Original Article
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Efficiency of 3D Implants with Bioactive Properties for Treatment of Extensive Bone Defects: Experimental Study

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Abstract

Background. The problem of replacing extensive bone defects remains relevant. The use of implant structures with bioactive properties can stimulate osteogenesis, which will improve the final treatment result. ***The aim of the study.*** In an in vivo experiment, to study the possibility of replacing an extensive defect in the bone diaphysis with a personal bioactive cellular 3D implant and evaluate the long-term results of its use. ***Materials and Methods.*** In an in vivo experiment, adult large mongrel dogs (n = 8) were modeled with an extensive segmental defect of the tibial diaphysis measuring 4 cm. The defect was replaced with a cellular bioactive 3D implant made of titanium alloy Ti6Al4V, manufactured using the additive technology. The diameter of the cells was 1.5 mm on average. The walls of the implant had pores of 100–300 μm in size. The inner and outer surfaces were coated with a calcium phosphate layer formed by micro-arc oxidation. The primary fixation was provided with the Ilizarov apparatus. In the early postoperative period, antibiotic prophylaxis with broad-spectrum drugs was performed. Clinical, X-ray, histological and statistical methods were used to analyze the results. The main control points were considered: the end of external fixation with the Ilizarov apparatus, after 180 days and 1 year after the termination of external fixation. ***Results.*** During the experiment, the death of animals and complications were not observed. The spatial location of the implant was preserved. The formation of a strong bone-implantation block occurred 37.2±6.3 days after the operation. During this period, the external fixation apparatus was dismantled. Osseointegration was provided under conditions of sufficient primary mechanical stability, due to the cellular structure of the implant, the presence of pores on its walls, and the osteoinductive properties of the applied calcium phosphate coating. The achieved degree of osseointegration persisted in long-term periods (6 months and 1 year after the termination of external fixation). The osteoinductive properties of the calcium phosphate coating were confirmed by the expression of osteopontin cells at all stages of the experiment. Outflow of Ca and P from bone fragments was not observed. An elastic sheath was formed on the surface of the implant, similar in structure to the periosteum. The implant cells were filled with a well-vascularized bone substrate. In the projection of the intermediate zone, compact bone tissue was formed, and in the projection of the medullary canal — reticulofibrous bone marrow. This indicates the possibility of organotypic remodeling of bone structures inside the implant. ***Conclusion.*** The results of the study showed the effectiveness of using a bioactive cellular 3D implant to replace an extensive defect in the shaft of the bone. The architectonics and osteoinductive properties of the implant surface contributed to the formation of complete osseointegration in a short time, while maintaining the achieved result in long-term periods.

Keywords: bone defect, 3D implant, additive technologies, bioactive coating, osseointegration, Ilizarov apparatus.

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Introduction

In the Russian Federation, the consequences of injuries take the first place among the causes of primary disability among citizens of working age, without having a downward trend, and are accompanied by huge economic losses for the state [1]. Literature data indicate that pseudoarthrosis and bone defects are preferred to be treated by open surgical methods using metallosteosynthesis, bone autografting, and microsurgical techniques [2]. However, unsatisfactory outcomes of treatment with free allo-and autografts are due to the lack of full-fledged intraosseous blood circulation [3].

The most effective methods in the treatment of pseudoarthrosis and bone defects are the methods of transosseous osteosynthesis developed by academician G. A. Ilizarov and based on the atraumatic nature of the surgical intervention, the stability of fixation, the dosed stress of the formative processes, full blood supply and functional load [4]. However, the duration (from 3 to 11 months. depending on the local status) and the multi-stage inpatient treatment, complexity of osteosynthesis, the need for constant supervision throughout the entire period of treatment and rehabilitation measures, and decreasing the quality of patients life problem remains unresolved [5, 6].

Pathological processes of bone formation disorders in a number of systemic and chronic infectious lesions are accompanied by the formation of bone areas with weakened mechanical properties, which can be observed in intraosseous fibrous, chondromatous foci, the presence of cysts. Similar changes are also determined during the marginal formation of the distraction regenerate. It also requires a long time for spontaneous bone defect replacement, even with the formation of the cortical bone section.[7, 8, 9, 10]. An important problem is the loss of bone mass due to traumatic lesions with subsequent consolidation of bone fragments, but with the preservation of defects in the epimetaphyseal zone or longitudinal defects of long bones. This leads to a serious decrease of the bone strength characteristics and its shape violation[11, 12, 13]. To solve such problems, additive technologies are used for manufacturing medical devices from various materials of a given shape and surface structure. It allows to take into account the individual anatomical patients features and leads to better functional results of treatment [14, 15, 16, 17]. Many researchers note the increased effectiveness of implants with osteoconductive and osteoinductive properties. In this direction, active developments are being carried out both in Russia and abroad [18, 19, 20, 21]. It is obvious that specialists are most interested in the long-term preclinical and clinical trials results of such products.

The aim of the study was to analyze the possibility of replacing large defect in the bone diaphysis with a personal bioactive cellular 3D implant in an in vivo experiment and to evaluate the long-term results of its use.

Materials and Methods

Research design

An in vivo study was conducted on 8 adult mongrel dogs of both sexes, who underwent replacement of the defect in the lower leg bones shafts with a height of 40.0 mm with an individual bioactive cellular 3D-implant. The defect was considered as critical, since its size was more than twice the diameter of the replaced bone area.

Compliance criteria

All animals were clinically healthy, their age varied in the range of 1.0-2.5 years, the length of the tibia was 18.1±0.6 cm, and the diameter in the middle of the diaphysis was 15.4±1.5 mm. These data were the main criteria for the inclusion of animals in the study. Before starting the experiment, the exclusion criteria were determined. These included: the death of an animal and other pathological conditions that are not related

to the conditions of the experiment; errors made during surgery that are not related to the characteristics of the tested product.

Terms of realization

The animals were kept in vivarium conditions in individual boxes (one individual per box), where the same temperature regime and lighting conditions were provided. The diet included clean drinking water without restrictions and nutritionally balanced identical feed. All surgical manipulations were performed in the operating room by one surgical team. When replacing extensive defects with the tested products, the bone fragments were fixed with the Ilizarov apparatus until the formation of a weight-bearing bone-implantation block. To prevent the occurrence of infectious complications, all animals underwent antibiotic prophylaxis with drugs of the cephalosporin class of the first generation according to a shortened scheme in the recommended doses (intravenously once, simultaneously with the administration of an anesthetic drug, and then intramuscularly for 2 days after surgery). After surgery, the wounds were treated with antiseptic drugs daily until sutures were removed.

Before use, all tested products were sterilized in an autoclave according to the following procedure: 121°C for 30 minutes, then dried for at least 1 hour.

Duration of the study

The total duration of the experiment exceeded 1 year and was 400-407 days after the surgical intervention.

The following experiment periods were determined: the end of fixation by the Ilizarov apparatus (EF), 180 days and 1 year after the end of fixation.

During these periods, the animals were euthanized by intravenous administration of lethal doses of sodium thiopental with preliminary premedication with conventional pharmacological drugs.

Tested products characteristics

For each animal, implants were made using additive technology by laser sintering of Ti6Al4V titanium alloy powders with subsequent application of a calcium-phosphate coating (utility model patent RU 171823 U1). To do this, before the start of the experiment, the animals underwent a computed tomography of the right lower leg. According to the three-dimensional segment images, individual customized implants of the specified sizes were made.

The implants were in the form of cylinders, which consisted of a central part and two external parts (ends). The central part (the body) was a lattice structure with a height of 40 mm. Its interior space was divided by cells. The walls forming the cells were 1.5 mm in size, 0.5 mm thick, and had pores of 300-500 microns in size. The implants ends were in the form of closed rims with a height of 10 mm each, with holes with a diameter of 1.5 mm for the possibility of fixing the implant to the bone fragments ends with screws. In all products, the wall of the ends was 1 mm thick and had pores of 100-300 microns in size. All the implant surfaces (external and internal) were covered with a bioactive layer of hydroxyapatite formed by microarc oxidation technology at the National Research Tomsk Polytechnic University.

Surgical technique

Before the surgery, the fur on the experimental limb was removed. After that, they were drugged. As an anesthetic, sodium thiopental was used in the recommended dosages, which was administered intravenously.

The animals were fixed on the operating table in a lateral position. The Ilizarov apparatus was mounted on the experimental lower leg according to the previously described technology [22]. The only fundamental difference was that at the second and third functional levels (closer to the ends of the bone fragments), one k-wire was passed

through each fragment. Accordingly, the total number of transosseous k-wires was 6, instead of 8 (in the classic version of fixation). This variant of fixation was chosen to avoid traumatization of the anterior and lateral muscle groups with k-wires.

After that, a longitudinal incision of soft tissues, including the periosteum, was made with a scalpel from the medial surface of the tibia, in the area of the tibial shaf middle third. The periosteum was carefully peeled off from the compact bone layer with a surgical dissector and, together with the adjacent muscles, was moved laterally in order to prevent their damage during the osteotomy. Then, using an oscillating saw, a section of the diaphysis with a length of 40.0 mm was cut out. A sterile individual cellular bioactive 3D implant was inserted into the resulting defect in such a way that the bone fragments ends were embedded in the ends of the implant (in the rims) (Fig. 1). In all cases, the periosteum and other soft tissues were sutured in layers with interrupted sutures.

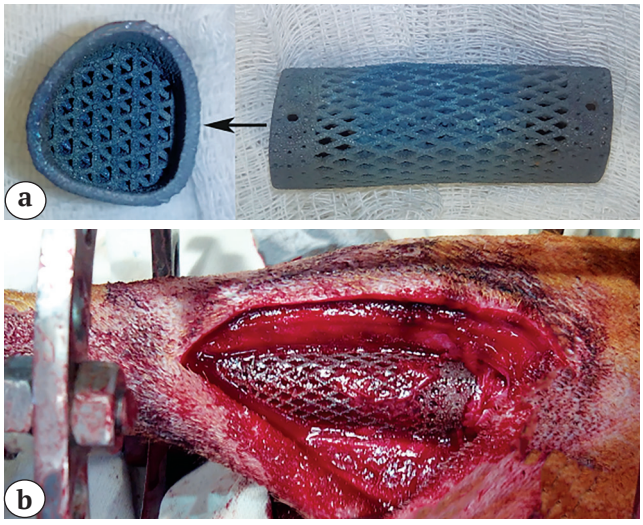

Figure 1. Cellular bioactive 3D implant for replacing a defect in the diaphysis of the bone (a); the stage of the operation (after installing the implant into the defect of the diaphysis of the tibia of a dog measuring 4 cm) (b)

The main outcome of the study

To achieve the goal, during the periods indicated by the control points, we noted:

- the weight-bearing function of the limb;
- position of the implant relative to the longitudinal axis of the segment;
- terms of formation of a weight-bearing bone-implantation block (the end of fixation period by the external device);
- signs of tissue integration on the surface and inside the implant.

Additional study outcomes

Additionally, the general condition of the animals and the local condition of the soft tissues in the area of the implants location were evaluated.

Methods for outcomes registration

To obtain the main results of the study, one of the methods was clinical examination, which was used to palpate and visually assess the axis of the experimental limb and its weight-bearing function.

The formation of strong bone-implantation block was recorded on the basis of the clinical test and X-ray examination results. For the clinical probe, the rods connecting the subsystems of the Ilizarov apparatus were removed. Flexion and rotational loads were applied to the bone fragments by hand. The absence of pathological mobility and pain in the area of the implant with bone fragments contact indicated the achieved osseointegration. If tight pathological mobility was determined, the threaded rods were mounted back and fixation with the apparatus continued.

Radiography was performed in direct and lateral projections using the VEP X Technology Premium VET X-ray machine (Spain). The technical conditions of the radiography were the same. The focal length was 97 cm, the amperage was 2.5 — 3.2 mA, and the tube voltage was 44-46 kV. The radio-

graphs showed: the implant location in relation to the longitudinal axis of the segment; signs of bone formation on the implant surface, bone fragments and in the area of visible contact of the implant end sections with the ends of the bone fragments. The presence or absence of signs of osteosclerosis, bone destruction and pathological changes in the cortical bone were noted.

The features of bone formation on the implant surface and inside the cells were evaluated by histological methods: light and scanning electron microscopy. These methods were used to study bone-implantation blocks obtained during the periods: EF (n = 3), 180 days (n = 3) and 1 year (n = 2) after end of fixation.

After animals euthanasia, bone-implantation blocks were cut out, which were fixed in a 10% solution of neutral formalin for 14 days. Then they were cut into fragments in the transverse and longitudinal planes on the precision cutting machine IsoMet 4000 (Buehler, USA). On one half of the cut, tissue fragments were cut from the implant surface and from its cells, dehydrated in alcohols of ascending concentration (from 70 to 100), poured into paraffin and epoxy resin. Paraffin histological sections were prepared using a microtome from Reichert (Austria), stained with hematoxylin and eosin and according to Van Gieson, as well as with an immunohistochemical reaction using polyclonal antibodies against ostepontin (protocol and reagents from Abcam, England). Semi-thin sections were prepared using the ultramicrotome LKB Bromma Ultrotome Nova (Sweden)and stained with methylene blue with a preliminary PAS-reaction. Histological sections were examined using an AxioScope stereomicroscope.A1 and AxioCam ICc 5 digital cameras complete with Zen blue software (Carl Zeiss, Germany).

The second half of the bone-implantation block was dried according to the original technology and examined using an energy-dispersive X-ray spectrometer for electron microscopy QUANTEX EDS (Bruker, USA), mounted on the basis of an EVO 18 scanning electron microscope (Carl Zeiss, Germany). The content of calcium and phosphorus, as well as their ratio in the tissue matrix inside the cells and on the surface of the implant, were determined. 6 fields of vision were analyzed for each study area of one bone-implantation block.

The values obtained in the study of intact diaphyses of 10 similar age dogs and kept in the same conditions were used as the norm.

Additional outcomes were determined by the clinical method. For this purpose, during the periods indicated by the control points, the behavioral reactions of the animals, the intensity of food and water intake, the presence or absence of infectious and neurological complications were noted. The skin condition in the area of operative approach was visually monitored.

Statistical analysis

Quantitative data were subjected to statistical processing using the AtteStat 13.1 program (Russia). The sets of quantitative indicators were described using the values of the median (Me) and the lower and upper quartiles (Q1-Q3). The Mann–Whitney U-test was used to compare independent populations. The differences were considered statistically significant at a significance level of $p<0.05$.

Results

Main results of the study

When evaluating function of the experimental segment, after 2-3 days the animals began to weight-bearing the limb. By the 7th day of the experiment, they were using a limb, but there was a limp of the leaning type, the signs of which almost completely disappeared by the end of the fixation period. During the experiment, visual and palpatory violations of the segment axis (the formation of deformities) were not determined.

Immediately after the operation, the X-ray images showed that the segment axis was correct. The implant was clearly visualized. The first signs of the bone-implantation block formation appeared after 14 days of fixation. They were in the form of thin indistinct shadows of periosteal origin (up to 2 mm thick, up to 3 mm long). These strands connected the bone surface to the end sections of the implant. Shadows of the endosteal reaction were observed in the projection of the bone marrow canal of the fragments. In subsequent periods, the thickness, length, and intensity of the periosteal shadows increased. They were formed not only in the area of contact of the implant with the bone, but also on the surface of the fragments.

By the end of the the Ilizarov device fixation period, the shadows of the periosteal layers acquired a more compact and clear structure and combined the end sections of the implants with the cortical layer of the bone in the form of "sleeve". The endosteal reaction in the bone fragments was preserved. The duration of hardware fixation averaged 37.2±6.3 days, including 28-35 days. - in 5 observations, in other animals-42-47 days. (Fig. 2 a). Areas of osteosclerosis or bone destruction were not detected either in this period or later after end of hardware fixation (180 days and 1 year after end of fixation).

In the area of visible contact of the implant ends with the bone, there were no signs of pathological changes in the cortical bone (Fig. 2b).

Histological examination revealed the formation of loose fibrous connective tissue on the surface of the implant, which was similar in structure to the periosteum, in which vessels and nerve were determined, when a strong bone-implantation block was formed (EF period). In the newly formed periosteum, the presence of cells expressing osteopontin was noted, which were detected both perivascularly and in areas far from the vessels (Fig. 3a). During the SEM study, integration of the tissue component with the implant walls in all areas was determined on the X-ray electron probe microanalysis maps. More mineralized tissue was observed near the implant ends. A large number of microvessels were observed to grow into the cells, and the presence of bone substrate was determined on the surface of these vessels. No resorption of the cortical bone on the fragments ends was detected (Fig. 3b). By this time, the implant cells were filled with a bone, similar in density to the spongy bone (Fig. 3c).

180 days after removal of the apparatus, the outer surface of the implant was covered with a white opaque elastic shell, which, according to the histostructural organization, represents the periosteum. Its external layer was well vascularized. Perivascular cells had osteogenic potency, as evidenced by the expression of osteopontin by some of them. When examining the implants by scanning electron microscopy and X-ray electron probe microanalysis, it was found that fragments of small-cell spongy bone were found in the cells close

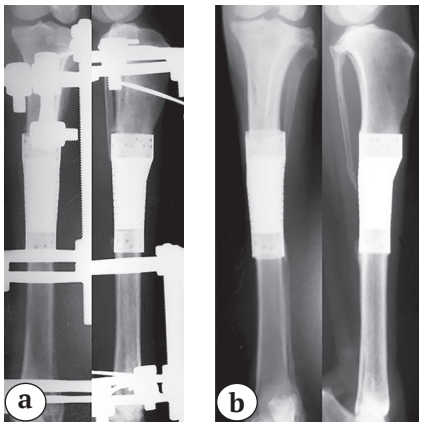

Figure 2. X-rays of the experimental segment:
a — during the period of termination of hardware fixation (35 days after surgery);
b — 1 year after the termination of external hardware fixation

to the bone ends at a distance of 5-7 mm from the contact zone (Fig. 4a, b). In projection of the implant intermediate zone, mineralizing bone tissue was formed inside the cells, as evidenced by the intense PAS-positive staining of the intercellular matrix of non-calcined semi-thin sections (Fig. 4c). In the cells located inside the middle part of the implant, by this period, fibroreticular bone marrow with microvessels contained in it and immature bone trabeculae were detected (Fig. 4 d).

A year after the removal of the apparatus, the bone fragments were tightly attached to the implant. Bone resorption was not detected (Fig. 5a). In the implant cells on the side of bone fragments for up to 8-10 mm in the projection of the intermediate zone, more mineralized bone tissue of the lamellar type was found than in the previous period. In the projection of the bone marrow canal, the tissue matrix in the cells was poorly mineralized (Fig. 5 b).

The areas combination of reticular, fibrous, and mineralizing bone tissue was determined on semi-thin sections of tissue extracted from the cells (Fig. 5c). This pattern indicates the possibility of organotypic rearrangement of the newly formed bone within the implant structures. This, in our opinion, occurs as a result of greater biomechanical load impact on the areas of the implant that are close to the bone fragments and in contact with them. In the all zones of the implant middle areas, the tissue substrate was less mineralized. Studies of tissue detritis formed on the implant surface showed the presence of an osteogenic periosteum, producing a layer of bone substance, which by this period acquired the structure of lamellar bone, which is confirmed by immunohistochemical studies with the detection of osteopontin expression (Fig. 5 d).

The data of quantitative X-ray electron probe microanalysis showed that the most mineralized bone substrate was formed in the implant cells in the projection of the bone sections by the end of the fixation period in the area of the bone marrow canal, and in later periods — in the projection of the intermediate zone (Table 1). The outflow of mineral components (Ca and P) from the bone fragments was not observed in all periods of the experiment. Their content did not differ statistically from the indicators in the norm.

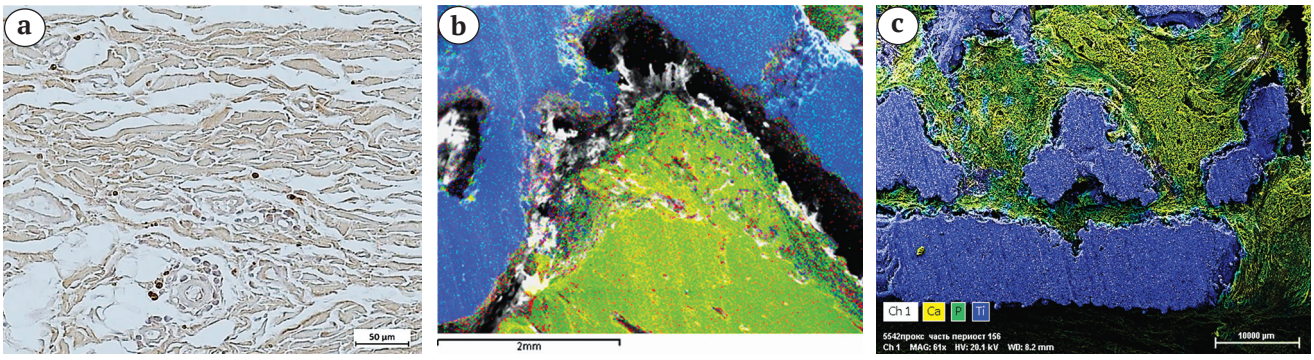

Figure 3. Structural features and mineralization of the tissue substrate within the implant cell at the end of the fixation period:
a — expression of osteopontin in the perivascular cells of the periosteum, which forms on the surface of the proximal part of the implant (immunohistochemical staining using polyconal rabbit antibodies against osteopontin); b — contact between the distal fragment and the implant; c — integration of the tissue substrate into the cells of the implant; b, c — aligned maps of X-ray electron probe microanalysis obtained in the characteristic radiation of Ca, P, Ti. Blue — Ti, light green — mixed overlay of Ca and P.
Mag.: a — ×400; b, c — ×50

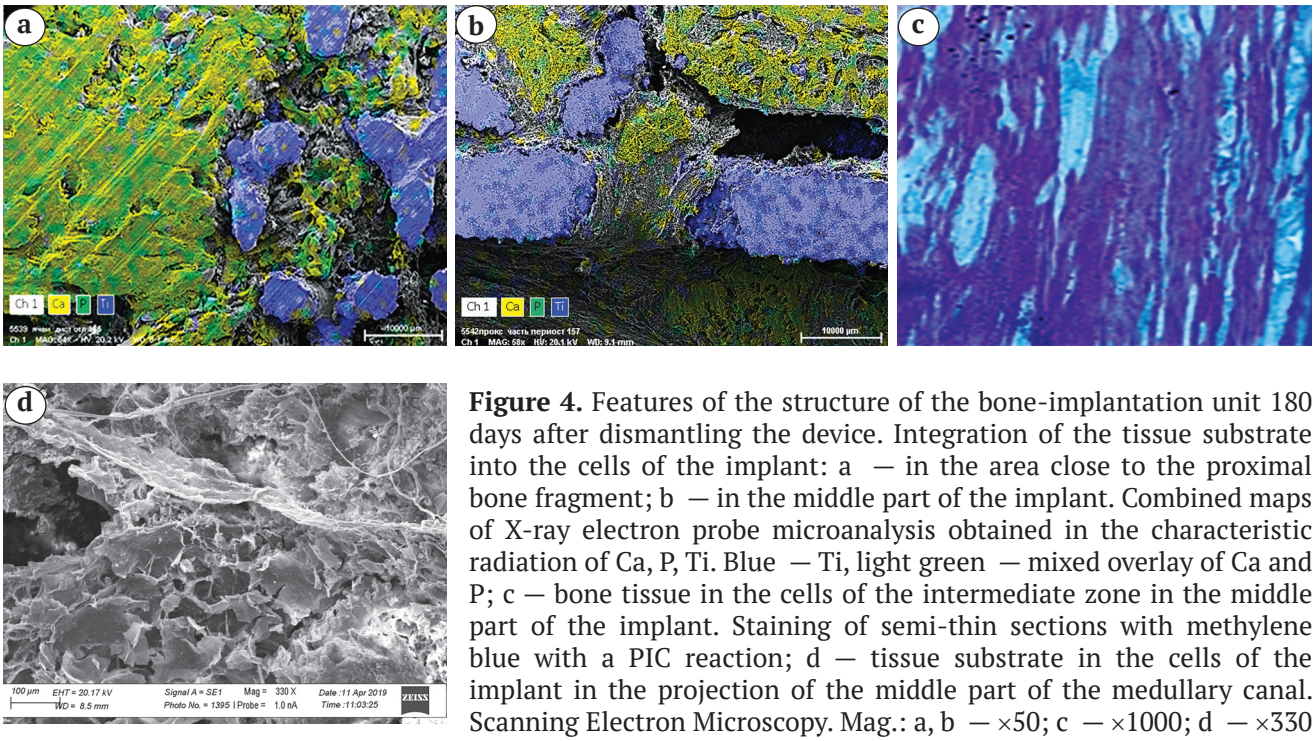

Figure 4. Features of the structure of the bone-implantation unit 180 days after dismantling the device. Integration of the tissue substrate into the cells of the implant: a — in the area close to the proximal bone fragment; b — in the middle part of the implant. Combined maps of X-ray electron probe microanalysis obtained in the characteristic radiation of Ca, P, Ti. Blue — Ti, light green — mixed overlay of Ca and P; c — bone tissue in the cells of the intermediate zone in the middle part of the implant. Staining of semi-thin sections with methylene blue with a PIC reaction; d — tissue substrate in the cells of the implant in the projection of the middle part of the medullary canal. Scanning Electron Microscopy. Mag.: a, b — ×50; c — ×1000; d — ×330

Table 1

The content of Ca and P in the cellular implant tissue matrix in different periods of the experiment (Me (Q1-Q3))

Research area of bone-implantation block	Content (W) in weight.%		
	Experiment period	Ca	P
Tissue matrix in the cells of the implant proximal part in the projection of the intermediate zone	End of fixation	3,68 (3,55–3,79)*	1,65 (1,58–1,67)*
	180 days without the apparatus	14,31 (13,83–14,75)*	6,70 (6,47–7,00)*
	1 year without the apparatus	18,43 (17,76–21,1)*	7,49 (7,11–7,93)*
Tissue matrix in the cells of the implant central part in the projection of the intermediate zone	End of fixation	3,00 (2,86–3,13)*	1,43 (1,39–1,52)*
	180 days without the apparatus	2,32 (2,26–2,41)*	0,48 (0,45–0,50)*
	1 year without the apparatus	1,71 (1,40–2,00)*	0,50 (0,41–0,53)*
Tissue matrix in the cells of the implant dostal part in the projection of the intermediate zone	End of fixation	3,64 (3,54–3,78)*	1,67 (1,59–1,74)*
	180 days without the apparatus	12,30 (10,13–14,70)*	5,01 (5,07–5,47)*
	1 year without the apparatus	14,77 (14,29–15,20)*	7,00 (6,58–7,46)*

End of table 1

Tissue matrix in the cells of the implant proximal part in the projection of the bone marrow canal	End of fixation	6,58 (6,54–6,61)*	2,61 (2,47–2,72)*
	180 days without the apparatus	2,30 (2,23–2,35)*	0,49 (0,46–0,51)*
	1 year without the apparatus	1,70 (1,63–1,78)**	0,50 (0,49–0,52)*
Tissue matrix in the cells of the implant central part in the projection of the bone marrow canal	End of fixation	3,20 (3,07–3,36)*	1,53 (1,48–1,57)*
	180 days without the apparatus	2,22 (2,09–2,35)*	0,170 (0,168–0,172)*
	1 year without the apparatus	1,81 (1,73–1,86)**	0,260 (0,257–0,270)*
Tissue matrix in the cells of the implant distal part in the projection of the bone marrow canal	End of fixation	3,20 (3,08–3,33)*	1,28 (1,24–1,31)*
	180 days without the apparatus	1,67 (1,59–1,74)**	0,52 (0,49–0,53)*
	1 year without the apparatus	1,55 (1,49–1,67)**	0,49 (0,46–0,50)*
Cortical bone of the proximal fragment	End of fixation	21,84 (20,98–22,24)**	9,35 (8,99–10,15)**
	180 days without the apparatus	21,93 (20,73–22,31)**	7,01 (6,98–7,18)*
	1 year without the apparatus	22,15 (21,95–22,33)**	10,6 (9,97–10,86)**
Research area of bone-implantation block	Content (W) in weight.%		
	Experiment period	Ca	P
Cortical bone of the distal fragment	End of fixation	20,53 (20,15–21,2)**	8,91 (8,12–9,99)**
	180 days without the apparatus	19,26 (18,60–20,10)**	10,68 (9,71–11,7)**
	1 year without the apparatus	21,47 (21,41–21,69)**	7,10 (6,96–9,170)**
Bone marrow canal of the proximal fragment	End of fixation	9,97 (9,73–10,12)*	4,57 (4,37–4,91)*
	180 days without the apparatus	4,21 (4,00–4,50)*	1,90 (1,85–2,00)*
	1 year without the apparatus	2,00 (1,91–2,31)*	0,83 (0,79–0,86)*
Bone marrow canal of the distal fragment	End of fixation	9,48 (9,09–10,66)*	3,90 (3,78–4,20)*
	180 days without the apparatus	3,84 (3,71–3,92)*	1,62 (1,53–1,69)*
	1 year without the apparatus	2,30 (2,26–2,35)*	0,885 (0,885–0,905)*
Bone marrow canal of the intact animals (norm)	–	1,72 (1,67–1,75)	0,71 (0,68–0,75)
Cortical bone of the intact animals (norm)	–	22,14 (21,12–22,72)	9,79 (9,64–10,10)

* — the values are statistically significantly different from the norm ($p<0.05$);

** — the values are not significantly different from the norm ($p=0.05$).

Additional research results

During the experiment, no cases of animal deaths and any neurological and infectious complications were recorded.

No pathological conditions were detected in the main organ systems. The color of the mucous membranes and skin did not change. Behavioral responses both immediately after surgery and during the experiment corresponded to the expected clinical condition. During periods of rest and during movement, the dogs assumed a natural physiological position (see Fig. 2).

Epithelization of postoperative wound occurred without any special features. The sutures were removed in the usual time (7-10 days after the surgery). During this period, a pink or red scar was formed in the area of the soft tissue incision. Subsequently, it acquired the color of the surrounding soft tissues and was almost not visually determined.

Discussion

Summary of the main research result

When replacing extensive defects of the bone diaphysis with a personal bioactive cellular 3D implant of the original design, the formation of a strong bone-implantation block occurred 37.2±6.3 days after the surgery. The achieved effect was maintained in the long-term period (after 6 months and 1 year). The weight-bearing function of the limb was not disturbed. Osseointegration was achieved under conditions of adequate primary mechanical stability due to the cellular structure of the implant, the presence of pores on its walls, and the osteoinductive properties of the applied calcium-phosphate coating. There were no infectious or neurological complications.

Discussion of the main research result

It is known that the effectiveness of various biomaterials types usage in traumatology and orthopedics depends on many factors. D. L. Williams and B. M. Isaacson in their study summarized these factors and identified 5 main signs that affect the clinical results of treatment. These include: the biocompatibility of the materials used to make the implants; the surgical technique used during their installation; the design of the implant and its primary mechanical stability after installation, as well as the prevention of infections [23].

One of the biocompatible, biologically inert materials used for the manufacture of medical devices is titanium and its alloys [24]. Modern additive technologies allow the use of powders of these materials to create surfaces with a given topography (roughness, porosity, etc.), with mechanical properties comparable to the spongy bone of humans and animals. The preference is often given to the Ti6Al4V titanium alloy, the cytocompatibility of which has been experimentally proven [25, 26, 27].

As noted earlier, the success of using the products being developed depends on their design. It is shown that cellular or mesh structures with the presence of micropores on their surface have the best osteoconductive properties. A certain balance must be achieved between these parameters, since structures with large cells and high porosity have reduced mechanical strength.

At the same time, such characteristics promote better ingrowth of well-vascularized bone tissue without prior formation of cartilage. In this context, pores of 100-300 microns or more are considered optimal [28, 29]. The presence of pores gives the surface of the implants a roughness that allows it to physically bind (integrate) not only the adjacent bone tissue, but also, if necessary, soft tissues.

The latter property of porous materials is currently poorly understood. It was discovered by a group of Russian scientists.

In experiment, R. M. Tikhilov and co-authors demonstrated the possibility of soft tissues integrating, in particular muscle tissue,

into porous metal structures. The authors emphasize the high clinical significance of the results obtained. This applies to cases when localization of bone defects captures the areas of muscles and tendon-ligamentous apparatus attachment[30].

Additive manufacturing technologies for medical devices allow to accurately reproduce a three-dimensional prototype of lost or replacing bone area. As a rule, the effectiveness and safety of the use of such implants is pre-evaluated by performing preclinical tests in vivo. It is believed that in traumatology and orthopedics, large animals (sheep, dogs, pigs, primates) are the most suitable for solving most problems. This is due to a certain similarity of their musculoskeletal system tissues(in size, structure, features of metabolism, and others) with those of humans. It also becomes possible to simulate clinical situations as accurately as possible and to study long-term (6 months or more) experimental results [31, 32].

In our study, the experiments were performed on adult mongrel dogs of large size with a lower leg length of at least 17 cm. This made it possible to simulate a large defect of the tibial diaphysis of 4 cm in size, which was at least 22% of the segment total length. In the manufacture of implants, the positive properties of cellular and porous structures were taken into account. The test samples were made of biocompatible titanium alloy Ti6Al4V and were cellular cylinders, the shape of which corresponded to the configuration of the replacing bone area. For better adhesion of osteogenic cells, the walls of external and internal surfaces of the implants additionally had optimal pores (from 300 microns).

Many studies have shown that it is possible to improve the surface characteristics of implantation materials by giving them osteoinductive properties. For this purpose, products are most often covered with a layer of bioactive substances, which include calcium-phosphate compounds [33, 34]. There are different ways of applying such coatings on a metal base [35, 36]. In the study, a bioactive calcium-phosphate layer was formed on a porous surface by microarc oxidation, the advantages and effectiveness of which were proved and presented in previous publications and in other literature sources [37, 38, 39].

According to the literature, the primary fixation of 3D-products is performed in different ways. More often, this function is carried out by structural elements or additional internal fixators made in the form of beams with holes, screws [40, 41, 42]. A. M. Crovace and co-authors in experiment on sheep when replacing a 5 cm tibial defect with 3D-biomimetic porous titanium frames, as fixators, they successfully used bone plates and cortical screws. However, to remove them after 9 months after implantation, additional surgical intervention was required [43]. E. Kon and co-authors used external devices for a similar purpose [44].

Other authors used implants to replace a bone defect in both forearm bones after resection of a malignant tumor in a patient, the design of which, among other elements, included cuffs with a height of 10 mm and a thickness of 3 mm, into which the ends of bone fragments were embedded. From the cuffs there were elements similar to bone plates, which were attached to the bone fragments with screws. No complications were observed within 12 months after the operation [45]. Despite the positive result of the treatment, when using implants of a similar design to replace bone defects in the lower extremities, such fixation may not be enough. This is due to the fact that the vertical axial loads on the segment experienced when walking can create prerequisites for the formation of hardware instability.

In our study, the primary stability of the tested products was provided as follows. First of all, the ends of the fragments were inserted into the end closed rims of the implants to a depth of 1 cm proximally and distally. This

height of the rims was sufficient to prevent the mobility of bone fragments under flexion loads. Secondly, the external ring device was additionally fixed, this excluded rotational movements. To dismantle the external device, no repeated surgical intervention was required. Despite the fact that the tested implants had holes at their ends for additional fixing with screws, in this study such fixation was not performed.

The animals maintained the weight-bearingt function of the operated limb throughout the experiment. The location of the implant did not change. There were no infectious complications, which were prevented by antibiotic prophylaxis with broad-spectrum drugs. The presence of bioactive layer on a rough (porous) surface provided the products with not only osteoconductive, but also osteoinductive properties, which was confirmed by the expression of osteopontin cells both in the early and long-term periods of the experiment. Some authors believe that the presence of pores and a coating of calcium phosphate on the walls of implants is a nessesary condition for the successful course of bone formation processes [46].

In the experiments, the optimal characteristics of the implant surface contributed to complete osseointegration in a fairly short time. A weight-bearing bone-implantation block was formed after 1.0–1.5 months, which allowed to dismantle the external fixator. All cells were filled with a well-vascularized bone substrate. After 6 months the formed bone-implantation block by its histological structure acquired some properties characteristic of a normal part of the bone, namely: 1) its surface was covered with an elastic membrane, similar in structure to the periosteum; 2) in the projection of the bone fragments ends contact with the implant, a lamellar bone was formed; 3) the cells located inside the middle part of the implant were filled with a tissue substrate, similar in composition to the fibroreticular bone marrow with microvessels and bone trabeculae located in it. The achieved positive result was maintained in a more distant period, in particular, one year after the end of external fixation, which is confirmed by clinical, radiological and histological studies.

Other authors in experiments on sheep when replacing a large defect in the shaft of the lower leg bones with similar implants characteristics obtained similar results. A year after the surgery, the bone fragments were combined with the implant by the bone tissue of the lamellar structure. A well-vascularized bone matrix was formed inside the cellular structure. Signs of bone remodeling were determined. No signs of immunotoxic tissue reactions were detected [43].

Limitations of the study

The main limitation of the performed study can be considered the absence of a control experimental group (replacement of similar defects with implants without bioactive coating).

When developing the design of the experiment, we primarily relied on the results of our own previously performed studies and the availability of known literature data, which studied in detail the effectiveness and safety of implants made of Ti6Al4V titanium alloy. The possibility of replacing extensive bone defects with 3D-cellular products made of this material is shown both in experiments on large animals and in clinical trials [25, 26, 27, 37, 43, 45].

Despite the fact that in traumatology, orthopedics and dentistry, products made of Ti6Al4V titanium alloy are often preferred, the presence of vanadium and aluminum in its composition does not exclude the possibility of toxic effects of these elements on the tissue. There is evidence that even titanium ions and nanoparticles can cause a mutagenic effect in cells [47]. It is possible to minimize the impact of metal ions on the tissues by applying protective coatings to the surface of the implanted products [48]. It can be assumed that in the study performed, the calcium-

phosphate coating applied to the surface of the Ti6Al4V titanium alloy implants additionally performed the necessary protective function. A year after the surgery, no pathological changes were detected in the peri-implant tissues. However, it is obvious that more research is needed to confirm this assumption.

Conclusion

The results of the performed study are comparable with the known literature data and demonstrated the effectiveness of using a domestic cellular bioactive structured 3D-implant with specified characteristics to replace a large segmental defect of the bone diaphysis. A strong bone-implantation block was formed due to the early integration of bone tissue with the metal surface of the tested products (within 1.0-1.5 months). This was due to the biocompatibility of the titanium alloy used for the manufacture of the latter, their cellular structure with the presence of optimal size micropores and bioactive calcium-phosphate layer, as well as due to the provision of sufficient primary mechanical stability.

Ethical approval

Before the experiment began, a positive decision was received from the local ethics committee to conduct it. When performing experiments, the principles of humane treatment of animals were observed in accordance with the requirements of the European Convention for the Protection of Vertebrates Used for Experiments and Other Scientific Purposes and Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes.

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All the authors read and approved the final version of the manuscript. All authors agree to be responsible for all aspects of the work to ensure that all possible issues related to the correctness and reliability of any part of the work are properly considered and resolved.

Conflict of interest:
The authors declare that there is no conflict of interest.