments was added a suspension of human fibroblasts in DMEM medium (Dulbecco's Modified Eagle Medium - basal medium for supporting the growth of various mammalian cells) with 10% fetal serum of cattle “Gibco” at the rate of 150x103 cells per well and incubated at + 37 ° C in a CO2 incubator with a CO2 concentration of 5%. After 3 days, trypoflavin solution was added to the wells to count cells at the bottom of the well and determine their adhesion to the graft, and microscopic examination of the fragments was carried out using a Nicon Eclipse 80i fluorescence microscope at x50 and x200 magnifications.

After evaluating the graft for biocompatibility and with the permission of the Ethics Committee (Protocol No. 3-19 dated August 19, 2019), we operated on 10 patients with 4-fragment fractures of the proximal humerus (according to Neer 1970). The patients' age is from 35 to 88 years old. All of them developed a fracture as a result of a low-energy injury - a fall on a flat surface. Among the patients there were 2 men and 8 women. All of them underwent osteosynthesis of the proximal humerus with a Philos plate (De Puy-Synthes). During the operation, the proximal humerus was augmented with the developed graft. Before use, the graft was rehydrated with saline for 15 minutes. After that, the graft was inserted into the medullary canal of the humerus from the side of the fracture so that the head of the graft fills the defect in the head of the humerus. If necessary, the head of the graft was formed with Liston nippers. After placing the graft, the fragments were repositioned and fixed with a plate and screws.

A direct projection X-ray of the operated joint was performed the next day after the operation, 6 weeks, 6 months, and one year after the operation. When evaluating radiographs, the angle between the head and diaphysis of the humerus was determined and compared in dynamics. At the same time, multislice computed tomography was performed, the presence of the border between the graft and own bone, the dynamics of the decrease in the thickness of the diaphyseal part of the graft, the quality of the own bone, and signs of graft restructuring were assessed.

The observation period is from 6 to 18 months after the operation.

Of the 10 operated patients, in one case, 9 months after the operation, the fracture did not heal, and therefore, the shoulder joint was replaced with a reversible endoprosthesis. The removed fragment of the humerus (head) containing the graft was examined morphologically to assess bone remodeling.

The head of the humerus was sawn off. After fixation of the material in a 10% solution of neutral formalin, decalcification and embedding in paraffin, the sections were stained with hematoxylin and eosin, picrofuchsin according to Van Gieson and Mallory.

RESULTS AND DISCUSSION

When assessing the osteoconductive effect in cell culture, it was revealed that on the 3rd day of cultivation, human fibroblasts on control samples of bone grafts (not saturated with collagen) were practically not detected (Fig. 1A). At the same time, the content of attached cells on the bone surface was 5–8 thousand / cm^2 on samples of the combined graft saturated with collagen (experiment 1) (Fig. 1B). On a sample saturated with plasma and saturated with growth factors (experiment 2), the content of attached cells on the bone surface was 16–18 thousand / cm^2 (Fig. 1C). At the same time, in all wells, the number of attached cells on the plastic (outside the bone) was 18–20 thousand / cm^2, that is, the presence of bone grafts in the culture wells did not affect the viability of human fibroblasts. Also, there was no increase in the number of deformed or apoptotic cells.

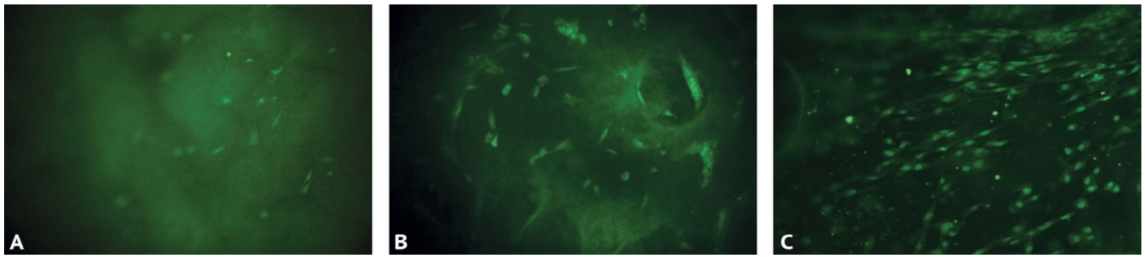


Fig. 1. Colonization of bone grafts by fibroblasts. A — bone graft (control); B — combined bone graft saturated with the type 1 collagen (experiment 1); C — combined bone graft impregnated with plasma saturated with growth factors (experiment 2)

Thus, the studied bone grafts were biocompatible and non-toxic for fibroblast culture. A minimal number of cells adhered to the surface of the bone graft without collagen, which is associated with the absence of specific adhesion ligands. Saturation of the bone graft with type I collagen promoted an increase in the adhesive activity of cells on the bone matrix, which indicates the presence of an osteoconductive effect. The addition of plasma saturated with growth factors increased the number of adherent cells compared to the first two samples. This proves that the combined graft can be successfully used as a carrier of biologically active substances.

Of the 10 operated patients, complications developed in 3 cases:

1. In one patient, the fracture did not heal, which required shoulder arthroplasty. The resected bone fragment was examined histologically as described below.

2. In one observation, 6 months after the operation, the screw eruption was revealed approximately 1 mm behind the subchondral plate, associated with a moderate collapse of the humeral head. Clinically, this did not manifest itself in any way, and the patient still refuses to remove the retainer.

3. In one case, deep suppuration developed, which was managed without removing the fixator and graft. Suppuration did not affect the union of the fracture. The fracture healed.

Control radiography in the postoperative period did not reveal a secondary varus displacement of the humerus head in any case (except for a patient with an ununited fracture). Control multislice computed tomography (MSCT) shows as early as 6 weeks after the operation that the border between the bone tissue of the graft head and the bone tissue of the humerus head is blurred. Six months after the operation, the border between the cancellous bone of the graft and the cancellous bone of the head of the shoulder is no longer defined, which indirectly indicates the restructuring of the graft, that is, the osteoconductive property of the graft. The cortical layer of the diaphyseal part of the graft becomes thinner after 12 months, which is clearly seen on MSCT images over time (Fig. 2, 3).

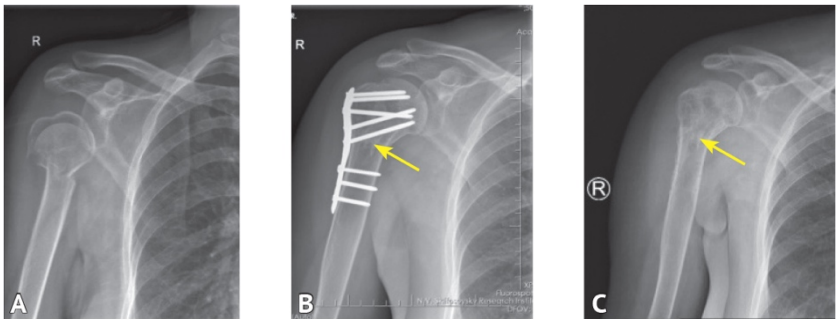


Fig. 2. Radiographs of the right shoulder joint. A — 3-fragment fracture of the proximal metaepiphysis of the right humerus; B — one year after plate osteosynthesis with allograft augmentation (the arrow indicates the residue cortical layer of the graft); C — after removing the plate (one year after the operation). The arrow indicates the residue of the graft cortex

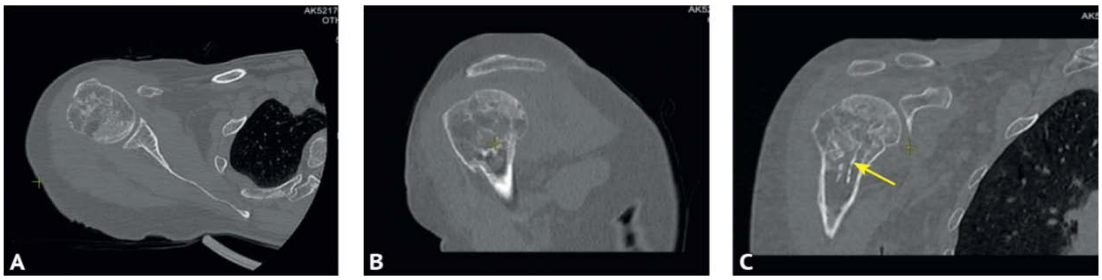


Fig. 3. Multislice computed tomography of the right shoulder joint after plate removal. A — axial slice; B — sagittal reformation; C — front plane reformation (the arrow indicates the residue cortical graft layer)

During the morphological examination of the removed humeral head, it was sawn into fragments. The cut shows a homogeneous spongy bone, dense to the touch. It was not possible to clearly differentiate the tissue of the allograft from its own bone tissue. Histological examination revealed only trabeculae of the own bone in the areas in contact with the cortical zone. Trabeculae had normal morphology, the intensity of autofluorescence of collagen fibers in their composition reached 30–40 foot-candles, which corresponded to the norm (Fig. 4). On the contrary, in the area of the cancellous bone, it was possible to identify both areas of the patient's own bone and fragments of a bone graft. Some fragments of the graft had a basophilic appearance, which made it possible to distinguish them from normal trabeculae in transmitted light. Most of the fragments of the graft had a weakly basophilic coloration, the fibers in their composition did not have dignificant damage when stained with hematoxylin-eosin. However, luminescence analysis showed that the collagen fibers of the graft had a much lower level of autofluorescence compared to healthy bone (Fig. 5, 6).

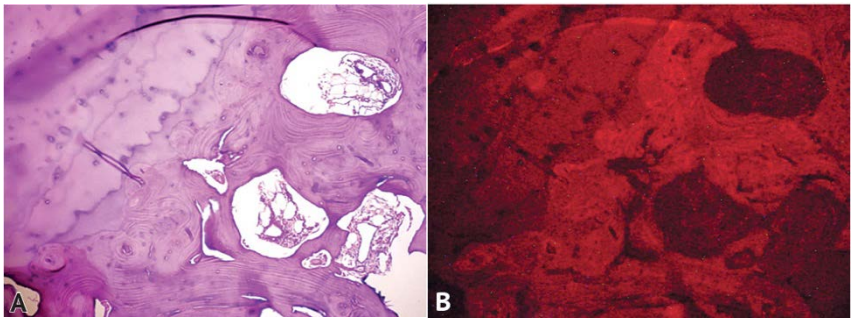


Fig. 4. Zone of the bone defect in the area of contact between the spongy and cortical bones. Magnification x100. A — staining with hematoxylineosin; B — autofluorescence of collagen fibers. Fluorescence level is normal

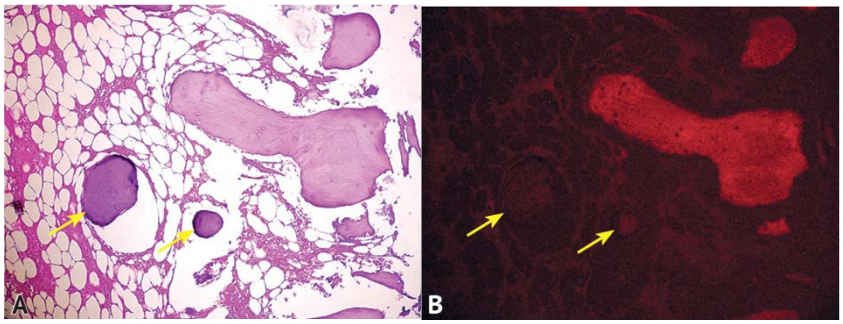
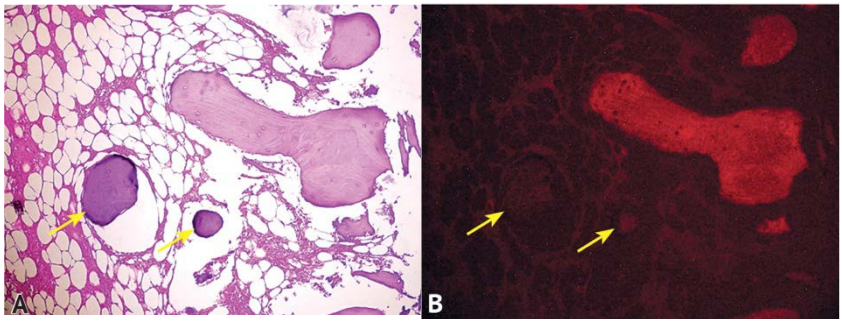


Fig. 5. Identification of bone graft fragments in the area of the cancellous bone. Magnification x100. A — staining with hematoxylin and eosin; B — autofluorescence of collagen fibers. Arrows show the fragments of the graft. In transmitted light, such trabeculae have a basophilic or weakly basophilic appearance, while their luminescence level is very low



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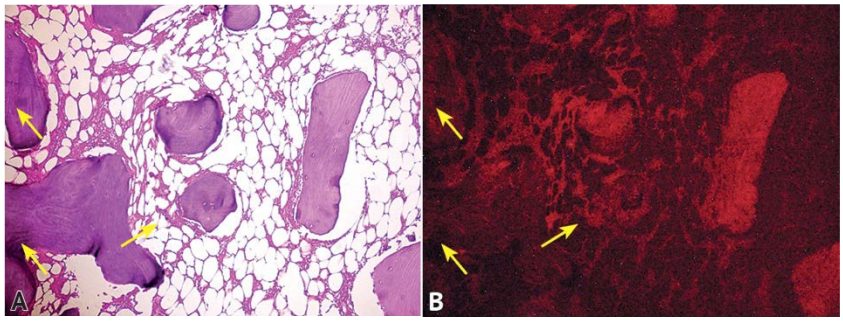

Fig. 6. Identification of bone graft fragments in the area of the cancellous bone (the other part of the head). Magnification x100. A — staining with hematoxylin and eosin; B — autofluorescence of collagen fibers. Arrows indicate graft fragments. In transmitted light, such trabeculae have a basophilic or weakly basophilic appearance, while the level of their luminescence is very low

If in transmitted light the fragments of the bone graft were clearly distinguishable, then in the analysis of autofluorescence these areas had a very low brightness (less than 10 foot-candles), comparable to the background luminescence of the bone marrow surrounding the trabeculae. On different preparations, the fragments of the graft occupied a different proportion of the area and were incorporated into their own bone tissue. According to our observations, under conditions of sharp degradation, the level of autofluorescence of collagen fibers, as a rule, significantly increases and exceeds 60 foot-candles. However, no such structures with very high autofluorescence were found in the graft fibers. The absence of cellular infiltration around fragments of allogeneic bone tissue should be noted, which indicates the absence of the development of a rejection reaction.

CONCLUSION

Thus, the developed combined allogeneic graft from the head of the fibula, saturated with type I collagen, is non-toxic, does not possess immunogenicity, while it has more pronounced osteoconductive properties compared to native bone allografts, which contributes to its colonization with cells. The graft can serve as a carrier of biologically active substances. The use of such a graft in clinical practice makes it possible to achieve fracture fusion, prevent secondary displacement of fragments and the development of collapse of the humerus head in most cases.

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