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Preclinical evaluation of the efficacy and safety of a new osteoplastic material of xenogenic origin containing vancomycin or meropenem

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Abstract

Bone xenomatrix is an available material for plasty due to its availability and possible significant modification. **The purpose** was comparative evaluation of the efficacy and safety of xenogenic bone graft material impregnated with antibiotics, vancomycin or meropenem, in an experiment on a model of long bone defect healing in rabbits. **Methods** The study was performed on 28 male rabbits aged from 8 months to 1.2 years. All animals were modeled with a cavity defect of the right and left distal femoral metaphysis measuring 4 × 4 × 6 mm. Bone matrix blocks of the same size were implanted into the defect cavity. Animals of group 1 (n = 8, control) were implanted with a free "clean" bone block. Animals of group 2 (n = 10) with a bone block saturated with vancomycin. Animals of group 3 (n = 10) with a bone block impregnated with meropenem. To assess the effectiveness and safety of the material, clinical, radiological, pathomorphological, histological, and laboratory methods were used. **Results** X-ray signs of substitution of the studied materials in the defect in animals of group 1 were noted by 182 days, in group 2 – by 84 days, in group 3 – about 182 days. In each group, there was a complication, arthrosis of the knee joint (one animal in each group). According to the histological study, it was found that in groups 2 and 3, a complete elimination of xenomaterial in the middle part of the defect and its replacement with trabecular bone was noted by 182 days after implantation. The severity of irritating action of the materials in the animals of groups 2 and 3 did not exceed the control value. The laboratory blood tests in the animals of groups 2 and 3 also did not reveal significant differences with group 1. **Conclusion** The developed osteoplastic materials based on bovine bone xenomatrix, impregnated with vancomycin or meropenem, have acceptable safety and efficacy characteristics.

Keywords: bone xenomaterial, vancomycin, meropenem, long bone defect

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INTRODUCTION

The growing need for osteoplastic materials in traumatology and orthopedics has currently resulted in the increased use of the materials of xenogenic origin in clinical practice [1, 2]. Clinical experience with the application of such materials indicates that their efficacy and safety are quite comparable with the materials of allogenic origin [3-5]. Among the main detected disadvantages of xenomaterials is their insufficient osteogenic activity and possible infectious complications that occur in the post-implantation period [6]. The frequency of the latter, according to a number of data sources, can reach 10 to 16 % [7–9].

These shortcomings can be resolved by modifying the material. The main direction of such modification is the use of the xenomatrix as a carrier for various stimulants [10, 11]. The following directions exist in the modification: 1) xenomatrix impregnation with biologically active substances [12, 13]; 2) creation of tissue engineering structures based on the xenomatrix [14–16]; 3) chemical modification of the mineral and organic components of the xenomatrix [17, 18]. Among the variants of the first direction, the most popular is the modification of xenomaterial with substances that prevent infection [19]. In this regard, we have developed a xenogenic osteoplastic material impregnated with antibiotics, vancomycin and meropenem.

Purpose of the study was comparative evaluation of the efficacy and safety of xenogenic bone graft material impregnated with antibiotics, vancomycin and meropenem, in an experiment on a model of long bone defect healing in rabbits.

MATERIAL AND METHODS

Study design The study was performed on 28 male rabbits aged from 8 months to 1.2 years, weighing from 3 to 4.5 kg. A cavity defect of the right and left distal femoral metaphysis measuring 4 × 4 × 6 mm was modeled in all the animals. Bone matrix blocks of the same size were implanted into the defect cavity for implementation of the tests. The material was bovine bone tissue processed with the technology described by the authors in the patents (utility model No. 191700, inventions No. 2708639, No. 2712701). To clean the material and impregnate antibiotics, the technology of supercritical fluid extraction was used.

Animals of group 1 (n = 8, control) were implanted with a free "clean" bone block (xenogenic material Matrix

osteoplastic "Bio-Ost", RZN 2015/3086). Animals of group 2 (n = 10) received bone blocks impregnated with vancomycin. Animals of group 3 (n = 10) had bone block saturated with meropenem.

The period of planned euthanasia was days 84 and 182 after implantation (half of the animals in each group were euthanized at each period).

Defect modeling In the operation room, under general anesthesia, a cavity defect of the distal metaphysis of the femur was modeled in animals. For sedation, solutions of diphenhydramine 1 % (0.02 mg/kg), atropine sulfate 0.1 % (0.02 mg/kg), meditin 1 % (0.35 mg/kg) were used, and propofol emulsion 1 % (4 mg/kg/min) for anesthesia. Surgical approach to the distal metaphysis of the femur was from the lateral side. A longitudinal incision of the skin and subcutaneous tissue was made on the lateral surface of the distal metaphysis of the femur, 2.5 to 3.0 cm long. The muscles were separated, and the surface of the metaphysis was skeletonized. Further, a non-through cavity defect in the form of a quadrangular prism 4 × 4 × 6 mm in size was formed with a dental bur. After that, an implant was placed in the defect. The implant was installed tightly with a light hammering. Next, the surgical wound was tightly sutured in layers. After the operation, to achieve an analgesic effect, a single injection of a 10 % ketoprofen solution at a dose of 0.02 ml/kg was administered.

To prevent complications of postoperative hypothermia after anesthetic sleep, rabbits were heated under an infrared lamp for 1–3 hours at a temperature of 25–28 °C on the body surface, until the animal fully awakened. Animals in the postimplantation period were kept in individual cages. The rabbits were fed once a day according to the standard diet; water was given without restrictions.

Lifetime examination The general condition of the animals, the peculiarities of their behavior, the intensity and nature of physical activity, the condition of their hair and skin, the color of the mucous membranes, and the consumption of food and water were examined. Local soft tissue changes, postoperative wound healing, changes in the functional state of the involved segment were assessed.

Radiographic studies X-rays of the implantation zones were taken in frontal and lateral projections before surgery, on days 14, 28, 56, 84, 112, 140, and 182 of the experiment. Toshiba (Rotanode) Model E7239.N:10G749 radiographic system was used (Japan). The current was 2.5–3.2 mA, the voltage was 43–44 kV, the focal length was 90 cm; and the shutter speed was set automatically.

Pathological studies Scheduled euthanasia of rabbits was carried out after premedication with a solution of Diphenhydramine 1 % (0.02 mg/kg) and Rometar 2 % (5 mg/kg), followed by the introduction of a lethal dose of barbiturates. After euthanasia, an autopsy of the animal was performed; a macroscopic description of their organs and tissues, the site of implant insertion, and the relative mass of internal organs were determined. The material was collected for histological examination.

Histological studies The proximal metaepiphyses of the experimental femurs were harvested after 84 and 182 days of implantation. The bone fragments were fixed in neutral 10 % formalin, decalcified, dehydrated, and embedded in celloidin-paraffin. Histological sections of the obtained blocks, 5-7 μm thick, were stained with hematoxylin and eosin. Automated digitization of histological preparations was performed in a scanning microscope for laboratory studies Pannoramic Midi II BF (3DHISTECH Ltd., Hungary) using the "Whole-slide imaging" technology. The study of digital histological preparations, semi-quantitative and quantitative assessment of tissue and cell components in the area of defect filling with osteoplastic materials was performed using the Pannoramic Viewer software, version 2.4. (3DHISTECH Ltd., Hungary). In digital images of histological sections in the area of implantation, the ratio of the areas of osteoplastic material, newly formed bone and connective tissues, and bone marrow was histomorphometrically evaluated.

To assess the biological effect of the materials, their irritating effect and the response of cells and tissues were determined in accordance with the method recommended by the GOST ISO 10993-6-2011 standard. All measurements were made in the middle part of the defects.

Laboratory studies included hematological and biochemical tests that were performed before surgery, on days 14, 30, 84, and 182 after the implantation. Hematological parameters were determined on an ABX Pentra 60 analyzer (Horiba ABX, Japan). The leukocyte formula was calculated in blood smears stained according to Romanovsky-Giemsa under immersion at x100 magnification. Biochemical tests included the determination of serum levels of total protein, urea, C-reactive protein, glucose, aspartate aminotransferase and alanine aminotransferase activity. The activity of enzymesas well as the levels of total protein, urea, glucose, and C-reactive protein in blood serum, were determined on an automatic biochemical analyzer Hitachi/BM 902 (Japan) using reagent kits from Vital Diagnostic (St. Petersburg).

Statistical evaluation The results of quantitative signs are presented in tables as a median, 1-3 quartiles (Me; Q1-Q3). The normality of distribution in the samples was determined using the Shapiro-Wilk test. The procedure for statistical assessment of the significance of differences between the parameters at the time of the experiment with preoperative values was performed using the Wilcoxon W-test. The significance of intergroup differences was assessed using the nonparametric Kruskal-Wallis H-test. The minimum significance level (p) was taken equal to 0.05.

Regulatory standards The study was carried out in accordance with the following documents:

– GOST R ISO 10993-1-2011National standard of the Russian Federation. Medical products. Evaluation

of the biological effect of medical devices. Part 1. Evaluation and research;

– GOST R ISO 10993-6-2011 National standard of the Russian Federation. Medical products. Evaluation of the biological effect of medical devices. Part 6. Studies of local effects after implantation;

– GOST 33215-2014 Guidelines for the maintenance and care of laboratory animals. Rules for equipping premises and organizing procedures;

– GOST 33216-2014 Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of laboratory rodents and rabbits.

Ethical principles The study was carried out in compliance with the principles of humane treatment of laboratory animals in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes, and Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes. The study was approved by the Ethics Committee of the Federal State Budgetary Institution Ilizarov NMRC TO.

RESULTS

Clinical and radiographic studies The surgical wounds healed by primary intention, without septic complications in the animals of all experimental groups. In the postoperative period, the general condition of the animals of all groups was satisfactory. Hyperemia of the skin and slight swelling of the soft tissues were observed in the area of surgical interventions during the first 5 to 7 days. There were no further signs of soft-tissue inflammation. During the first three days, subfebrile body temperature of 39.5 °C was recorded in the animals, on the following days the body temperature was within the average values. The function of the limbs was preserved in full throughout the entire period of the experiment.

The implanted xenomatrix was well visualized in the radiographs on the day of surgery in animals of groups 1 and 3. Its radiographic density was similar to the density of the cortical plate of the maternal bone, and the spongy structure was clearly visible (Fig. 1, a, c). In group 2, the density of the implanted material was low and corresponded to the density of the surrounding cancellous bone tissue; therefore, the bone defect filled with xenomaterial was difficult to see (Fig. 1, b).

On day 84 of the experiment, the border between the implanted material and the maternal bone was smoothed in four (50 %) animals of group 1 (Fig. 2, a). In group 2, by that time, the xenomatrix was not visualized in 100 % of cases (Fig. 2, b). In group 3, the boundaries between the implanted material and the maternal bone erased, and the implant in most cases (60 %) had the shape of a cloud-like shadow of high intensity (Fig. 2, c).

On day 182 of the experiment, the implanted material looked like a faint cloud-like shadow in group 1 (Fig. 3, a). In group 2, by that time, the material was not visualized in 100 % of cases (Fig. 3, b). In group 3, in 5 (50 %) animals, the xenomatrix was visualized as a rounded blurred spot of increased radiographic density (Fig. 3, c).

Pathomorphological study All animals were euthanized according to plan; no death of animals due to other reasons was recorded. Pathoanatomical examination of the internal organs did not reveal pathological changes in all experimental animals. The relative mass of organs in animals of groups 2 and 3 did not differ significantly relative to the animals of group 1. The examination of the implantation zones revealed a dense fusion of the implant with the maternal bone, there were no fistulous tracts. However, in one animal from each group, the presence of cartilaginous outgrowth was noted, which was interpreted as developed arthrosis of the knee joint. This observation suggests that xenomaterial should be used with caution to replace bone defects located near the articular cavities.

Summary data on the rate of degradation of the material and clinical adverse events noted during the experiment are presented in Table 1. It shows that earlier substitution of the material was noted in the animals of group 2. In group 3, complete replacement of the material by the last time-point was not observed.

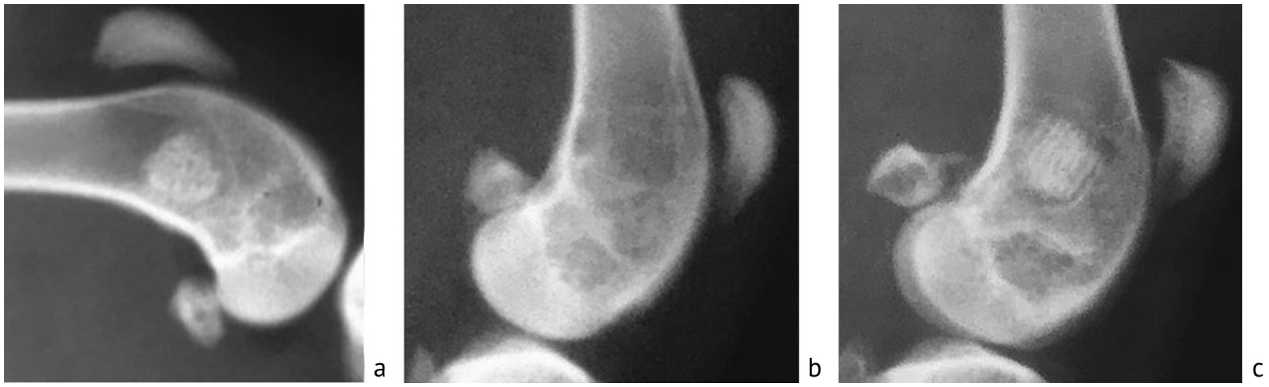

Fig. 1 X-rays of the defect after the implantation, lateral view: ***a*** group 1; ***b*** group 2; ***c*** group 3

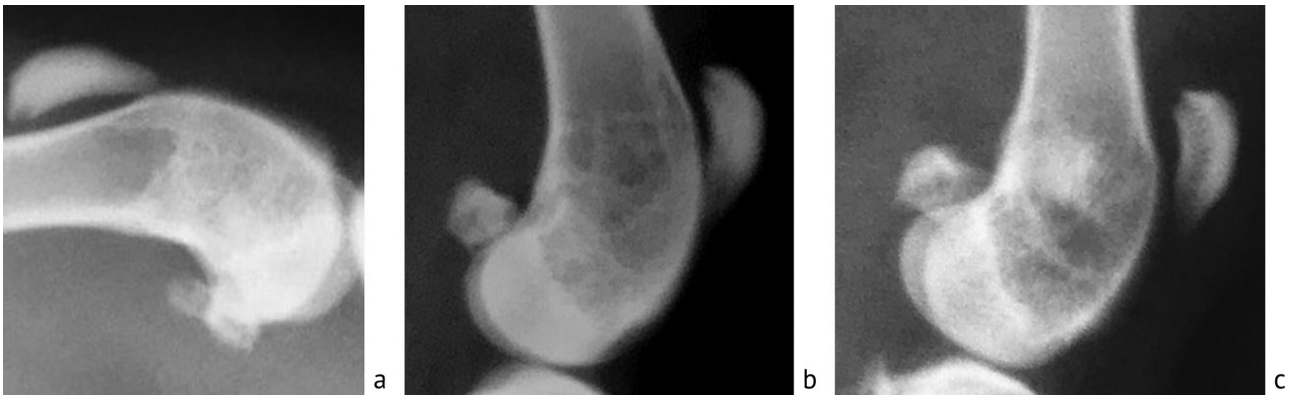

Fig. 2 X-rays the implantation area, day 84 of the experiment, lateral view: ***a*** group 1; ***b*** group 2; ***c*** group 3

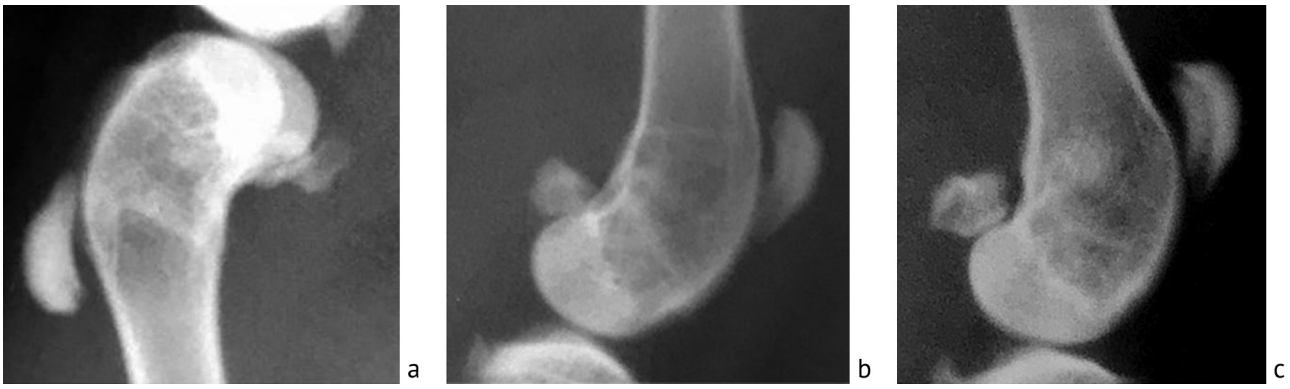

Fig. 3 X-rays of the implantation area on day 182 of the experiment, lateral view: ***a*** group 1; ***b*** group 2; ***c*** group 3

Table 1

Material degradation and complications in the experimental groups (portion from the total of animals)

Group	1	2	3
Degradation and substitution of the implant in the defect (in 100 % of cases), days	182	84	> 182
Knee joint arthrosis	1/8 (0.13)	1/10 (0.10)	1/10 (0.10)
Complications, total	1/8 (0.13)	1/10 (0.10)	1/10 (0.10)

Thus, clinical, radiological and pathoanatomical studies indicate that in all groups the implant material had acceptable biocompatibility. No cases of rejection and toxic manifestations (local and systemic) were found in any animal. In all groups, implantation of an osteoplastic matrix (both unmodified and modified) into the bone tissue of the metaphyses of tubular bones did not cause obvious signs of inflammation, sepsis, or the development of serious adverse reactions in the animals, which indicated an acceptable safety of the tested materials in general. However, different duration of degradation of the materials and the presence of certain complications result in the need to clarify the indications for their use, depending on the location of the defect.

Histological study On the 84th day of the experiment, the phenomenon of osteoconduction was recorded in the animals of all groups. It consisted in a close contact of the newly formed coarse fibrous bone matrix with the surface of bone xenomatrix fragments (Fig. 4). At the same time, the adhesion of newly formed trabeculae to implant materials was not strong, what led to the appearance of local artifacts in the form of their separation and the formation of voids on histological sections.

In this experimental period, active resorption of newly formed bone trabeculae was also noted. Both attached (Fig. 5, a, c) and detached (Fig. 5, b) osteoclasts with 2–10 nuclei were found. Attached osteoclasts had a well-developed corrugated border while the detached osteoclasts had a smoothed oval contour. The number of osteoclasts was about 1 to 2, rarely 3–5 per field of view.

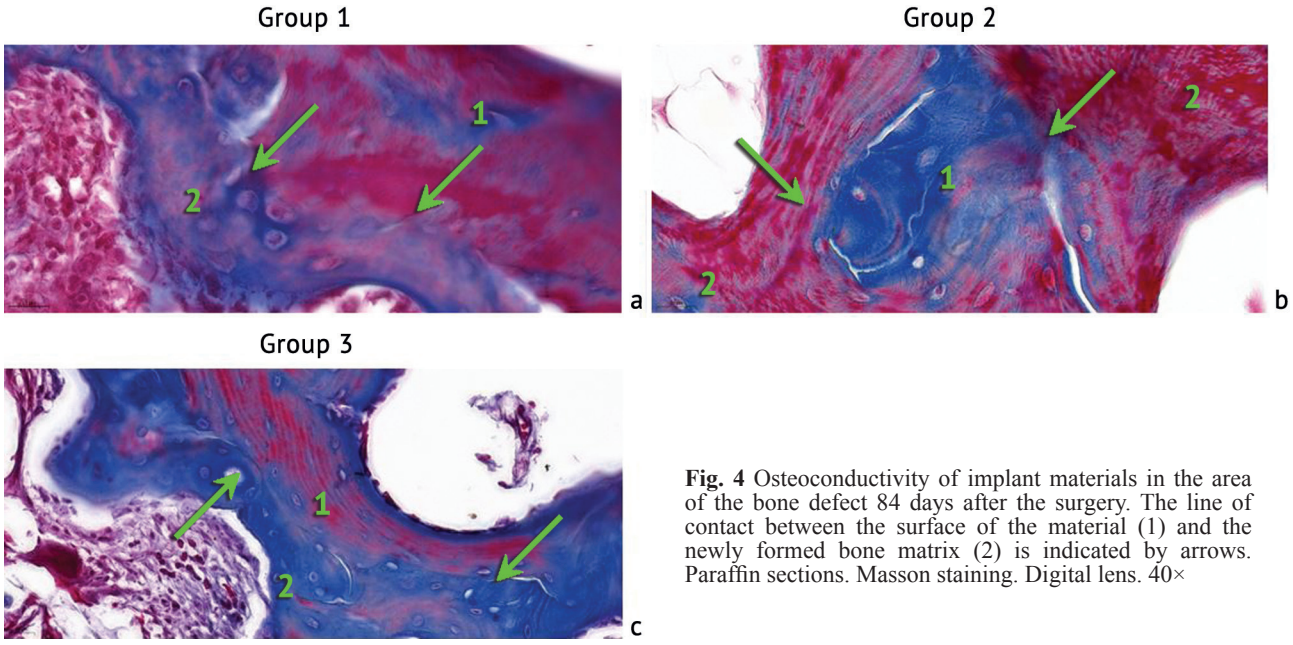

Fig. 4 Osteoconductivity of implant materials in the area of the bone defect 84 days after the surgery. The line of contact between the surface of the material (1) and the newly formed bone matrix (2) is indicated by arrows. Paraffin sections. Masson staining. Digital lens. 40×

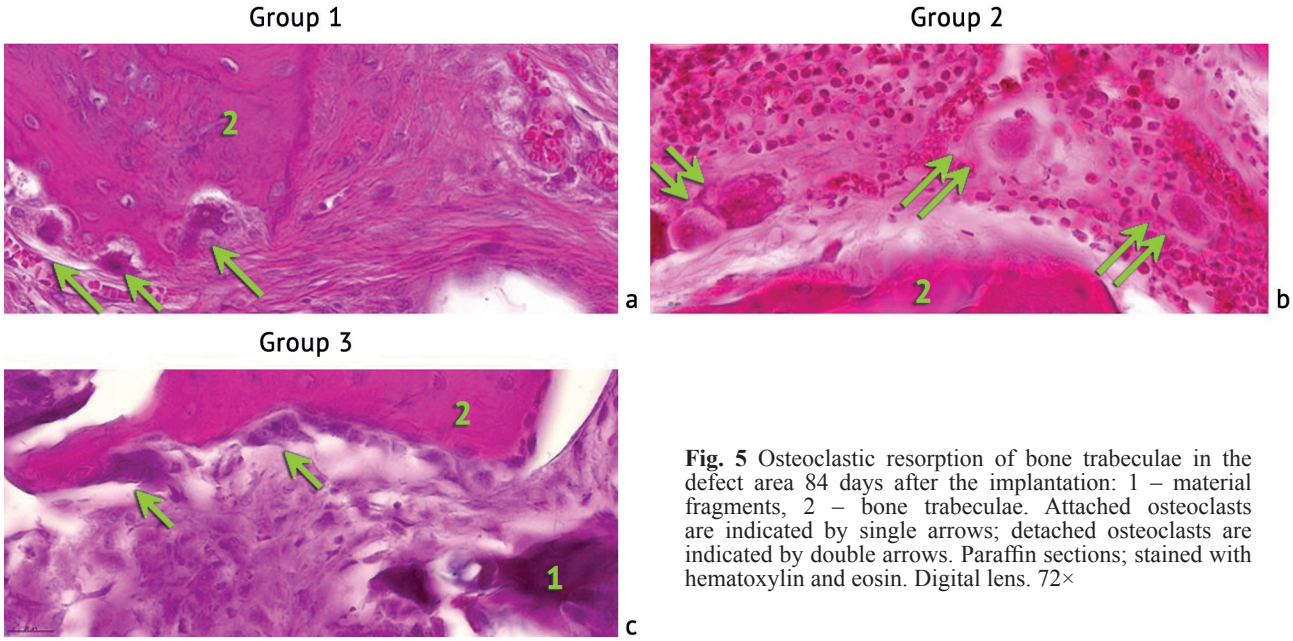

Fig. 5 Osteoclastic resorption of bone trabeculae in the defect area 84 days after the implantation: 1 – material fragments, 2 – bone trabeculae. Attached osteoclasts are indicated by single arrows; detached osteoclasts are indicated by double arrows. Paraffin sections; stained with hematoxylin and eosin. Digital lens. 72×

Restoration of the organotypic structure of the metaphyses of tubular bones in the area of defect modeling was observed 182 days after the implantation in the animals of all experimental groups (Fig. 6, a–c). In group 1, the implant material underwent biodegradation, being replaced by a newly formed network of massive bone trabeculae (Fig. 6, a). The intertrabecular spaces were filled with hematopoietic bone marrow, including adipocytes and foci of connective tissue. In groups 2 and 3, almost complete elimination of the xenomaterial and filling of the defect with spongy bone substance with a hypoplastic trabecular meshwork and extensive fields of red and yellow bone marrow were noted at that time-point of the experiment (Fig. 6, b, c).

Analysis of the quantitative ratio of tissue components in the middle part of the defect revealed that on the day 84 of the experiment in groups 2 and 3, the relative area of xenomatrix fragments and connective tissue in the histological sections was significantly lower compared to the control. The areas of bone tissue in the composition of newly formed bone trabeculae and bone marrow in the intertrabecular spaces, on the contrary, significantly exceeded the control values (Table 2).

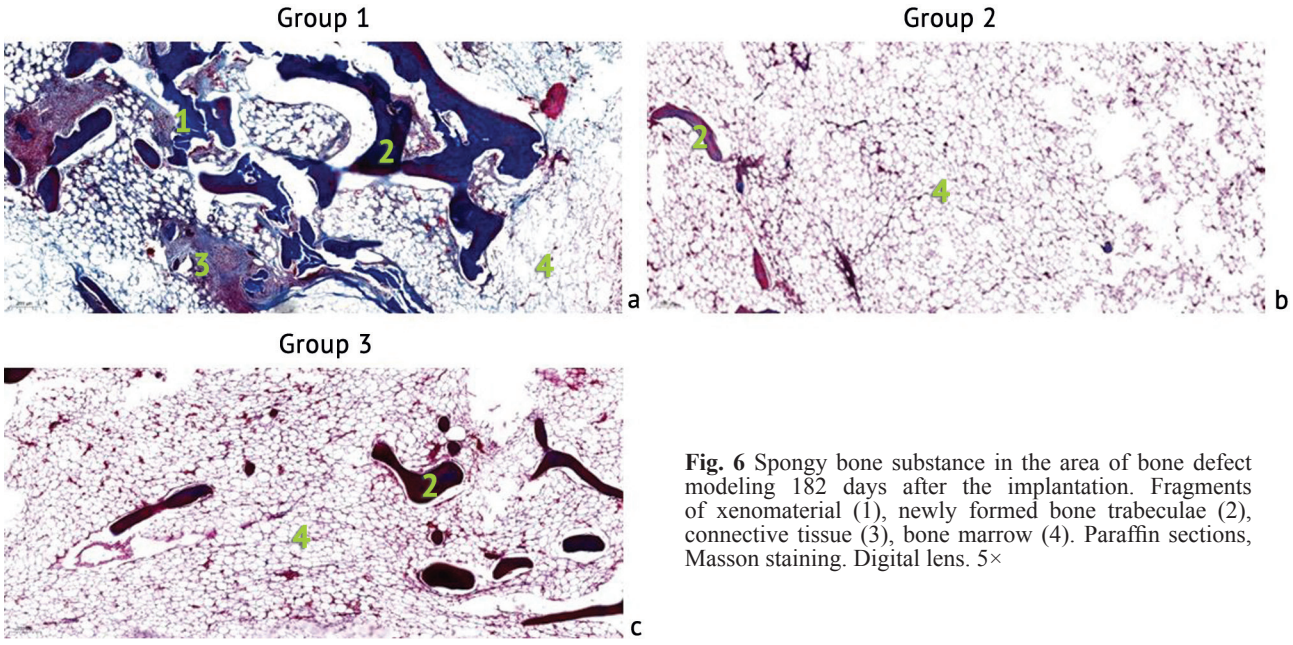

Fig. 6 Spongy bone substance in the area of bone defect modeling 182 days after the implantation. Fragments of xenomaterial (1), newly formed bone trabeculae (2), connective tissue (3), bone marrow (4). Paraffin sections, Masson staining. Digital lens. 5×

Table 2

Rate (%) of tissue components area in the region of bone defect modeling, median (1–3 quartile)

Time-point	Day 84			Day 182		
Group	1	2	3	1	2	3
Xenomatrix	14 (12–14)	5* (4–7)	5* (3–7)	4 (2–5)	0* (0–2)	0* (0–0)
Spongy bone substance	13 (12–16)	67* (52–83)	74* (54–80)	77 (68–83)	100* (98–100)	100* (99–100)
Connective tissue	73 (72–74)	33* (18–38)	22* (15–37)	17 (15–19)	0* (0–0)	0* (0–0)

Note: * – differences of groups 2 and 3 with the group 1 in the experiment time-point significant at $p< 0.05$

On day 182 after the operation in group 1, a threefold decrease in the area of the implant material and a fourfold decrease in the area of the connective tissue were noted. The total area of trabecular bone tissue slightly increased in comparison with the previous period of the experiment. In groups 2 and 3, almost complete elimination of xenomaterial in the middle part of the defect and its replacement by trabecular bone with bone marrow were noted.

The analysis of the irritating effect of the studied materials showed that on day 84 after the implantation, the indicators of cell and tissue reactions, as well as the resulting total indicator in groups 2 and 3, did not differ from those of group 1 (Table 3). On day 182 of the experiment, the index of cell reaction in the area of the defect in the animals of groups 2, 3 was reduced in comparison with the control group, which determined a significant decrease in the irritating effect of the materials.

Analysis of the histological findings showed that the implantation of xenogenic bone matrix and its modifications impregnated with vancomycin or meropenem does not prevent the filling of bone defects with cancellous bone substance with a developed trabecular structure. The studied materials exhibit the properties of bioresorption and osteoconduction. Osteoplastic materials with vancomycin and meropenem have better biocompatibility characteristics in comparison with the control. At the same time, on the 182nd day after implantation, the materials impregnated with vancomycin and meropenem were less irritating compared to the control material. The severity of signs of tissue and cell reactions decreases in proportion to the elimination of osteoplastic material.

Table 3

Evaluation of irritating effect of the implantation materials (points), median (1-3 quartile)

Time-point	Group	Cell reaction (CR)	Tissue reaction (TR)	Grade of irritating effect (CR + TR)
Day 84	1	12 (12–12)	3 (3–3)	15 (15–15)
	2	14 (12–14)	2 (2–2)	16 (14–16)
	3	12 (12–14)	2 (2–2)	14 (14–16)
Day 182	1	4 (4–7)	2 (2–2)	6 (6–10)
	2	0 (0–0)*	2 (2–2)	2 (2–2)*
	3	0 (0–0)*	0 (0–0)*	0 (0–0)*

Note: * – differences of groups 2 and 3 with the group 1 in the experiment time-point significant at $p < 0.05$

Laboratory tests A significant increase in the average values of the level of leukocytes was noted on the 30th day of the experiment in animals of group 2 (Table 4), a decrease in the level of erythrocytes was noted in animals of group 1. In the animals of all groups, an increase in the number of platelets was noted on days 14-30 after implantation. The average values of C-reactive protein in the blood serum of the animals of all groups did not differ significantly. The level of total protein, glucose and urea, as well as the activity of transaminases in the blood serum of the animals of all groups did not differ significantly during the experiment.

Evaluation of individual deviations of laboratory parameters showed that one animal (frequency 0.13) of group 1 showed signs of an inflammatory reaction at the time-points of the experiment: an increase in the level of C-reactive protein on the 30th day and leukocytopenia with thrombocytosis on the 84th day after implantation. Clinically, that animal was found to have dermatitis on both hind legs. The relationship of this event with the implantation of the material was assessed as unlikely, because the cause of dermatitis was probably corns on the paws from the grate of the trolley due to a large weight of that animal. In groups 2 and 3, two animals in each group (frequency 0.20) had a single increase in the level of C-reactive protein. Thus, the results of the laboratory study also demonstrate the acceptable safety of the studied xenomaterials.

Table 4

Laboratory counts at different time-points after implantation, median (1–3 quartile)

Parameter	Group	Before operation	Day 14	Day 30	Day 84	Day 182
Leucocytes, 10^9/l	1	7.8 (7.2–8.1)	7.5 (7.2–7.9)	7.0 (6.8–7.4)	7.3 (5.5–9.3)	6.9 (6.4–7.3)
	2	8.0 (7.0–8.3)	9.0 (7.9–10.4)	10.6* (9.9–11.5)	9.2 (8.9–9.4)	7.8 (6.8–9.1)
	3	8.3 (7.6–10.0)	9.2 (8.5–10.8)	7.9 (7.2–8.8)	8.2 (7.8–9.0)	7.8 (6.9–8.0)
Erythrocytes, 10^{12}/l	1	6.5 (6.1–6.9)	5.9* (5.7–6.0)	6.7 (6.3–6.9)	6.9 (6.6–7.0)	6.5 (6.3–6.5)
	2	6.6 (4.9–5.9)	6.2 (5.8–6.5)	5.9 (5.4–6.0)	5.6 (5.4–6.1)	6.7 (6.4–6.8)
	3	6.4 (5.1–6.5)	6.0 (5.1–6.1)	6.4 (6.1–6.6)	6.3 (6.0–6.9)	6.7 (6.3–7.0)
Platelets, 10^9/l	1	369 (293–455)	559* (526–592)	406 (395–497)	417 (362–481)	395 (390–428)
	2	348 (299–360)	509* (434–632)	473* (451–616)	418 (305–465)	405 (353–434)
	3	365 (320–383)	440 (412– 475)	564* (488–615)	428 (360–490)	389 (356–421)
CRP, mg/l	1	0.0 (0.0–0.9)	0.0 (0.0–2.4)	0.4 (0.0–2.5)	0.0 (0.0–1.9)	0.0 (0.0–1.0)
	2	0.0 (0.0–0.5)	0.0 (0.0–3.6)	0.0 (0.0–2.5)	0.0 (0.0–5.0)	0.9 (0.0–7.0)
	3	0.0 (0.0–1.3)	0.0 (0.0–1.6)	0.0 (0.0–2.0)	0.8 (0.2–3.3)	0.8 (0.0–.9)

Note: * – significance of differences compared with preoperative values at $p < 0.05$.

DISCUSSION

Xenomaterials seem to be a promising alternative to autografts and allografts for bone grafting, [20, 21]. However, the ambiguity of the evidence of the effectiveness of xenomaterials currently leads to the need to improve and expand the biological characteristics of the xenomatrix, including imparting antimicrobial activity to the material [22–24].

In this direction, it was previously shown that the bone xenomatrix is the optimal material for loading antibiotics, both in terms of the availability of raw materials and as a material with osteoinductive and biodegradable properties [25].

According to the results of this experiment, we have shown acceptable characteristics of the original bone xenomaterials containing antibiotics. It was established that these materials had sufficient biocompatibility. The rate of degradation and replacement of cavity defects with the material containing vancomycin even exceeded the characteristics of pure xenomaterial. Within the framework of the study design, one can only tentatively speak about the antimicrobial activity of the material (no cases of infection were noted). Nevertheless, we have previously demonstrated the antibacterial characteristics of this material against S. aureus in in vitro experiments [26].

Analyzing the effectiveness of the tested xenomatrices, it is important to note that most of its performance characteristics (biocompatibility, resorption rate, osteoconduction and osteoinduction) seem to depend on the compatibility of the bone base and the antibiotic, as well as on the impregnation technology of the latter. According to the study, it is clear that these conditions in our experiment, most likely, did not affect the efficacy and safety of the material with antibiotics in comparison with the pure material. The literature data also support this. Thus, it has been shown that the impregnation technology we used does not affect the biocompatibility of the bone material [27], and the addition of vancomycin does not have a significant effect on the structure of the bone scaffold [28].

However, the literature data are rather ambiguous in terms of assessing the rate of remodeling of xenomaterials impregnated with antibiotics. According to some data, both an increase in the remodeling rate and its decrease relative to the pure material were observed [29, 30]. In this regard, we must agree with the authors of [31], who

note that the local effect of antibiotics on bone repair is a poorly studied process that depends on the type of antibiotics, their combination, and concentration. This is also consistent with our radiographic data, when the rate of bone remodeling in the area of implantation of the material with vancomycin was higher, and that of the material with meropenem was lower relative to the rate of remodeling of the pure material. Though, the data of histological studies indicate an accelerated degradation of the xenomatrix modified with antibiotics in both experimental series.

Our experiments also indicate a sufficiently acceptable safety of the studied material with antibiotics (no inflammation, infection, serious adverse events, etc.) relative to control samples. In general, sufficient safety of the xenomaterial was also observed in other works [32, 33].

Thus, our data on the efficacy and safety of bone xenomaterials impregnated with vancomycin or meropenem indicate the possibility of their use in clinical practice. In support of this, there is information on the positive experience of the clinical use of xenomaterials with antibiotics, in particular in patients with post-traumatic bone infection of lower limb long bones [34]. Obviously, some characteristics of the studied material, namely, the possibility of preventing infectious complications, can be studied with a high level of evidence only in clinical practice. However, the comparable rate of replacement of the material with native bone, its safety relative to pure xenomaterial will allow the use of the material with antibiotics for indications applicable to the use of pure material. Moreover, the use of xenomatrix as a means of local delivery of antibiotics, according to some researchers, is quite promising not only for the prevention of infection, but also for the repair of defects in the treatment of osteomyelitis [35]. In addition, it should be noted that the use of its stepwise (multiple) sterilization looks promising for the prevention of xenomaterial infection. However, these procedures can significantly reduce its characteristics [36].

CONCLUSION

The developed osteoplastic materials based on bovine bone xenomatrix, impregnated with vancomycin or meropenem, have acceptable safety and efficacy characteristics both in terms of defect filling and infection prevention for bone grafting. Their further approval in clinical practice is required.

REFERENCES

1. Stogov M.V., Smolentsev D.V., Kireeva E.A. Kostnye ksenomaterialy v travmatologii i ortopedii (analiticheskii obzor literatury) [Bone xenomaterials in traumatology and orthopedics (analytical review of the literature)]. *Travmatologiia i Ortopediia Rossii* [Traumatology and Orthopedics of Russia], 2020, vol. 26, no. 1, pp. 181-189. (in Russian) DOI: 10.21823/2311-2905-2020-26-1-181-189.
2. Bracey D.N., Cignetti N.E., Jinnah A.H., Stone A.V., Gyr B.M., Whitlock P.W., Scott A.T. Bone xenotransplantation: A review of the history, orthopedic clinical literature, and a single-center case series. *Xenotransplantation*, 2020, vol. 27, no. 5, pp. e12600. DOI: 10.1111/xen.12600.
3. Diesel C.V., Ribeiro T.A., Guimarães M.R., Macedo C.A.S., Galia C.R. Acetabular revision in total hip arthroplasty with tantalum augmentation and lyophilized bovine xenograft. *Rev. Bras. Ortop*., 2017, vol. 52, no. Suppl. 1, pp. 46-51. DOI: 10.1016/j.rboe.2017.08.009.
4. Jinnah A.H., Whitlock P., Willey J.S., Danelson K., Kerr B.A., Hassan O.A., Emory C.L., Smith T.L., Bracey D.N. Improved osseointegration using porcine xenograft compared to demineralized bone matrix for the treatment of critical defects in a small animal model. *Xenotransplantation*, 2021, vol. 28, no. 2, pp. e12662. DOI: 10.1111/xen.12662.
5. Rhodes J., Mansour A., Frickman A., Pritchard B., Flynn K., Pan Z., Chang F., Miller N. Comparison of Allograft and Bovine Xenograft in Calcaneal Lengthening Osteotomy for Flatfoot Deformity in Cerebral Palsy. *J. Pediatr. Orthop*., 2017, vol. 37, no. 3, pp. e202-e208. DOI: 10.1097/BPO.0000000000000822.
6. Tang G., Liu Z., Liu Y., Yu J., Wang X., Tan Z., Ye X. Recent Trends in the Development of Bone Regenerative Biomaterials. *Front. Cell Dev. Biol*., 2021, vol. 9, pp. 665813. DOI: 10.3389/fcell.2021.665813.
7. Kutepov S.M., Volokitina E.A., Gilev M.V., Antoniadi Iu.V., Pomogaeva E.V. Augmentation of distal tibial defects with synthetic b-tricalcium phasphate and Osteomatrix xenoplastic material in surgical treatment of intra-articular impression fractures. *Genij Ortopedii*, 2016, no. 3, pp. 14-20. (in Russian) DOI: 10.18019/1028-4427-2016-3-14-20.
8. Iakimov L.A., Sliniakov L.Iu., Bobrov D.S., Kalinskii E.B., Liakhov E.V., Losik A.L. Biodegradiruemye implanty. Stanovlenie i razvitie. Preimushchestva i nedostatki. (Obzor literatury) [Biodegradable implants. Formation and development. Advantages and disadvantages. (Review of the literature)]. *Kafedra Travmatologii i Ortopedii* [Department of Traumatology and Orthopedics], 2017, no. 1 (21), pp. 47-52. (in Russian)
9. Kubosch E.J., Bernstein A., Wolf L., Fretwurst T., Nelson K., Schmal H. Clinical trial and in-vitro study comparing the efficacy of treating bony lesions with allografts versus synthetic or highly-processed xenogeneic bone grafts. *BMC Musculoskelet. Disord*., 2016, vol. 17, pp. 118. DOI: 10.1186/s12891-016-0930-1.
10. Bracey D.N., Jinnah A.H., Willey J.S., Seyler T.M., Hutchinson I.D., Whitlock P.W., Smith T.L., Danelson K.A., Emory C.L., Kerr B.A. Investigating the Osteoinductive Potential of a Decellularized Xenograft Bone Substitute. *Cells Tissues Organs*, 2019, vol. 207, no. 2, pp. 97-113. DOI: 10.1159/000503280.
11. Jin Y.Z., Zheng G.B., Lee J.H., Han S.H. Comparison of demineralized bone matrix and hydroxyapatite as carriers of Escherichia coli recombinant human BMP-2. *Biomater. Res*., 2021, vol. 25, no. 1. DOI: 10.1186/s40824-021-00225-7.
12. Liu F., Wells J.W., Porter R.M., Glatt V., Shen Z., Schinhan M., Ivkovic A., Vrahas M.S., Evans C.H., Ferreira E. Interaction between living bone particles and rhBMP-2 in large segmental defect healing in the rat femur. *J. Orthop. Res*., 2016, vol. 34, no. 12, pp. 2137-2145. DOI: 10.1002/jor.23255.
13. Zhu H., Blahnová V.H., Perale G., Xiao J., Betge F., Boniolo F., Filová E., Lyngstadaas S.P., Haugen H.J. Xeno-Hybrid Bone Graft Releasing Biomimetic Proteins Promotes Osteogenic Differentiation of hMSCs. *Front. Cell Dev. Biol*., 2020, vol. 8, pp. 619111. DOI: 10.3389/fcell.2020.619111.
14. Chen M., Xu Y., Zhang T., Ma Y., Liu J., Yuan B., Chen X., Zhou P., Zhao X., Pang F., Liang W. Mesenchymal stem cell sheets: a new cell-based strategy for bone repair and regeneration. *Biotechnol. Lett*., 2019, vol. 41, no. 3, pp. 305-318. DOI: 10.1007/s10529-019-02649-7.
15. Xu G., Hu X., Han L., Zhao Y., Li Z. The construction of a novel xenograft bovine bone scaffold, (DSS)6-liposome/CKIP-1 siRNA/calcine bone and its osteogenesis evaluation on skull defect in rats. *J. Orthop. Translat*., 2021, vol. 28, pp. 74-82. DOI: 10.1016/j.jot.2021.02.001.

16. Zhang Y., Wu D., Zhao X., Pakvasa M., Tucker A.B., Luo H., Qin K.H., Hu D.A., Wang E.J., Li A.J., Zhang M., Mao Y., Sabharwal M., He F., Niu C., Wang H., Huang L., Shi D., Liu Q., Ni N., Fu K., Chen C., Wagstaff W., Reid R.R., Athiviraham A., Ho S., Lee M.J., Hynes K., Strelzow J., He T.-C., El Dafrawy M. Stem Cell-Friendly Scaffold Biomaterials: Applications for Bone Tissue Engineering and Regenerative Medicine. *Front. Bioeng. Biotechnol*., 2020, vol. 8, pp. 598607. DOI: 10.3389/fbioe.2020.598607.
17. Samirah, Budiatin A.S., Mahyudin F., Khotib J. Fabrication and characterization of bovine hydroxyapatite-gelatin-alendronate scaffold cross-linked by glutaraldehyde for bone regeneration. *J. Basic Clin. Physiol. Pharmacol*., 2021, vol. 32, no. 4, pp. 555-560. DOI: 10.1515/jbcpp-2020-0422.
18. Qiao W., Liu R., Li Z., Luo X., Huang B., Liu Q., Chen Z., Tsoi J.K.H., Su Y.X., Cheung K.M.C., Matinlinna J.P., Yeung K.W.K., Chen Z. Contribution of the in situ release of endogenous cations from xenograft bone driven by fluoride incorporation toward enhanced bone regeneration. *Biomater. Sci*., 2018, vol. 6, no. 11, pp. 2951-2964. DOI: 10.1039/c8bm00910d.
19. Lewis C.S., Katz J., Baker M.I., Supronowicz P.R., Gill E., Cobb R.R. Local antibiotic delivery with bovine cancellous chips. *J. Biomater. Appl*., 2011, vol. 26, no. 4, pp. 491-506. DOI: 10.1177/0885328210375729.
20. Gashtasbi F., Hasannia S., Hasannia S., Mahdi Dehghan M., Sarkarat F., Shali A. Comparative study of impact of animal source on physical, structural, and biological properties of bone xenograft. *Xenotransplantation*, 2020, vol. 27, no. 6, pp. e12628. DOI: 10.1111/xen.12628.
21. Haugen H.J., Lyngstadaas S.P., Rossi F., Perale G. Bone grafts: which is the ideal biomaterial? *J. Clin. Periodontol*., 2019, vol. 46, no. Suppl. 21, pp. 92-102. DOI: 10.1111/jcpe.13058.
22. Nakoskin A.N., Diuriagina O.V., Kovinka M.A. Ksenoimplantatsiia matriksa kostnoi tkani pri zameshchenii defektov kosti u krolikov [Xenoimplantation of the bone tissue matrix in the replacement of bone defects in rabbits]. *Veterinariia Kubani*, 2016, no. 6, pp. 19-21. (in Russian)
23. Gilev M.V., Volokitina E.A., Antropova I.P., Bazarny V.V., Kutepov S.M. Bone remodeling markers after experimental augmentation of trabecular bone defects with resorbable and non-resorbable osteoplastic materials in rabbits. *Genij Ortopedii*, 2020, vol. 26, no. 2, pp. 222-227. DOI: 10.18019/1028-4427-2020-26-2-222-227.
24. Rahimnia A., Hesarikia H., Rahimi A., Karami S., Kaviani K. Evaluation and comparison of synthesised hydroxyapatite in bone regeneration: As an in vivo study. *J. Taibah Univ. Med. Sci*., 2021, vol. 16, no. 6, pp. 878-886. DOI: 10.1016/j.jtumed.2021.06.006.
25. Lewis C.S., Supronowicz P.R., Zhukauskas R.M., Gill E., Cobb R.R. Local antibiotic delivery with demineralized bone matrix. *Cell Tissue Bank*, 2012, vol. 13, no. 1, pp. 119-127. DOI: 10.1007/s10561-010-9236-y.
26. Stogov M.V., Smolentsev D.V., Naumenko Z.S., Godovykh N.V., Gurin M.V., Kireeva E.A., Lukianov A.E., Diuriagina O.V., Tushina N.V. In vitro assessment of antimicrobial activity of modified bone xenomaterials. *Genij Ortopedii*, 2019, T. 25, No 2, pp. 226-231. DOI: 10.18019/1028-4427-2019-25-2-226-231.
27. Smolentsev D.V., Gurin M.V., Venediktov A.A., Evdokimov S.V., Fadeev R.S. Poluchenie ksenogennoi kostnoi kroshki dlia implantatsii s pomoshchiu sverkhkriticheskoi fliuidnoi ekstraktsii [Obtaining xenogenic bone chips for implantation using supercritical fluid extraction]. *Meditsinskaia Tekhnika* [Medical Equipment], 2019, no. 4 (316), pp. 8-10. (in Russian)
28. Beenken K.E., Campbell M.J., Ramirez A.M., Alghazali K., Walker C.M., Jackson B., Griffin C., King W., Bourdo S.E., Rifkin R., Hecht S., Meeker D.G., Anderson D.E., Biris A.S., Smeltzer M.S. Evaluation of a bone filler scaffold for local antibiotic delivery to prevent Staphylococcus aureus infection in a contaminated bone defect. *Sci. Rep*., 2021, vol. 11, no. 1, pp. 10254. DOI: 10.1038/s41598-021-89830-z.
29. Gao J., Huang G., Liu G., Liu Y., Chen Q., Ren L., Chen C., Ding Z. A biodegradable antibiotic-eluting PLGA nanofiber-loaded deproteinized bone for treatment of infected rabbit bone defects. *J. Biomater. Appl*., 2016, vol. 31, no. 2, pp. 241-249. DOI: 10.1177/0885328216654424.
30. Shiels S.M., Raut V.P., Patterson P.B., Barnes B.R., Wenke J.C. Antibiotic-loaded bone graft for reduction of surgical site infection in spinal fusion. *Spine J*., 2017, vol. 17, no. 12, pp. 1917-1925. DOI: 10.1016/j.spinee.2017.06.039.
31. Han W., Zhang L., Yu L.J., Wang J.Q. Effect of Local Delivery of Vancomycin and Tobramycin on Bone Regeneration. *Orthop. Surg*., 2021, vol. 13, no. 5, pp. 1654-1661. DOI: 10.1111/os.13020.
32. Nakoskin A.N., Kovinka M.A., Talashova I.A., Tushina N.V., Luneva S.N. Biokhimicheskie markery osteogeneza i vospaleniia v syvorotke krovi pri ksenoimplantatsii [Biochemical markers of osteogenesis and inflammation in blood serum during xenoimplantation]. *Meditsinskii Vestnik Severnogo Kavkaza* [Medical Bulletin of the North Caucasus], 2018. vol. 13, no. 1-1, pp. 82-85. (in Russian)
33. Nakoskin A.N., Silanteva T.A., Nakoskina N.V., Talashova I.A., Tushina N.V. Reparativnye protsessy pri allo- i ksenoimplantatsii vnekletochnogo matriksa kosti [Reparative processes in allo- and xenoimplantation of the bone extracellular matrix]. *Patologicheskaia fiziologiia i eksperimentalnaia terapiia* [Pathological Physiology and Experimental Therapy], 2018, vol. 62, no. 3, pp. 60-66. (in Russian) DOI: 10.25557/0031-2991.2018.03.60–66.
34. Wang F., Liu Y., Qiu X., Fei H., Liu W., Yuan K. Effect of Anti-infective reconstituted bone xenograft combined with external fixator on serum CRP and PCT levels and prognosis of patients with bone infection after lower extremity long bone trauma. *Evid. Based Complement. Alternat. Med*., 2021, vol. 2021, pp. 5979514. DOI: 10.1155/2021/5979514.
35. Wassif R.K., Elkayal M., Shamma R.N., Elkheshen S.A. Recent advances in the local antibiotics delivery systems for management of osteomyelitis. *Drug Deliv*., 2021, vol. 28, no. 1, pp. 2392-2414. DOI: 10.1080/10717544.2021.1998246.
36. Rozanov V.V., Matveichuk I.V., Cherniaev A.P., Nikolaeva N.A. Izmeneniia morfomekhanicheskikh kharakteristik kostnykh implantatov pri radiatsionnoi sterilizatsii [Changes in the morphomechanical characteristics of bone implants during radiation sterilization]. *Izvestiia Rossiiskoi Akademii Nauk. Seriia fizicheskaia* [News of the Russian Academy of Sciences. Physical Series], 2019, vol. 83, no. 10, pp. 1435-1440. (in Russian)

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