



## Evaluation of bone substitute material based on polyurethane polymers for treatment of chronic osteomyelitis (experimental morphological study)

A.S. Sudnitsyn, T.A. Stupina✉, O.V. Dyuryagina, N.V. Tushina, T.N. Varsegova, N.V. Kubrak

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

**Corresponding author:** Tatyana A. Stupina, [StupinaSTA@mail.ru](mailto:StupinaSTA@mail.ru)

### Abstract

**Introduction** Treatment of patients with post-osteomyelitic defects is one of the most challenging areas in traumatology and orthopedics.

**Purpose** To substantiate, on an experimental model, the potential of a partially bioresorbable material *Rekost* used to compensate for osteomyelitic cavities in combination with an antibiotic.

**Materials and methods** The study was performed on nine male Soviet Chinchilla rabbits (16–18 months old, 4.3–4.5 kg of weight), in which osteomyelitis was modeled in the proximal metaphysis of the tibia by producing a trepanation hole and installing an Osteolite™ containing a *Staphylococcus aureus* culture. The animals were divided into three groups. Animals in group 1 were withdrawn from the experiment after 21 days of osteomyelitis modeling. In groups 2 and 3, the second stage included sequestrectomy and substitution of the bone defect thus formed with *Rekost* material (OOO AikonLabGmbH, Nizhny Novgorod, RZN 2014/1646 dated 03.07.2014, for indefinite) impregnated with an antibiotic in group 3. After 21 days of implantation, the animals were withdrawn from the experiment. Clinical, radiological, biochemical and histological methods were used.

**Results** In Group 1, signs of osteomyelitis were recorded: necrotic fragments of bone trabeculae, inflammatory infiltrate, osteoclastic resorption and osteogenesis, bone marrow fibrosis, and pronounced CD15 expression. In groups 2 and 3, three weeks after implantation, a network of newly formed bone trabeculae at the bone-material interface was noted, and bone trabeculae were observed in the implant cavities. In the epiphysis of group 2, a HOES score of  $\geq 6$  points corresponded to active chronic osteomyelitis. In group 3, a median HOES score of 2 points indicated a remission of chronic osteomyelitis, and CD15 marker expression was less pronounced than in group 2. C-reactive protein levels continued to increase in Group 2 and significantly decreased in Group 3.

**Discussion** Bioresorbable implants, impregnated with antibiotics, provide gradual replacement of the material with bone tissue, eliminate the need for repeated surgeries, reduce the risk of chronic infection, and are biocompatible.

**Conclusion** In an in vivo experimental model, minimal degradation of the *Rekost* material was observed three weeks after implantation, manifested by highly frequent foreign-body-type giant multinucleated cells. Early data on the osteoplastic properties of the *Rekost* material and its ability to suppress infection and inflammation in combination with an antibiotic suggest the potential for further experimental studies of this product to be used in the treatment of patients with chronic osteomyelitis.

**Keywords:** coronary osteomyelitis, bone defects, Recost bone-substituting material, antibiotics, histology

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## INTRODUCTION

High incidence of chronic osteomyelitis that ranges from 3 to 25 % of all musculoskeletal diseases, its recurrence rates of up to 30–40 %, secondary amputations and functional limb disability in 10.3–57 % of cases required the search for new ways to improve the outcomes of its surgical treatment [1, 2, 3]. Regardless of pathophysiology, the majority of osteomyelitis cases are caused by the gram-positive bacterium *Staphylococcus aureus* [4, 5], which can persist in the Haversian canals, in the interlamellar spaces of osteons and trabeculae, and intracellularly in macrophages. This fact plays a major role in chronic bone infection, characterized by persistent inflammation, destruction of bone tissue, and impaired regeneration [6, 7]. Osteomyelitis triggers pathological bone remodeling, which isolates foci of infection from the effectors of innate immunity [5]. Current approaches to treating patients with chronic osteomyelitis include antibiotic therapy, surgical resection of infected areas, and compensation of bone defects. Bone compensation is a key step, as insufficient bone tissue lead to recurrent infections and musculoskeletal dysfunction [8].

Surgical intervention in the management of osteomyelitis allows for the targeted delivery of high-dose antibiotics to the site of inflammation, reduces the toxic and side effects of drugs through the use of various types of implants and materials for substitution in bone cavities, which is an important component of the therapy [9, 10, 11]. Particular efforts are directed at the development of new antimicrobial biomaterials that not only eliminate infection but also promote the subsequent process of bone tissue restoration [2, 9]. The use of local drug carriers based on polymethyl methacrylate [12, 13], carbon, ceramics [14, 15] and biopolymers [16, 17, 18] enables arrest of infection, compensation of defects, support of soft tissue tension and stimulation of bone tissue regeneration, which has been confirmed by a number of studies [19, 20, 21, 22]. However, non-biodegradable materials do not provide prolonged release of antibiotics [23, 24]; their main disadvantages are incompatibility with many antimicrobial drugs [25] and the need for repeated surgery to remove such a spacer [5, 26].

The use of a partially bioresorbable material impregnated with an antibiotic is a promising option for treating infection and restoring bone in a single surgical procedure, thus reducing costs and shortening the length of hospital stay [27, 28, 29].

The advance in surgical treatment methods for patients with chronic osteomyelitis requires experimental studies aimed at modeling the chronic process, developing effective technologies, and identifying materials that combine antibacterial and osteoinductive properties [30, 31, 32].

**Purpose** To substantiate, on an experimental model, the potential of a partially bioresorbable material *Rekost* used to compensate for osteomyelitic cavities in combination with an antibiotic.

## MATERIAL AND METHODS

### *Study design*

The study was conducted on nine male laboratory rabbits of the Soviet Chinchilla breed, aged 16–18 months, weighing 4.3–4.5 kg, which were randomly divided into three groups of three animals each.

**Inclusion criteria:** Clinically healthy rabbits of Soviet Chinchilla breed, free from musculoskeletal diseases.

**Exclusion criteria:** Animals with somatic diseases and pathologies of the musculoskeletal system.

At the first stage, chronic osteomyelitis of the proximal metaphysis of the right tibia through infection of the bone marrow cavity with a culture of *Staphylococcus aureus* (MSSA, reference strain,  $1 \times 10^6$  CFU). A block of bone matrix *Osteolite*® measuring  $4 \times 4$  mm was used as a carrier

of the infectious agent (RZN 2023/19470 Lyophilized bone bioimplants, sterile for traumatology and orthopedics *Osteolite*®, Russia). Before the operation, the bone matrix block *Osteolite*® was placed for 5–7 minutes in an aqueous suspension of a 24-hour culture of *Staphylococcus aureus* for soaking. The volume of absorbed culture was 0.05 ml (0.0386 g).

A surgical approach to the bone surface was performed on the medial surface of the proximal tibial metaphysis. A bone burr was used to create a hole in the cortex corresponding to the size of the infectious agent carrier. A bone matrix block soaked in the infectious agent culture was placed in the metaphysis cavity so that its outer surface was within the burr hole but did not protrude above its surface. The burr hole was then hermetically sealed with Knochenschwamm bone wax (AESCULAP AG, Germany). The surgical wound was sutured with single-layer interrupted PDS 4/0 sutures (Ethicon, Johnson & Johnson, USA). The waiting time for osteomyelitic process to develop was 21 days. At that point, animals from Group 1 were withdrawn from the experiment for morphological confirmation of osteomyelitic process.

In the second stage, animals in Group 2 and Group 3 underwent sequester necrectomy and bone defect filling with *Rekost* polymer bone substitute material (OOO AikonLabGmbH, Nizhny Novgorod, RZN 2014/1646 dated July 3, 2014, indefinite). Fistula tract tissue was excised, and exostoses on the cortical surface around the trepanation hole were removed. Using spherical bone burrs (Ø 1 and 4 mm), the osteomyelitic lesion was opened, removal of sequestra followed along with granulation tissue and purulent exudate, with cleaning of the endosteal surface of the metaphysis down to the blood-supplied areas of the bone. Next, according to the instructions, the components of the *Rekost* bone substitution polymer material were mixed and animals in Group 3 received Vancomycin antibiotic powder at a ratio of 1 g of active ingredient per 10 ml of bone cement. The resulting mixture was drawn into a 1 ml syringe with a cut-off needle cone and evenly filled into the bone defect cavity, avoiding air pockets. After partial polymerization, the excessive material was trimmed with scissors to the level of the cortex. The surgical wound was closed with single-layer interrupted sutures using PDS 4/0 (Ethicon, Johnson & Johnson, USA) on the skin. In Groups 2 and Group 3, the study continued 21 days after defect repair, after which the animals were withdrawn from the study.

Surgical interventions were performed under general anesthesia. For premedication, a solution of diphenhydramine 1 % (0.02 mg/kg), atropine sulfate 0.1 % (0.02 mg/kg) and meditin 1 % (0.35 mg/kg) was used. For anesthesia, a propofol emulsion 1 % (4 mg/kg/min) was used. Thirty minutes before the start of the operation, a solution of ethamsylate 12.5 % (10–12 mg/kg) was administered to prevent bleeding and a solution of ketoprofen 10 % (2 mg/kg) as an analgesic and anti-inflammatory agent. Antibiotic therapy was not used. Euthanasia was performed by intravenous administration of high doses of anesthetics under general anesthesia.

### **Ethical approval**

The study was carried out in accordance with the rules of the European Convention (ETS No. 123) for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and interstate standards (GOST 33044-2014), and was approved by the local ethics board (protocol dated 11/29/2024 No. 1 (76)).

### **Clinical and laboratory methods**

The general condition of the animals, the condition of the skin, the color of the mucous membranes, food and water consumption, the condition of the implantation area (local changes in soft tissues, healing of the postoperative wound) were assessed. The general body temperature was measured

rectally for 1 minute using a DT-623 thermometer Vega Technologies Inc (China). Animals were weighed on a Shtrikh-MIB 15–2.5 desktop electronic scales (Russia). Radiography of the implantation site was performed in frontal and lateral views at the stages of surgical intervention and on the 21<sup>st</sup> day after defect filling using a Toshiba (Rotanode) Model E7239 system; N: 10G749 (Japan). The current was 2.5–3.2 mA, the voltage was 43–44 kV, the focal length was 90 cm: the shutter speed was set automatically.

### **Biochemical method**

C-reactive protein (CRP) levels were determined in the serum of the experimental animals. The studies were performed on a Hitachi/BM 902 automated biochemical analyzer (F. Hoffmann-La Roche Ltd., Italy) using Vital Diagnostics reagent kits (Russia).

### **Histological method**

The object of the study was the proximal metaepiphysis of the tibia.

The material harvested was fixed in 10 % neutral formalin, decalcified, and subjected to standard histological processing using a HISTOSAFE™ INFILTRA™ tissue vacuum processing apparatus (ErgoProduction LLC, Russia). Paraffin sections (5–7 µm) were prepared on a Thermo Scientific HM 450 microtome (USA) and stained with hematoxylin and eosin and Masson's three-color staining. An AxioScope.A1 microscope with an AxioCam ICc 5 camera and Zenblue software (Carl Zeiss Micro Imaging GmbH, Germany) was used to obtain digital images of the specimens.

To assess the infectious and inflammatory process, the semi-quantitative HOES score developed by A. Tiemann et al. was used, including an analysis of the severity of soft tissue necrosis, osteonecrosis, bone neogenesis and fibrosis, and inflammatory infiltrate in points (0 — no changes, 1 — mild, 2 — moderate, 3 — severe) [33]. Five to 10 fields of view were analyzed in each animal at a magnification of ×100.

Granulocytes were detected by immunohistochemistry using mouse polyclonal antibodies to CD15 (ab233869) (Abcam, UK) and a peroxidase detection system with diaminobenzidine and micropolymer (ab236469, Rabbit-specific HRP/DAB Detection IHC Detection Kit-Micropolymer, Abcam, UK). All steps were performed according to the protocol recommended by the antibody manufacturer. Sections were counterstained with hematoxylin.

### **Statistical methods**

The study was conducted on small samples (three animals per group). Table 1 presents the primary data. Table 2 presents the data as medians and quartiles (Me ( $p_{25};p_{75}$ )). Statistical processing was performed in Microsoft Excel spreadsheets using the Attestat program (version 9.3.1, author I.P. Gaidyshev, Rospatent Certificate No. 2002611109). For comparative analysis, the nonparametric Mann – Whitney test was used, which allows for the detection of differences between small samples at a significance level of  $p < 0.05$ .

## **RESULTS**

In Group 1 animals, slight edema, moderate hyperemia, and scant to moderate purulent exudate were observed at the site of inoculation of the infectious agent. The general condition was characterized by a poorer appetite and a decrease in body weight by an average of 13.5 %. The general body temperature remained within the physiological norm throughout the entire stage of osteomyelitis modeling. In animals of Group 2, soft tissue edema, diffuse hyperemia, moderate to profuse purulent discharge were observed at the surgical site after defect filling, and focal dermatitis and phlegmon

were observed in the perioperative area. The body weight decreased relative to the preoperative values by 25.8 % on average. The general body temperature was above normal physiological values. In Group 3, after defect filling, the general condition was assessed as good in two cases, and the healing of the surgical wound was uneventful. The body weight decreased relative to preoperative values by an average of 17.8 %. The general body temperature was within normal physiological reference limits. In one case, the general condition and local symptoms corresponded to the clinical picture of osteomyelitis.

In the radiographs taken after the procedure of the infectious agent insertion into the proximal metaphysis of the tibia, the edges of the trepanation hole and the *Osteolite*® bone matrix block were clearly visible (Fig. 1 a). Twenty-one days after infection, multiple rounded areas of bone tissue lysis were observed in the intervention area. The edges of the trepanation hole were indistinct, the bone tissue was partially sclerotic and surrounded by a lysis zone, and the boundary between the bone matrix block and the bone was smoothed. Dense periosteal exostoses were noted on the cortical plate (Fig. 1 b). During sequester necrectomy, the pathologically altered tissue was removed, and the bone defect cavity was filled with *Rekost* bone substitute material, which was non-radiopaque in radiographs, including after the addition of vancomycin (Fig. 1 c).

In Group 2, by day 21 after sequester necrectomy and defect repair, radiographs showed signs of total inflammatory destruction of the metaphyseal bone tissue. The central metaphysis had low optical density, and the boundaries of the trepanation hole and bone defect were blurred. The defect cavity projection showed randomly distributed structures of medium and low intensity, varying in size and with an unclear outline. The cortex had a blurred outline and a heterogeneous structure. Weak, thin, cloud-like periosteal deposits were noted on its lateral surface (Fig. 1 d).

In Group 3, the proximal tibial metaphysis had smooth contours and a homogeneous bone structure. The radiographic density of bone tissue in the defect area was identical to that of the parent bone. The boundaries of the bone defect cavity were undetectable. No foci of bone destruction or lysis were observed. Periosteal layers were dense, organized, and located around the trepanation hole. The metaphyseal cortex had a clear contour, uniform structure, and average radiographic density (Fig. 1 e). In one animal with purulent exudate discharge from the surgical wound in the postimplantation period, radiographs showed signs of chronic osteomyelitis manifested by focal bone destruction in the epiphyseal and distal parts of the metaphysis and weak periosteal layers around the trepanation hole (Fig. 1 f).

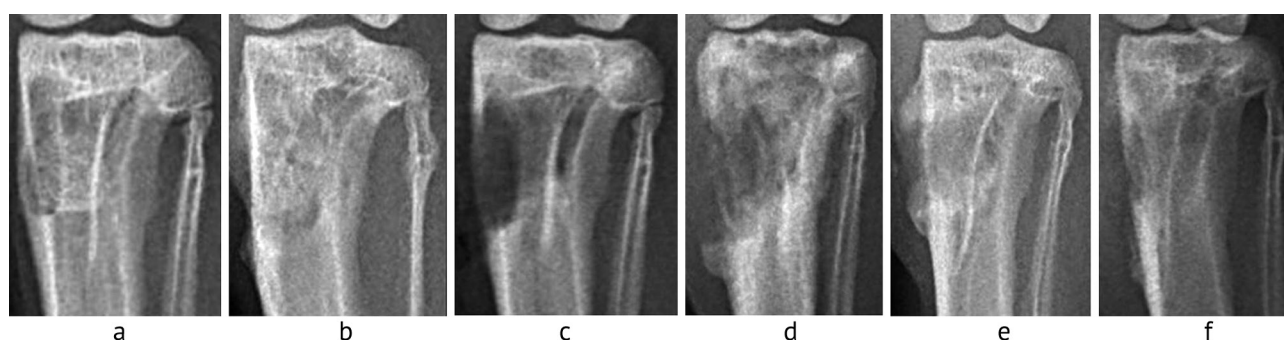


Fig. 1 Radiographs of the proximal metaphysis of the tibia of rabbits (anteroposterior view) at the stages of the study: (a) introduction of an infectious agent, the day of surgery; (b) 21 days after introduction of infection; (c) sequester necrectomy and filling of the bone defect, the day of surgery; (d) Group 2, 21 days after defect filling; (e) Group 3, 21 days after defect filling, fair result; (f) Group 3, 21 days after defect filling, poor result

The CRP level in the blood serum of all animals in all groups increased significantly on the 21<sup>st</sup> day after infection agent introduction (Table 1).

Table 1

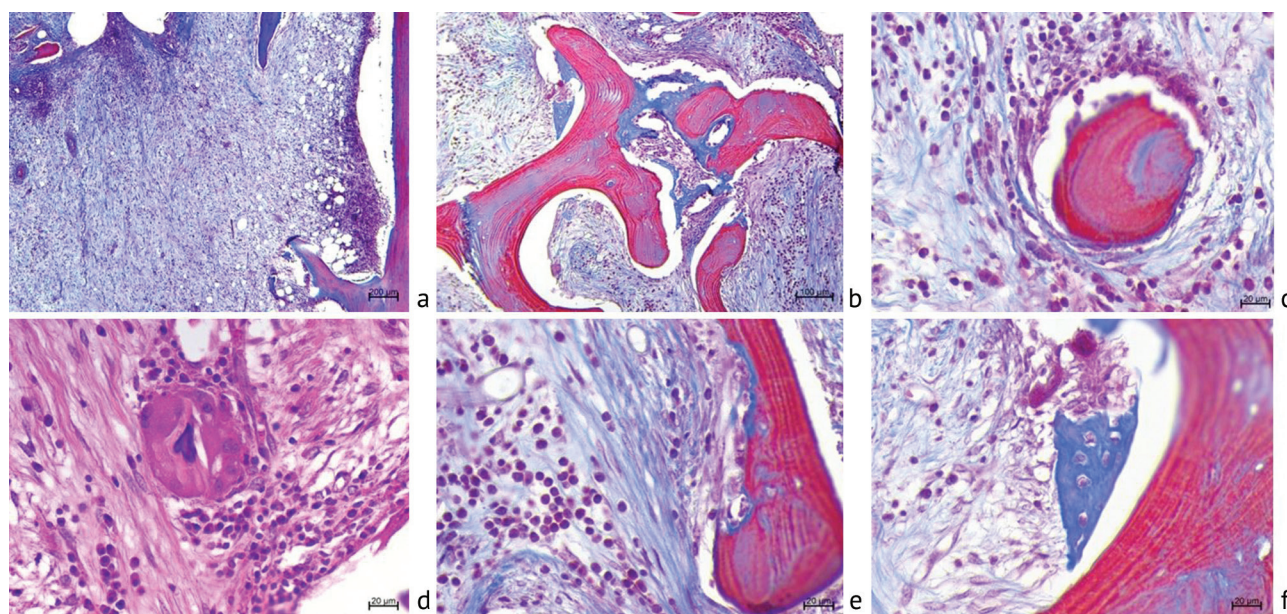
Concentration of C-reactive protein in the blood serum of the animals in the experimental groups

| Groups / animals |   | Концентрация СРБ (мкг/л) |         |                          |
|------------------|---|--------------------------|---------|--------------------------|
|                  |   | 0 days                   | 21 days | 42 days                  |
| Group 1          | 1 | 0                        | 11.8    | –                        |
|                  | 2 | 0                        | 15.2    |                          |
|                  | 3 | 1.8                      | 30.7    |                          |
| Group 2          | 1 | 0                        | 12.7    | 25.3                     |
|                  | 2 | 0                        | 18.0    | 50.4                     |
|                  | 3 | 0                        | 25.3    | 31.6                     |
| Group 3          | 1 | 0                        | 10.0    | 0                        |
|                  | 2 | 0                        | 18.3    | 7.6                      |
|                  | 3 | 0                        | 23,4    | 20.5; $p^{2-3} = 0.0495$ |

Note:  $p$  — level of significance of differences after comparing Groups 2 and 3 using the Mann – Whitney test  $p < 0,05$ .

In Group 2, CRP levels increased in all animals 21 days after implantation of the material (day 42 of the experiment), indicating further development of the infectious process. In Group 3, CRP levels decreased significantly in two animals 21 days after implantation of the antibiotic-impregnated material, and less significantly in one.

Light-optical investigation of histological group preparations revealed extensive fields of granulation and connective tissue in the proximal metaphysis of the tibia (Fig. 2 a), bone trabeculae with signs of necrosis (Fig. 2 b), bone microsequestrum (Fig. 2 c), inflammatory infiltrate of focal and diffuse types of distribution (Fig. 2 c, d) containing neutrophils, plasma cells, macrophages, and single giant multinucleated cells of the foreign body type (Fig. 2 e). Signs of osteoclastic resorption (Fig. 2 e) and reparative osteogenesis (Fig. 2 b, f) were recorded. The median score on the HOES scale [33] of 6 points corresponded to the active inflammatory phase of chronic osteomyelitis (Table 2).



**Fig. 2** Fragments of images of the proximal metaphysis of the tibia of Group 1: (a) extensive fields of granulation and connective tissue in the metaphysis; (b) trabeculae with empty osteocyte lacunae and newly formed bone trabeculae; (c) bone microsequestrum; (d) giant multinucleated cell, inflammatory infiltrate; (e) thinning of the bone trabecula, "corrugated edge" resulting from of osteoclastic resorption; (f) in the center, a newly formed area of bone tissue (the matrix is stained blue). Paraffin sections, stained with Masson's three-color method (a, b, c, e, f), hematoxylin and eosin (d). Magnification  $\times 40$  (a),  $\times 100$  (b),  $\times 400$  (c, d, e, f)

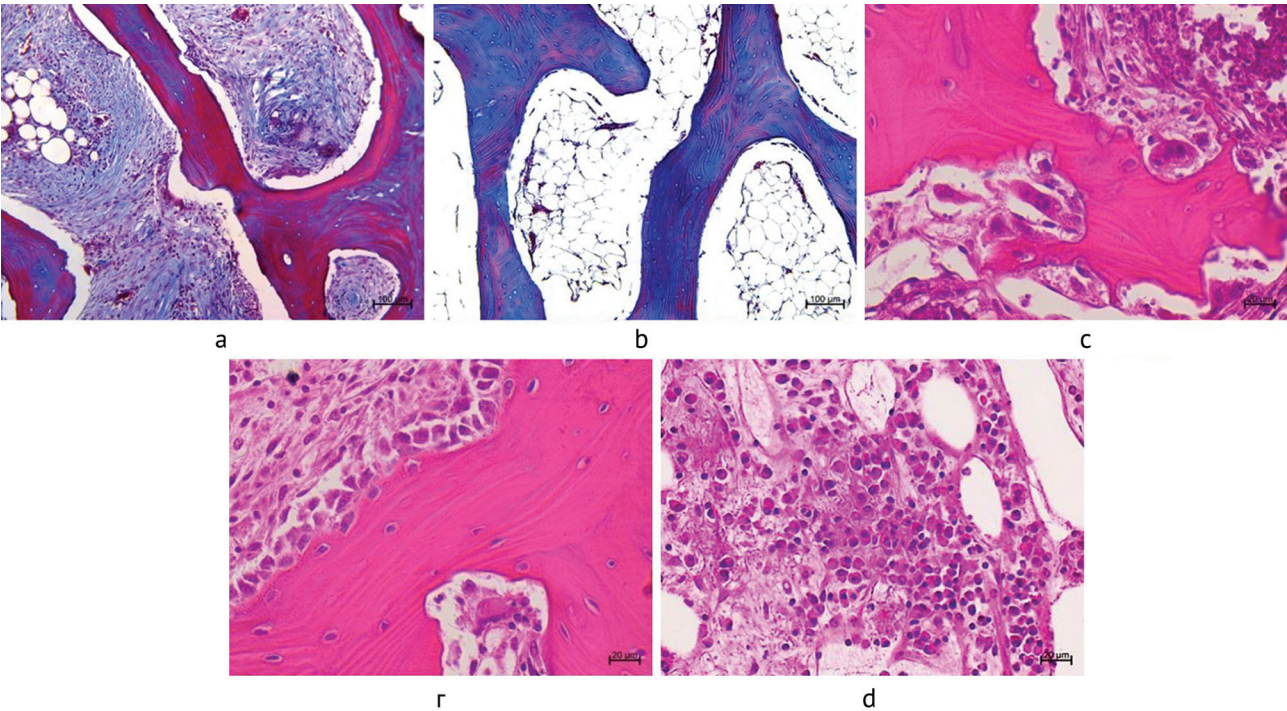
Table 2

Semiquantitative assessment of chronic osteomyelitis using the HOES scale

| Groups  | HOES score, points, Me ( <i>p</i> 25; <i>p</i> 75) | Chronic osteomyelitis stage |
|---------|----------------------------------------------------|-----------------------------|
| Group 1 | 6 (5;6)                                            | CAO                         |
| Group 2 | 7 (6;7); $p^{1-2} = 0.0165$                        | CAO                         |
| Group 3 | 2 (1;3); $p^{1-3} = 0.0001$ ; $p^{2-3} = 0.0001$   | COR                         |

Notes: COR — chronic osteomyelitis remission; CAO — chronic osteomyelitis active; *p* — level of significance by comparing Groups 1, 2 and 3 Mann – Whitney test at *p* < 0.05

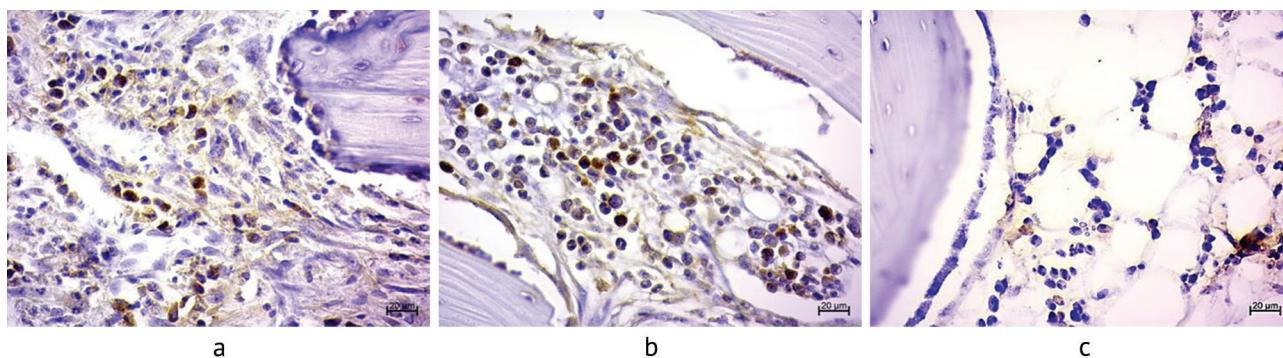
In Group 2, histological signs of chronic osteomyelitis were noted in the epiphysis, including replacement of bone marrow with connective tissue (Fig. 3 a), intense signs of bone trabeculae remodeling, osteoclastic resorption (numerous osteoclasts resorbing the ground substance, trabeculae with ulcerated surfaces, with interstitial osteonecrosis), reparative osteogenesis (numerous osteoblasts lining the bone trabeculae) (Fig. 3 c, d), and lymphohistiocytic infiltrate containing neutrophils of focal and diffuse distribution (Fig. 3 e). The median semiquantitative HOES score [33] of 7 points corresponded to the active inflammatory phase of chronic osteomyelitis (Table 2).



**Fig. 3** Fragments of images of the proximal tibial epiphysis: (a) Group 2, replacement of bone marrow with connective tissue; (b) Group 3, fatty bone marrow in the intertrabecular spaces; (c) Group 2, numerous osteoclasts; (d) Group 2, osteoblasts on the surface of the trabecula, osteoclasts below; (e) Group 2, lymphohistiocytic infiltrate of diffuse distribution. Paraffin sections, stained with Masson's three-color method (a, b), hematoxylin and eosin (c, d, e). Magnification ×100 (a, b), ×400 (c, d, e)

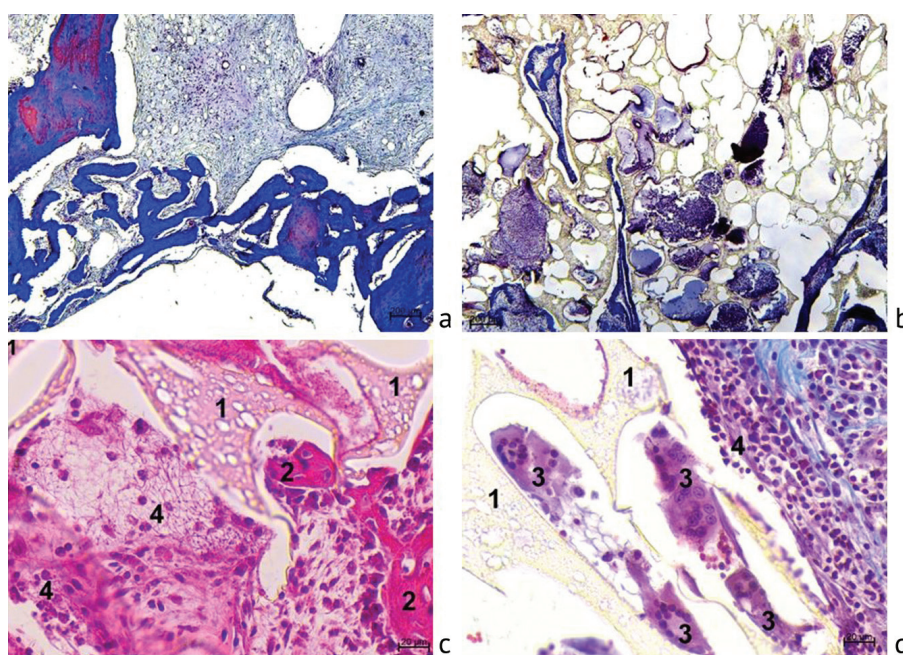
In Group 3, histological signs of chronic osteomyelitis were not detected in the epiphysis of two animals; the intertrabecular spaces were filled with fatty bone marrow (Fig. 3 b). The median HOES score [33] of 2 points indicated remission of chronic osteomyelitis (Table 2). Focal bone marrow fibrosis was seen in one animal; rare arrangement of bone trabeculae, disruption of their architecture, and a lymphohistiocytic infiltrate with solitary neutrophils of diffuse distribution were observed in the intertrabecular spaces.

Immunohistochemical study showed that the intensity of CD15 expression was highest in Group 2 and lowest in Group 3 (Fig. 4).



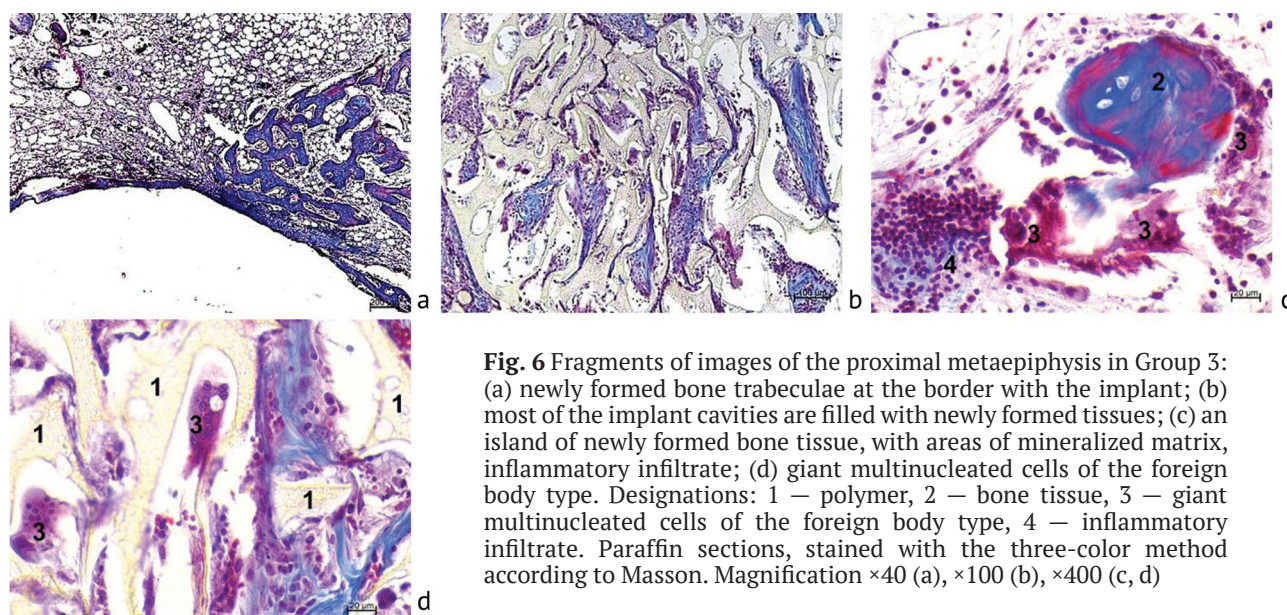
**Fig. 4** Fragments of images of the proximal tibial epiphysis, CD15 expression: (a) Group 1; (b) Group 2; (c) Group 3. Paraffin sections. Magnification  $\times 400$

Filling the defect between the epiphysis and the implant in Group 2 featured extensive fields of loose connective tissue (Fig. 5 a); in Group 3 there was predominantly fatty bone marrow (Fig. 6 a). At the border with the implant, the formation of a network of newly formed bone trabeculae was noted in all cases. In the partially bioresorbable implant in Group 2, part of the polymer cavities was filled with blood cells and primary matrix, loose connective tissue, newly formed bone trabeculae (Fig. 5 b) with numerous active osteoblasts (Fig. 5 c); giant multinucleated cells of the foreign body type (Fig. 5 d), dense, diffusely distributed lymphohistiocytic infiltrate with single neutrophils (Fig. 5, c, d) were noted.



**Fig. 5** Fragments of images of the proximal metaphysis in Group 2: (a) at the border with the implant there is a network of newly formed bone trabeculae; (b) implant cavities are empty and filled with newly formed tissues; (c) osteoblasts on the surface of trabeculae, diffuse lymphohistiocytic infiltrate; (d) giant multinucleated cells of the foreign body type, inflammatory infiltrate. Designations: 1 – polymer, 2 – bone tissue, 3 – giant multinucleated cells of the foreign body type, 4 – inflammatory infiltrate. Paraffin sections, stained with the three-color method according to Masson (a, b, d), hematoxylin and eosin (c). Magnification  $\times 40$  (a),  $\times 100$  (b),  $\times 400$  (c, d)

In Group 3, most of the polymer cavities were filled with erythrocytes, granulation and connective tissue, and newly formed bone trabeculae (Fig. 6 b). Masson's three-color staining revealed fuchsinophilic areas of the bone matrix (Fig. 6 c). Highly frequent foreign-body-type giant multinucleated cells (Fig. 6 d) and focal and diffuse lymphohistiocytic infiltrate were observed (Fig. 6 c).



**Fig. 6** Fragments of images of the proximal metaepiphysis in Group 3: (a) newly formed bone trabeculae at the border with the implant; (b) most of the implant cavities are filled with newly formed tissues; (c) an island of newly formed bone tissue, with areas of mineralized matrix, inflammatory infiltrate; (d) giant multinucleated cells of the foreign body type. Designations: 1 — polymer, 2 — bone tissue, 3 — giant multinucleated cells of the foreign body type, 4 — inflammatory infiltrate. Paraffin sections, stained with the three-color method according to Masson. Magnification  $\times 40$  (a),  $\times 100$  (b),  $\times 400$  (c, d)

## DISCUSSION

Treatment of patients with chronic osteomyelitis is based on surgical debridement of infected necrotic bone (radical sequestrectomy) and antibacterial therapy. Local antibiotic delivery systems are frequently used. Systemic antibacterial therapy, in the absence of adequate blood supply to the sites of bone infection, fails to deliver and maintain effective concentrations of antibacterial drugs, leading to poor treatment outcomes. Local antibiotic delivery aims to achieve therapeutically effective drug concentrations at the site of infection while minimizing side effects [11, 34, 35].

One-stage technology for treating patients with osteomyelitic cavities is a more effective method compared to traditional methods due to a reduction in the number of surgical interventions, in rehabilitation periods, early functional recovery of patients and a reduction in the frequency of relapses of the purulent process [36, 37, 38].

The development and experimental testing of antibiotic-impregnated bioresorbable materials for the treatment of patients with osteomyelitis is a promising area. Bioresorbable implants, saturated with antibiotics, provide gradual replacement of the material with bone tissue, eliminate the need for repeated surgeries, reduce the risk of chronic infection, promote bone regeneration, are biocompatible, and allow for local administration of antibiotics [11, 35].

In 2014, a domestic partially bioresorbable bone substitute material *Rekost* (ZN 2014/1646, state registration date 03.07.2014, unlimited), was developed. It is a mixture of components: a polyurethane polymer prepolymer, calcium orthophosphate powder, and a polyol hardener [39]. *In vitro* studies have shown that *Rekost*, compared to polymethyl methacrylate, has advantages in terms of the duration of antibiotic release at therapeutic doses [40].

This *in vivo* study demonstrated that *Rekost* is a partially biodegradable material with osteoplastic properties. Our results are consistent with other experimental and clinical studies on the *Rekost* application in paranasal sinus wall reconstruction, as well as in the treatment of patients with bone defects after oncological diseases [41, 42].

The presented method of experimental modeling of osteomyelitis allows us to obtain a chronic inflammatory process in bone tissue 21 days after infection. In experiments on modeling the purulent-necrotic process in bone (Group 1), the main histological signs of osteomyelitis were seen: necrotic fragments of bone trabeculae surrounded by connective tissue and inflammatory infiltrate, active processes of osteoclastic resorption and reparative osteogenesis, bone marrow

fibrosis, and intensive CD15 expression. Neutrophilic granulocytes, as well as plasma cell aggregates, along with lymphohistiocytic infiltration against the background of fibrosis, and the presence of bone microsequestrers are diagnostic signs of chronic osteomyelitis [43].

In Groups 2 and 3, three weeks after implantation, the presence of a network of newly formed bone trabeculae at the bone-material interface demonstrated the osteoconductive properties of this polymer. In both groups, bone trabeculae were observed in the implant cavities, along with red blood cells and provisional matrix, granulation tissue, and connective tissue, confirming the osteoplastic properties of the polymer.

In the epiphysis of Group 2, histological signs of chronic osteomyelitis (bone marrow fibrosis, trabeculae with interstitial osteonecrosis) were observed; a HOES score of  $\geq 6$  points corresponded to an active course of chronic osteomyelitis. In Group 3, a median HOES score of 2 points indicated a remission of chronic osteomyelitis; expression of the CD15 marker was less pronounced compared to Group 2. The CRP level in Group 2 continued to increase in all animals three weeks after implantation, indicating further development of the infectious process. Persistence of inflammation for more than three weeks usually indicates the development of an infectious process [44].

In Group 3, the CRP level in two animals decreased significantly, and in one less intensively, which, however, allows us to speak about signs of infectious process arrest in this group.

The results of this study showed that the use of *Rekost* material to fill post-osteomyelitic cavities without the addition of an antibiotic in Group 2 led to further development of the infection and bone tissue destruction. The impregnation of the *Rekost* bone substitute with antibiotic vancomycin in Group 3 stopped the infection in two out of three animals (one case had active chronic osteomyelitis) and facilitated favorable organotypic bone tissue remodeling aimed at restoring bone structure.

According to literature data and our experience, the partially bioresorbable material *Rekost* allows for modeling of complex anatomical contour and, over time, is gradually replaced by bone tissue, while simultaneously acting as a local carrier of antibiotics, which meets modern requirements for bioresorbable implants in the correction of post-osteomyelitic defects [37, 41, 42].

It should be noted that the treatment outcome is influenced not only by the composition of the bone substitute material, but also by the quality of bone preparation during sequester necrectomy. Incomplete removal of necrotic and/or infected bone tissue after sequester necrectomy is the main cause of recurrence of chronic osteomyelitis [45].

In all observations, high frequent giant multinucleated cells of the foreign body type may be considered as a morphological manifestation of the polymer biodegradation process (the giant cell reaction is one of the markers of tissue reactions to the implant) and as a pathomorphological component of persistent inflammation in the conditions of chronic osteomyelitis [44].

### **Limitations**

The design of this study has limitations. It was conducted on a small sample of animals at early stages of the experiment. The duration of implanted degradable/resorbable sample tests depends on the time and rate of their degradation. We plan to conduct further studies on a larger sample for a longer experiment term.

### **CONCLUSION**

An experimental in vivo model showed minimal degradation of the *Rekost* material at three weeks after the implantation, manifested by highly frequent foreign-body-type giant multinucleated cells. Early data on the osteoplastic properties of the *Rekost* material and its ability to suppress infection and inflammation in combination with an antibiotic suggest the potential for further experimental studies of this product for the treatment of patients with chronic osteomyelitis.

**Conflict of interests** The authors declare no conflict of interest.

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**Ethical statement** The study was conducted in accordance with the European Convention (ETS No. 123) for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and interstate standards (GOST 33044-2014) and was approved by the institutional ethics committee (protocol dated November 29, 2024, No. 1(76)).

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#### Information about the authors:

Anatolii S. Sudnitsyn — Candidate of Medical Sciences, Orthopaedic Surgeon, Head of the Scientific Laboratory, anatol\_anatol@mail.ru, <https://orcid.org/0000-0002-2602-2457>;

Tatyana A. Stupina — Doctor of Biological Sciences, Leading Researcher, StupinaSTA@mail.ru, <https://orcid.org/0000-0003-3434-0372>;

Olga V. Dyuryagina — Candidate of Veterinary Sciences, Head of the Experimental Laboratory, diuriagina@mail.ru, <https://orcid.org/0000-0001-9974-2204>;

Natalya V. Tushina — Candidate of Biological Sciences, Senior Researcher, ntushina76@mail.ru, <https://orcid.org/0000-0002-1322-608X>;

Tatyana N. Varsegova — Candidate of Biological Sciences, Leading Researcher, varstn@mail.ru, <https://orcid.org/0000-0001-5430-2045>;

Nadezhda V. Kubrak — Senior Researcher, kubrak2@mail.ru, <https://orcid.org/0000-0002-7494-8342>.