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Morphological assessment of osteointegration of various implants for management of long bone defects (experimental study)

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Purpose To assess osseointegration of implants made of various osteoconductive materials in an animal experiment by management of a long-bone diaphysis defect complicated with chronic osteomyelitis. **Materials and methods** Experimental studies were conducted on 24 healthy outbred adult rabbits for six weeks. Diaphyseal defects were managed with allo-, ceramic, and carbon implants. **Results and discussion** With carbon material, an adhesive interaction between the implant and the maternal bone tissue was of intercalation type. Osteointegration was evidenced by the presence of microfragments of an implant in bone tissue extracted for morphological study from the peri-implant zone, with areas of numerous osteoid islets. With a ceramic implant in the defect, no signs of remodeling and osteointegration in the implant extracted for morphological examination of bone tissue were detected. In the experiment with allograft, the area of implantation was isolated by a fibrous capsule, in which individual osteo-osteoid areas were seen without typical bone structure formation, and complete bone fusion was not achieved. **Conclusion** Morphological data on the regeneration processes prove the advantages of a carbon nanostructured implant relative to the other osteoplastic materials used in this animal experiment.
Keywords: experiment, long bone, osteomyelitis, bone defect, nanocarbon implant, allograft, ceramic implant

INTRODUCTION

Regeneration of organs and tissues which were lost due to the pathological process is one of the challenging problems of the contemporary medicine. Introduction of synthesized materials into the affected body area leads to disruption of homeostasis, the consequence of which is poor integration of the explant and even its rejection, as well as inability of its long-term functioning [1, 2].

Currently, various types of grafts have been widely used to manage bone defects. In particular, a demineralized allogenic material with the ability to stimulate osteogenesis, carbon composite materials and implants based on ceramics have been used [3, 4, 5]. The literature contains data on the study of osseointegration of carbon implants in an experiment on a native preparation. However, data on the integration of various implants and their morphological characteristics have been described insufficiently [6, 7]

The results of applying different options for surgical treatment of patients with bone defects complicated with chronic osteomyelitis are controversial; therefore the search for criteria for evaluating the effectiveness of using different types of bone grafting remains an objective of medical practice [8, 9].

Purpose Evaluate osteointegration of implants fabricated of various osteoconductive materials in an experiment using a model of chronic osteomyelitis and bone diaphysis defect.

MATERIAL AND METHODS

Experimental studies were carried out on 24 outbred adult rabbits, chosen according to the principle of analogues. The studies complied with the principles of humanity directives of the European Community (86/609 / EEC) and the Helsinki Declaration.

At the first stage, primary chronic fistulous osteomyelitis of the diaphysis of the radial bone was modelled in all the animals [1]. After two weeks, segmental necrsequestrectomy was performed to debride the inflammatory nidus, and then bone defects were replaced with various types of implants.

To study the processes of reparative bone regeneration in the resulting defect, the animals were randomly organized into groups (Table 1).

Evaluation of bone strength was performed on healthy intact animals of the control group.

Reparative regeneration was evaluated correlating the results of histological studies with X-ray data and the strength properties of the regenerate at the bone-to-implant interface. After six weeks, animals were sacrificed by intravenous administration of sodium thiopental at a dose of 200 mg/kg.

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Due to inability to have a solid bone microscopic preparation with the carbon implant, we carried out a preliminary extraction of the implant, after which we performed a morphological study of the remaining bone with ingrown elements. The implant was extracted from the bone tissue using a tensile testing machine R-05 UHL 4.2. The results of the experiment were analyzed from the point of view of the mechanical strength of the biological union formed at the bone-to-implant interface in Ns [4].

For morphological analysis, slices of 10–12 microns thick were prepared from pieces of bone tissue with a sledge microtome (Reichard, Germany) after decalcification in a 7 % solution of nitric acid and celloidinous filling. Next, the celloidin sections were stained with hematoxylin and eosin, using Masson's trichrome method, Schmorl thionin-picric acid. Later they were studied under a magnifying glass. Microphotographs were also taken under magnification using a lens 10 and eyepiece 12.5 (× 125). For microscopic examination, an AxioScope A1 stereomicroscope and an AxioCam ICc 5 digital camera were used, completed with Zen blue software (Carl Zeiss MicroImaging GmbH, Germany).

Table 1

Groups of animals according to implant used

Group	Implant type	Number of animals (n)
1 (index group)	Nanostructured carbon [7]	8
2 (comparison group)	Allogenic bone bioimplant	8
3 (comparison group)	Ceramic implant made of non-organic material fabricated by molding of thermoplastic ceramic slip into a silicone shape	8

RESULTS

With allogenic bone material in the defect (group 2), histological assessment of the reparative process detected the presence of staged morphogenetic manifestations resulting from the formation of a bone-implant conglomerate. The bone allograft underwent resorption with the formation of a cavity in which fragments of detritus were located (Fig. 1a). Fibrous and osseo-fibrous areas around the resorption cavity and the fibrous layer of the interfragmental regenerate were combined into a common regeneration complex. Alongside, pronounced restructuring processes were detected in the maternal bone adjacent to the implant with spongiosis areas, rarefaction of the dense bone matrix and formation of osteoid fields and sections of rough fibrous bone (Fig. 1b). When the material was stained with the Masson's trichromic method, fragments were obtained that showed predominantly immature rough fibrous bone fields with non-mineralized fields of chondroosteoid type, which were characterized by high cellularity. In red mineralized fields, the density of cellular elements was noticeably lower, and in some areas small fields were revealed featuring the initial lamellar structuring (Fig. 1c).

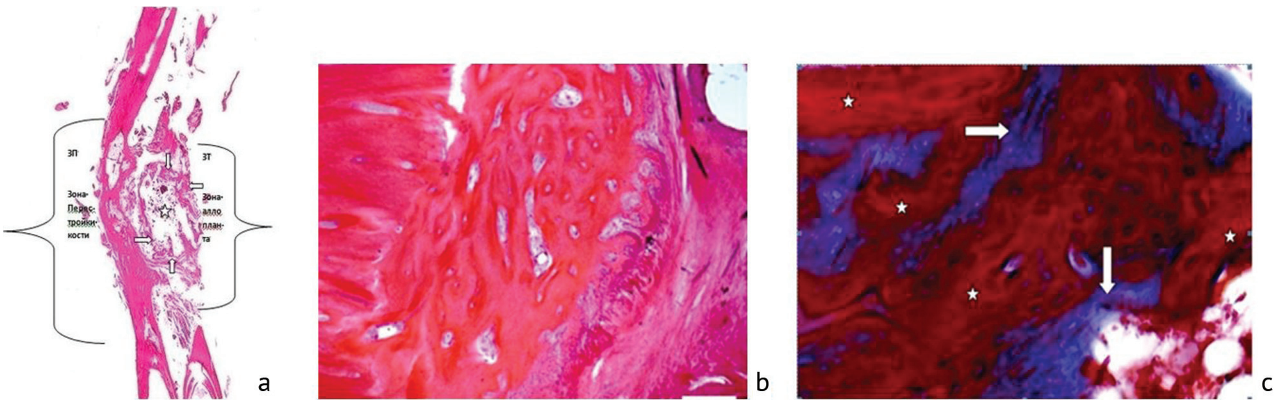

Fig. 1 Histological studies of the regeneration zone six weeks after implantation of the allograft: ***a*** histotopographic diagram of the regeneration zone; where ЗТ is the grafting area, ЗП is the area of the adjacent bone rearrangement, the asterisk is the area of allograft resorption; arrows is capsule around the resorption zone stained with hematoxylin and eosin; ***b*** interfragmental area; the field of coarserough fibrous bone, bordering on fibrous tissue (right) and a fragment of the cortical plate (left). Magnification: lens 10, eye-piece 12.5 (× 125), stained with hematoxylin and eosin; ***c*** area of formation and rearrangement of bone tissue (asterisks are the mineralized structures of the rough fibrous bone, formation of lamellar structures; arrows are non-mineralized chondroosteoid portions in the rough fibrous bone. Magnification; lens 10, eye-piece 12.5 (× 125), Masson Trichrome staining

In group 3 with a ceramic implant in bone defect, it was found that in axial projections from the location of this material there was a clutch-like outgrowth of spongy bone tissue included in the bone regenerate, which was formed according to the hyperplastic type. Bone-osteoid trabeculae of the regenerate were at the stage of unfolding mineralization (Fig. 2a). Single areas of the newly formed tissue were a network of bone-osteoid trabeculae, visualized as mineralized and non-mineralized bone-osteoid structures in the area of contact between the mother bone and ceramics. There were no signs of osteogenesis processes at the bone-to-implant interface (Fig. 2b). In all samples, the newly formed structures featured rough fibrous bone tissue of a spongy structure with areas of immature bone tissue in the trabeculae of the forming regenerate. Lamellar bone structures were not detected either. The prevalence of non-mineralized structures (blue) over mineralized ones (red) was convincingly proven by the preparations with the Masson trichromic staining (Fig. 2c).

Unlike the above two experimental groups, the formation of a single conglomerate was macroscopically observed in the experiments with the carbon implant (group 1) due to the envelope of the maternal bone on the implant periphery (Fig. 3a). After extraction of the implant from the conglomerate formed as a result of the regeneration process, the remaining bone contained carbon implant particles that maintained contact with the mother bone and were detected at bone-to-implant interface (Fig. 3b).

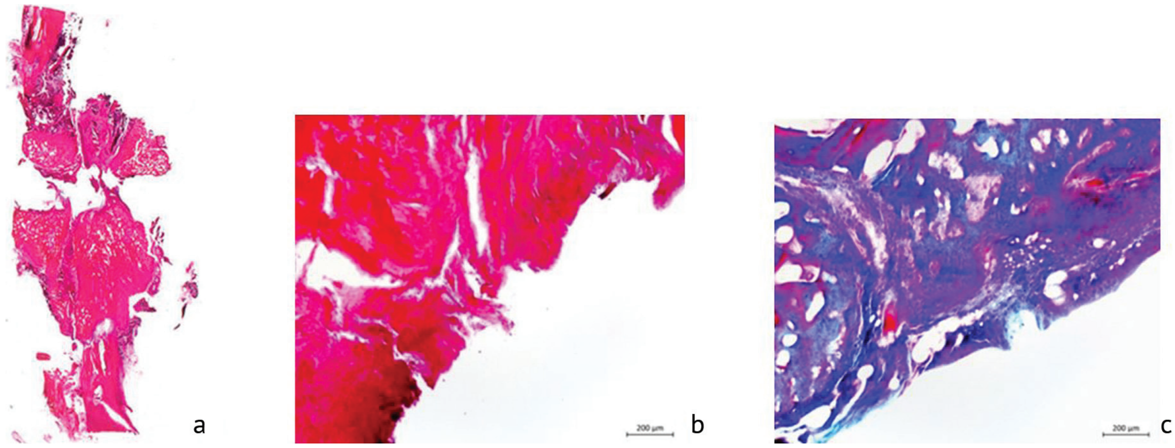

Fig. 2 Results of histological studies of the regeneration zone six weeks after the defect was replaced with a ceramic implant: ***a*** clutch-like outgrowths of spongy bone tissue, a celloidin histotogram stained with hematoxylin and eosin; ***b*** area of the connective tissue mass adjacent to the graft, the contact zone is lower right, stained with hematoxylin and eosin; Magnification – × 125 (lens 10, eye-piece 12.5); ***c*** the zone in the inferior contact with the implant, staining with the Masson trichromic method; magnification - × 125 (lens 10, eye-piece 12.5)

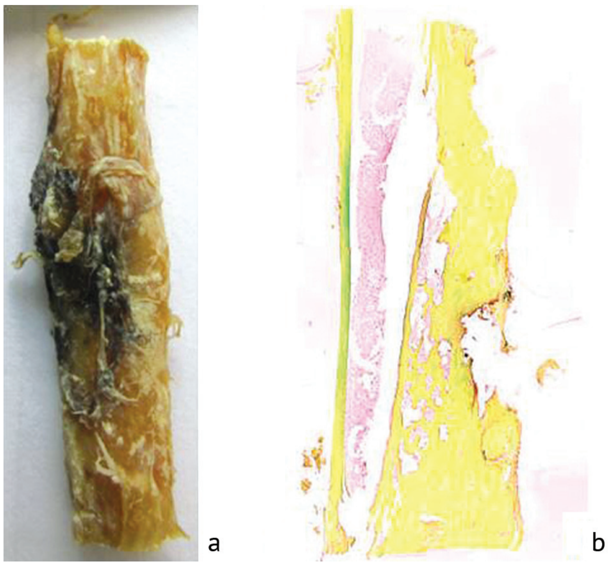

Fig. 3 Macropreparation: ***a*** conglomerate formed at the site of integration of the carbon implant, with bone tissue envelope (magnification lens); ***b*** histotogram showing carbon microparticles in the bone defect in the area of the extracted implant; magnification (lens), staining (thionine-picric acid staining according to Schmorl)

Histological study of the bone-to-implant border found periosteal osteogenesis around the implant. Adhesive cords and carbon microparticles, scattered in the bone structures, were located at the bone-to-implant border and at a distance from this zone (Fig. 4a); sections of the cortical plate reconstruction were seen as cortex rarefaction with expansion of the intraosseous spaces. We also observed a change in the orientation of the direction of accumulations of bone plates as they acquired a wavy configuration. In the thickness of the compact bone of the maternal bone cortical layer, the presence of young osteoid non-mineralized structures of an irregular configuration was revealed in the process of restructuring. They testified to the insertion or intercalation growth of bone structures in this experiment (Fig. 4b). When the samples were stained with thionine-picric acid according to Schmorl, osteocyte lacunae and blood vessels could be traced in a remodeling bone matrix (Fig. 4c).

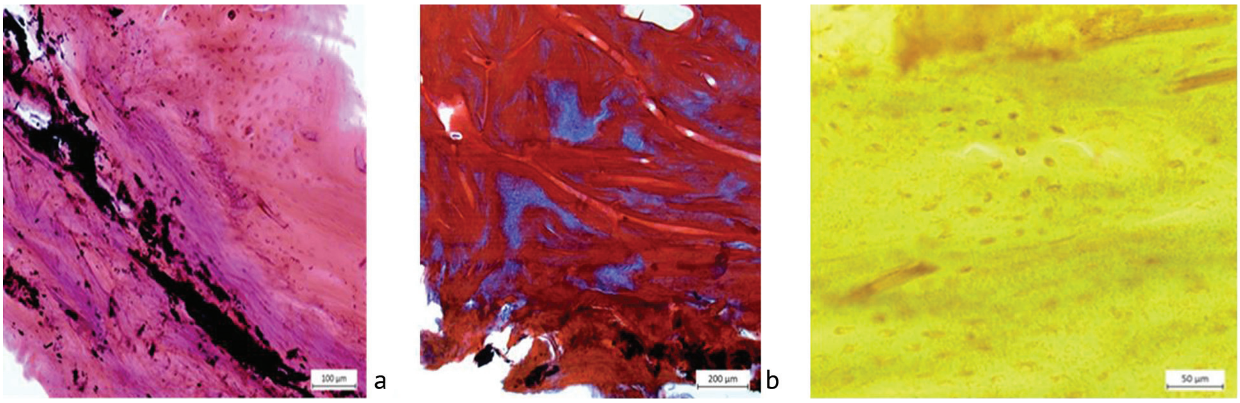

Fig. 4 Results of histological studies of the regeneration zone six weeks after a carbon implant was embedded in the defect: ***a*** pronounced osseointegration of the maternal bone with the implant, a section of the bone cortex near the implant with carbon microparticles between the bone structures; hematoxylin and eosin staining, magnification – × 250; ***b*** non-mineralized osteoid intercalates in the cortical bone with adjacent sections of anthracotic deposits; Masson trichromic staining; magnification × 125 (lens 10, eye-piece 12.5); ***c*** view of the remodeling cortex; Schmorl staining with thionine-picric acid, magnification × 500 (lens 40, eye-piece 12.5)

DISCUSSION

Surgical activity in chronic osteomyelitis is over 70 % [10]. In various types of the process, surgical debridement of the osteomyelitis nidus is only performed, or it is supplemented by soft tissue and bone defect management [11, 12]. It should be noted that the problem of bone defect management is rather complicated and means, first of all, that the infected bone defect cavity is bridged [13].

The most rational technique for compensating segmental bone defects is distraction regenerate using the Ilizarov method which is based on the idea of inducing and maintaining osteogenesis by creating tension stress in the osteogenic tissue under conditions of rigid fixation of bone fragments [10, 14].

The literature describes methods of combined techniques for treating long bone defects using transosseous and intramedullary osteosynthesis [15].

Autogenic bone is often used as a material for defect coverage [13, 16, 17]. In order to prevent local infection in the plasty area, antibiotics can be added to a free bone autograft [18].

Currently, the use of allografts has become widespread [8]. All materials used for defect filling can be divided into three types: designed for rejection or removal in the future, designed for resorption, and designed for integration and subsequent encapsulation. To non-resorbable fillings currently used, one can refer bone cement, which is of a complex polymer composition based on polyacrylate. It is possible to add an antibiotic to this composition in order to prevent the development of local infectious processes [8, 19].

The representative of modern bio-composite biodegradable materials is the combined preparation "Kollapan", "Kollatamp G", consisting of hydroxyapatite, collagen and various immobilized antimicrobial agents [8, 20].

Currently, the use of carbon composite materials and ceramic-based implants has become widespread [21, 22]. Hydroxyapatite has a porous structure comparable to the spongy bone, and functions as an effective osteoconductive matrix [7].

During the experiment, we obtained convincing data on the advantages of osseointegration when using a nanostructured carbon implant in comparison with allogeneic bone material and ceramic implant; it correlates with published data.

In the presence of a noticeable reparative reaction, complete bone fusion with the use of an allobone implant was not achieved. The resorption cavity at the implantation site was isolated by the emerging fibrous capsule, in which individual fragmentary bone-osteoid sites were determined without the formation of typical bone structures.

In observations using ceramic implants in the area of the bone defect, the absence of fusion of the maternal bone with ceramic material was noted. The periphery of the implant revealed the formation of connective tissue encapsulation. Newly-formed immature bone-osteoid structures of reactive bone formation were adjacent to the periphery of the forming connective tissue capsule. No signs of organotypic bone remodeling were observed in this group.

Morphological analysis in the experiment with the nano-carbon implant indicates formation of a conglomerate consisting of an implant and recipient bone tissue which type is envelope cover. Moreover, the portions of the connective tissue layer formed during the repair process were replaced by bone tissue. The presence of small angular fragments in the adjacent maternal bone tissue, including at a certain distance, indicates the processes of osseointegration. Numerous sites with osteiod islets were found in the matrix of the rarefied maternal bone, which indicated bone formation of the intercalation type. At the same time, we did not reveal a full-fledged “creeping” replacement according to Andersson of the newly formed bone tissue of the entire volume of the carbon implant.

In our earlier studies [4], it was proven that the maximum bone-implant conglomerate strength (0.097 ± 0.013 N/m) was reached by the end of six weeks in this experiment with carbon implants. It turned out to be comparable with the strength characteristics of a healthy bone (0.095 ± 0.008 N/m). According to the results of the X-ray examination in animals of the group with the implantation of carbon material, the segmental defect was bridged by this time due to the formation of a periosteal bone-to-implant union in comparison with animals of other groups, where an unbridged bone defect was preserved on the radiographs.

Thus, the results of the morphological study correlate with the available literature data, as well as the previously obtained results of biomechanical and radiological studies [4].

CONCLUSION

1. When assessing the condition of the implantation zone, the carbon implant was enveloped with bone tissue, and after its extraction, signs of osseointegration, including the presence of carbon microparticles in the maternal bone tissue were determined at the bone-to-implant border.

2. Morphological study confirmed the presence of rearrangement of the cortical layer area at the border with the carbon implant that featured formation of lacunae of osteocytes and blood vessels in the remodeling bone matrix.

3. Morphological data on the regeneration processes prove the advantages of a carbon nanostructured implant relative to the other osteoplastic materials used in this animal experiment, and also indicate the possibility of optimizing bone remodeling processes in the implantation zone.

REFERENCES

1. Yerofeyev S.A., Pritykin A.V., Gorodilov R.V. Kosteobrazovanie pri ispolzovanii elektromagnitnogo izlucheniia vysokoi chastoty v usloviiakh gnoinoi infektsii (eksperimentalnoe issledovanie) [Bone formation using electromagnetic radiation of high frequency under the conditions of purulent infection (An experimental study)]. *Genii Ortopedii*, 2009, no. 4, pp. 5-10. (in Russian)
2. Timchenko P.E., Zakharov V.P., Volova L.T. Mikroskopicheskii kontrol protsessa osteointegratsii implantatov [Microscopic controlling the process of implant osseointegration]. *Kompiuternaia Optika*, 2011, vol. 35, no. 2, pp. 183-187. (in Russian)
3. Irianov Iu.M., Borzunov D.Y., Chernov V.F., Diuriagina O.V., Aksenov D.I. Osteointegratsiia setchatykh konstruktsii nikelida titana i reparativnoe kosteobrazovanie pri ikh implantatsii [Osteointegration of titanium nickelide mesh constructs, and reparative osteogenesis for their implantation]. *Genij Ortopedii*, 2014, no. 4, pp. 76-80. (in Russian)
4. Reznik L.B., Stasenko I.V., Negrov D.A. Rezultaty primeneniia razlichnykh vidov implantov pri zameshchenii osteomieliticheskikh defektov dlinnykh kostei v eksperimente [Results of using various types of implants in experimental management of long bone osteomyelitic defects]. *Genij Ortopedii*, 2016, no. 4, pp. 81-87. (in Russian)

5. Tkachenko A.N., Markovichenko R.V., Khachatrian E.S. Khirurgicheskie tekhnologii zameshcheniia defektov kostei pri khronicheskom osteomielite [Surgical technologies of filling bone defects for chronic osteomyelitis]. *Mediko-biologicheskie i Sotsialno-psikhologicheskie Problemy Bezopasnosti v Chrezvychainykh Situatsiiakh*, 2012, no. 4, pp. 11-13. (in Russian)
6. Kononovich N.A., Shevtsov V.I., Gorbach Ye.N., Medik V.A., Stogov M.V., Borzunov D.Y., Stepanov M.A. Eksperimentalnoe issledovanie ispolzovaniia uglerodnykh nanostrukturnykh implantatov pri zameshchenii tsirkuliarnykh diafizarnykh defektov dlinnykh kostei [An experimental study of using carbon nano-structural inplants when filling circular shaft defects of long bones]. *Journal of Bone Reports & Recommendations* [Zhurnal Kostnykh Otchetov i Rekomendatsii], 2015, vol. 1, no. 1, pp. 7-14. (in Russian)
7. Egol K.A., Nauth A., Lee M., Pape H.C., Watson J.T., Borrelli J. Jr. Bone Grafting: sourcing, timing, strategies, and alternatives. *J. Orthop. Trauma*, 2015, vol. 29, no. Suppl. 12, pp. S10-S14. DOI: 10.1097/BOT.0000000000000460.
8. Mikulich E.V. Sovremennye printsipy lecheniia khronicheskogo osteomielita [Modern principles of chronic osteomyelitis treatment]. *Vestnik Novykh Meditsinskikh Tekhnologii*, 2012, vol. 19, no. 2, pp. 180-184. (in Russian)
9. Galpérine T., Ader F., Piriou P., Judet T., Perronne C., Bernard L. Outpatient parenteral antimicrobial therapy (OPAT) in bone and joint infections. *Med. Mal. Infect.*, 2006, vol. 36, no. 3, pp. 132-137. DOI: 10.1016/j.medmal.2006.01.002.
10. Nikitin G.D., Rak A.V., Linnik S.A., Saldun G.P., Kravtsov A.G., Agafonov I.A., Fakhrutdinov R.Z., Khaimin V.V. *Khirurgicheskoe lechenie osteomielita* [Surgical treatment of osteomyelitis]. SPb., Rus. Grafika, 2000, 288 p. (in Russian)
11. Cholakhian A.V. Sovremennye predstavleniia o khronicheskom posttravmaticheskom osteomielite [Modern conceptions of chronic posttraumatic osteomyelitis]. *Izvestiia Vysshikh Uchebnykh Zavedenii. Povolzhskii region. Meditsinskie Nauki*, 2013, no. 1 (25), pp. 113-123. (in Russian)
12. Parsons B., Strauss E. Surgical management of chronic osteomyelitis. *Am. J. Surg.*, 2004, vol. 188, no. 1A Suppl., pp. 57-66. DOI: 10.1016/S0002-9610(03)00292-7.
13. Vinnik Iu.S., Shishatskaia E.I., Markelova N.M., Zuev A.P. Khronicheskii osteomielit: diagnostika, lechenie, profilaktika (obzor literatury) [Chronic osteomyelitis: diagnosis, treatment, prevention (review of the literature)]. *Moskovskii Khirurgicheskii Zhurnal*, 2014, no. 2, pp. 50-53. (in Russian)
14. Desiaterik V.I., Chverkalik A.K., Dunai O.G., Gubarik V.P., Cherniak A.I., Suvorov O.E. Lechenie defektov dlinnykh kostei na fone khronicheskogo travmaticheskogo osteomielita [Treatment of long bone defects through chronic traumatic osteomyelitis]. *Travma*, 2010, vol. 11, no. 1. (in Russian) Available at: http://www.mif-ua.com/archive/article/19518 (accessed 05.07.2018).
15. Shchepkina E.A., Netylko G.I., Lebedkov I.V., Sushkov I.V., Anisimova L.O. Osobennosti formirovaniia i perestroiki distraktsionnogo regenerata pri posledovatelnom primenenii chreskostnogo i intramedulliarnogo osteosinteza v eksperimente [Peculiar features of forming and reorganizing the distraction regenerated bone in case of successive using transosseous and intramedullary osteosynthesis experimentally]. *Ilizarovskie Chteniia: «Kostnaia patologiia: ot teorii do praktiki»: materialy nauch.-prakt. konf. s mezhdunar. uchastiem* [Proc. Scientific-practical Conference Ilizarov Readings “Bone Pathology: from theory to practice”]. Kurgan, 2016, pp. 409-410. (in Russian)
16. Strelkov N.S., Gavrilov A.N., Petrova E.V. Novoe v lechenii osteomielita dlinnykh trubchatykh kostei. Materialy iubileinoi vseros. nauch.-prakt. konf. «Aktualnye voprosy travmatologii i ortopedii» [New in osteomyelitis treatment. Proc. Jubilee All-Russian Scientific-practical Conference “Current Issues of Traumatology and Orthopaedics”]. *Travmatologiia i Ortopediia Rossii*, 2006, no. 2, pp. 276-277. (in Russian)
17. Kanellakopoulou K., Giamarellos-Bourboulis E.J. Carrier systems for the local delivery of antibiotics in bone infections. *Drugs*, 2000, vol. 59, no. 6, pp. 1223-1232. DOI: 10.2165/00003495-200059060-00003.
18. Flint J.D., Saravana S. Tuberculous osteomyelitis of the midfoot: a case report. Cases J., 2009, vol. 2, pp. 6859. DOI: 10.4076/1757-1626-2-6859.
19. Raghuram T., Conway J.D. Antibiotic Cement-Coated Nails for the Treatment of Infected Nonunions and Segmental Bone Defects. *J. Bone Joint Surg. Am.*, 2008, vol. 90, pp. 163-174. DOI: 10.2106/JBJS.H.00753.
20. Mousset B., Benoit M.A., Delloye C., Bouillet R., Gillard J. Biodegradable implants for potential use in bone infection. An in vitro study of antibiotic-loaded calcium sulphate. *Int. Orthop.*, 1995, vol. 19, no. 3, pp. 157-161. DOI: 10.1007/bf00181861.
21. Skriabin V.L., Denisov A.S., comp. *Ispolzovanie uglerodnykh nanostrukturnykh implantov dlia zameshcheniia postrezektsionnykh defektov pri opukholevykh i kistoznykh porazheniiakh kostei: klinich. rek.* [The use of carbon nano-structural implants for filling postresection defects for tumoral and cystic bone lesions. Technique manual]. Perm, 2011, 19 p. (in Russian)
22. Čolović B., Milivojevic D., Babić-Stojić B., Jokanović V.R. Pore geometry of ceramic device: the key factor of drug release kinetics. *Science of Sintering*, 2013, vol. 45, no. 1, pp. 107-116. DOI: 10.2298/SOS1301107C.

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