doi: 10.21823/2311-2905-2020-26-2-98-108

Osseointegration of Titanium and Steel Additive Manufactured Implant in Rabbit Tibia under External Fixation: Comparative Study

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Abstract

Background. The main goals of successful prosthesis remain ensuring the osseointegration and infectious safety of implants. ***The purpose of the study*** — the comparative analysis of osseointegration of titanium and steel additive manufactured implants in the rabbit tibia under additional fixation by Ilizarov apparatus. ***Materials and Methods.*** The study was performed on 20 chinchilla male rabbits. The animals of the first group ($n = 8$) were implanted a stainless steel product EOS PH1 (EOS, Germany), the animals of the second group ($n = 12$) — a titanium alloy Ti6Al4V product. The implant was additionally fixed by Ilizarov apparatus. The implants were processed with additive technology by selective laser fusion at the EOSINT M 280 installation (EOS, Germany). The survival and safety of the implants were assessed using clinical, histological, laboratory and statistical methods. ***Results***. The implant fall due to chronic inflammation was found in 2 animals of group 1 and none in group 2. The formation of weakly mineralized bone tissue on the surface of the implant was noted in 3 weeks in all cases. The bone became more mineralized by the 12th week of the experiment. However, in group 2, the calcium content and Ca / P ratio of the newly formed bone tissue at the 3rd and 12th week after implantation were significantly higher than in the animals of group 1. This indicated the greater maturity of the bone tissue in animals of group 2 at all stages of the experiment. In group 1, the compact plate osteoporosis and calcium-phosphorus balance disturbance were greater. ***Conclusion***. The results of the study indicate that the survival rate (osseointegration) and safety of the product made of the titanium alloy were higher compared with the stainless steel product.

Keywords: additive technology, selective laser fusion, titanium implant, stainless steel implant, osseointegration, Ilizarov apparatus.

Cite as: Emanov A.A., Kuznetsov V.P., Gorbach E.N., Stogov M.V., Kireeva E.A., Ovchinnikov E.N. [Osseointegration of Titanium and Steel Additive Manufactured Implant in Rabbit Tibia under External Fixation: Comparative Study]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia]. 2020;26(2):98-108. (In Russian). doi: 10.21823/2311-2905-2020-26-2-98-108.

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Received: 02.03.2020. Accepted for publication: 21.04.2020.

Introduction

Currently, in orthopedics, a lot of research for solving prosthetics problems devoted to the fundamental and clinical justification of using osteointegrated transdermal implants of various design is carried out [1, 2, 3, 4, 5, 6]. The main goal in finding solutions to this problem remains to ensure the survival and infectious safety of such implants [7, 8, 9, 10, 11].

According to the literature, it was found that osseointegration of the percutaneous implants depends on various factors, including geometry, relief, topology [12, 13, 14] and biological peculiarities of the implant interaction with bone [15, 16, 17].

Recently, a personified approach employing the additive technologies (AT) has been used for the implants manufacturing. This allows taking into account the individual anatomical features of patients, controlling and setting the size characteristics and parameters of implant surface formation, which undoubtedly contributes to the improvement of treatment results [18, 19]. However, to date, the risk of complications remains quite high, which is often associated with the quality of alloys used for the implants manufacture [20]. It has been proved that biological fluids can be an aggressive environment for metal products. In this regard, the problems of the occurrence of metallosis and metal allergy are relevant [21, 22]. For the needs of traumatology and orthopedics, alloys of iron and titanium are most often used. Most authors note that the corrosion resistance of medical implants made of titanium alloys is relatively higher, and, accordingly, the tendency to the metallosis development is lower, than in the stainless steel products [23, 24, 25]. Nevertheless, we previously revealed that during prosthetics of the rabbit's stump with stainless steel implants made with AT, a bone-implantation block due to the structured surface could withstand a sufficient support load [26].

The purpose of this study was the comparative analysis of osseointegration of titanium and steel implants, manufactured using the AT, in the rabbit's tibia under conditions of additional fixation of the biomechanical system by Ilizarov's apparatus.

Material and Methods

Study design

Since a comparison group was present in the study to identify the effectiveness osseointegration and safety of the implants, and the animals were randomly assigned to the groups, this work can be attributed to the category of controlled randomized trials.

Animals

The experiment was performed on 20 chinchilla male rabbits. The age of rabbits was from 6 to 10 months, the average weight is 3.4±0.2 kg. Microbiological status was conventional animals.

Implants

The design of the implants was original. They were made by laser fusion using AT (RF patent 152558). The manufacture of implants by laser fusion created a complex geometry of the outer surface. The novelty of this implant was the concept of a screw-in immersion part with a combined geometry of a threaded surface consisted of a cutting-calibrating and supporting rectangular thread. A fundamentally new in the design of the implant for osseointegration was the support belt on the end face of the bone (Fig. 1).

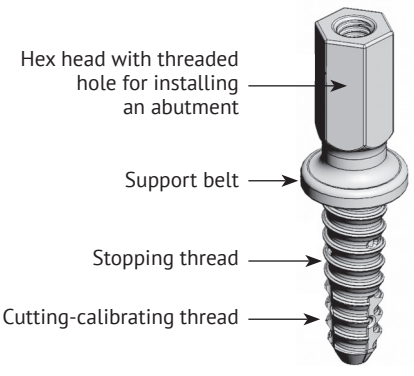

Fig. 1. Implant for a tubular bone stump.

The distal threaded part of the implant forms and calibrates the profile of the stopping thread, which ensures the stability of the implant in the tubular bone.

The roughness parameters of the implants made of two alloys were studied using a WYKO NT 1100 3D optical profiler (Veeco, USA) at three points of the groove of the cutting-calibrating part and three points of the stopping thread of five implants, 3 measurements at each point. The average values of the roughness parameters of the implant surface (Ra, Rz, and Rt) are presented in Table 1.

Experimental model

The surgery of rabbits was performed under general anesthesia. Tibia osteotomy was carried out at the border of the upper and middle third. The fibula was removed at the same level. After that, a channel was prepared with a diameter of 4.0 or 4.5 mm, into which an implant corresponding to a diameter of 4.5 or 5.0 mm was screwed. Then, soft tissues were excised at the level of the hock joint. A hole was made in the formed skin flap to exit the outer part of the implant and a stump was modeled. The soft tissues were sutured in layers. A prosthesis was attached to the implant with crosses of spokes, which were fixed on the Ilizarov apparatus (Fig 2). The Ilizarov apparatus was dismantled in 6 weeks.

Group 1 (n = 8) of the animals were implanted with the EOS PH1 stainless steel product (EOS, Germany). Group 2 (n = 12), the Ti6Al4V titanium alloy was placed. The implants were made by selective laser fusion using an EOSINT M 280 device (EOS, Germany).

Maintenance of the animals

The rabbits were kept in the vivarium of the Ilizarov National Medical Research Center of Traumatology and Orthopedics in cages without shelves, one animal in a cage. All cages were equipped with containers for food and water. Sawdust of coniferous trees was used as a bedding. The cages were cleaned daily. The feed was given to animals

Table 1

The average values of the roughness parameters of the implant surface, μm

Implant surface	Stainless steel, EOS PH1			Titanium Alloy, Ti6Al4V		
	Ra	Rz	Rt	Ra	Rz	Rt
Cutting-calibrating groove	18.48	128.71	137.53	23.50	137.57	182.74
Stopping thread	14.49	90.04	94.43	16.37	183.50	152.04

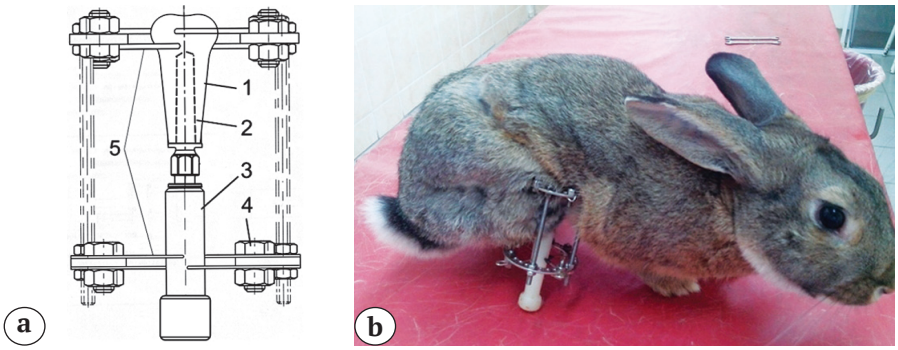

Fig. 2. Osseointegration of the implant in the tubular bone of a rabbit with biomechanical system fixation by Ilizarov apparatus:
a — scheme of the bone, implant and abutment fixation with a prosthesis (1 — tibia, 2 — implant, 3 — abutment with a prosthesis, 4 — Ilizarov apparatus, 5 — crossed pins);
b — the rabbit with Ilizarov apparatus.

once a day, clean drinking water was without restrictions. Before entering the experiment, the animals were quarantined for 21 days.

Euthanasia. In each group, half of the animals were withdrawn from the experiment in 3 weeks after implantation, the other part — in 12 weeks. Euthanasia was performed by administering a multiple exceeded dose of barbiturates.

Regulatory standards. The study was carried out in accordance with: ГОСТ ISO 10993-1-2011. Medical devices. Biological evaluation of medical devices. Part 1. Evaluation and testing. ГОСТ ISO 10993-6-2011. Medical devices. Biological evaluation of medical devices. Part 6. Tests for local effects after implantation.

The implant effectiveness was evaluated by clinical signs, such as limb function and implant survival.

To provide the evidence to the evaluation of the process of osseointegration, the location and concentration of osteotropic elements (calcium and phosphorus) in the tissue substrate adhered to the surface of the implants were determined by the INKA Energy 200 X-ray electron probe microanalyzer (Oxford Instruments Analytical, United Kingdom). Histologically, the tissues in the bone-implantation unit were evaluated by light and scanning electron microscopy. We used an AxioScope A1 stereo microscope (Carl Zeiss Microscopy GmbH, Germany) and an AxioCam ICc 5 digital camera (Carl Zeiss Microscopy GmbH, Germany) together with ZEN blue edition software (Carl Zeiss Microscopy GmbH, Germany), as well as scanning electronic JSM-840 microscope (JEOL, Japan). To further evaluate the effectiveness of the product, we determined the activity of the enzymes — markers of bone metabolism in the serum of experimental animals: alkaline phosphatase and tartrate-resistant acid phosphatase , as well as the concentration of total calcium and inorganic phosphate.

The safety assessment of the implants was carried out by intravital observation data (food intake, signs of chronic inflammation) and laboratory blood tests of the animals. Blood was taken from the marginal vein of the ear. The following indicators were used: total protein, C-reactive protein, glucose, urea, creatinine, and the activity of aminotransferases (ALT and AST). The activity of enzymes, as well as the concentration of substrates in the blood serum, was determined on a Hitachi / BM 902 automated biochemical analyzer (F. Hoffmann-La Roche Ltd./ Roche Diagnostics GmbH) with reagent kits from Vital Diagnostic (Russia).

Ethical principles

Prior to the study, approval was obtained from the local ethics committee. The study was conducted in compliance with the principles of humane treatment of laboratory animals in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes and the 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.

Statistical analysis

In the tables, the data are presented as arithmetic mean with standard deviation. The statistical significance of intergroup differences was determined using the nonparametric Kruskal-Wallis test. Differences were considered statistically significant with a minimum significance level of $p<0.05$. The statistical analysis comprised the data from all animals included in the study.

Results

Implants survival

During the experiment, the clinical condition of the animals in both groups was satisfactory. The limb support function was restored on the 4th to 5th day after implantation. In animals of group 1, there were two cases

of the implant falling out during the experiment (25% of all animals of the group). There were no such cases in group 2.

The histological studies in 21 days of the experiment showed the signs of osteoporosis in the distal and middle areas of the compact plate of the tibial stump in animals of both groups. In animals of group 1, the porosity of the compact plate was higher and larger. Besides, in animals of group 1, the presence of voluminous periosteal strata in the form of medium and large cellular cancellous bone was noted.

Due to the cone-shaped decrease in the proximal part of the implant diameter and the expansion of the bone diameter in this section, in the proximal part of the cuts of the bone-implantating blocks of the animals in both groups, an increase of the proximal direction gap between the implanted product and the compact bone layer was observed (Fig. 3).

A study performed by X-ray electron probe microanalysis showed that in both groups, amorphous hydroxyapatite was found on the surface of the implant. This was justified by the presence of calcium and phosphorus in the tissue substrate formed on the surface of the implant with a predominance of phosphorus. The surface roughness of both types of implant samples contributed to the adhesion of cells and microvessels.

12 weeks after implantation of the product into the rabbit's tibial bone marrow canal, mild signs of osteoporosis of the compact lamina in the distal part of the stump were present similar to the 21 days period. The wider Haversian channels filled with loose fibrous connective tissue were noted in the compact plate of group 1.

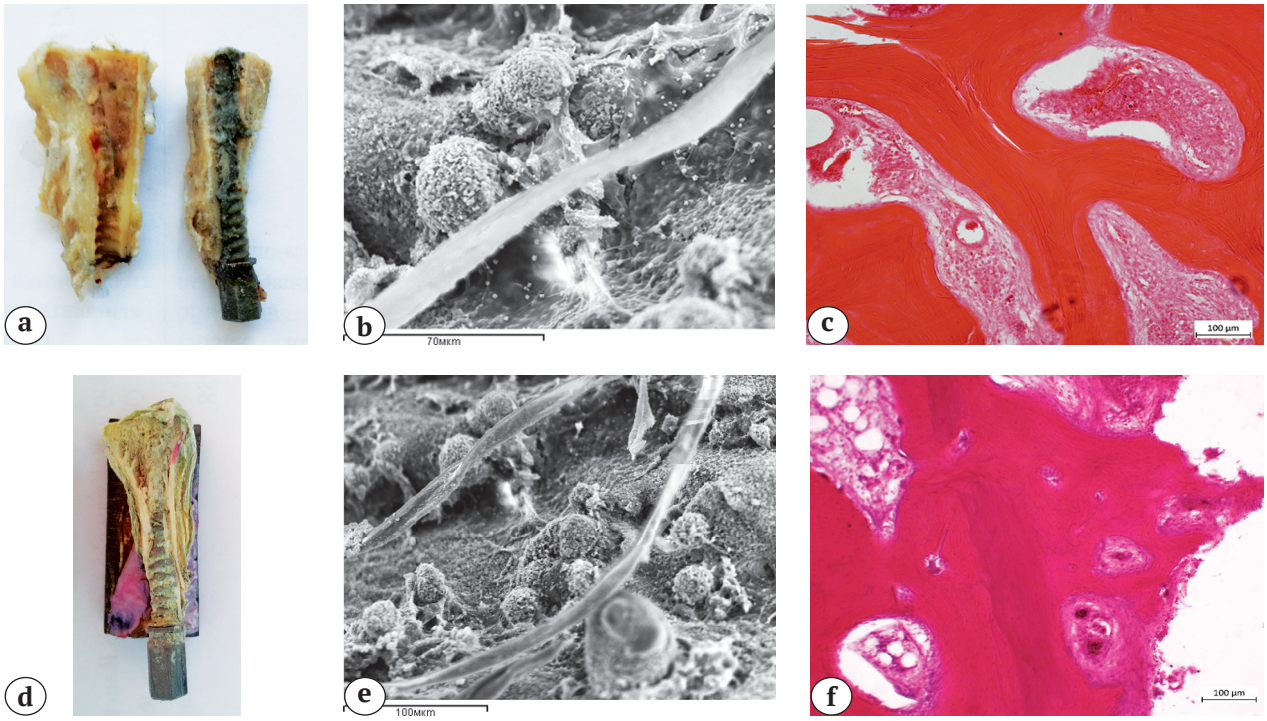

Fig. 3. Adhesion of the newly formed reticular fibrotic bone tissue on the surface of an implanted intraosseous metal structure, histological and structural changes in the tibia stump of a rabbit after 3 weeks implantation: a, b, c — group 1; d, e, f — group 2; a, d — cuts of rabbit tibia with an implant; b, e — the capillary type vessels and cells of the osteogenic line cells on the surface of the implant. Scanning electron microscopy, mag. ×700; c, f — osteogenesis in the area of contact with the implant. Staining: c — Van Gieson, f — hematoxylin and eosin, mag. ×100.

In other studied areas, a layer of trabecular bone between the compact layer of the tibia stump and the implanted product was formed in the animals of both groups. This layer consolidated both the stump and the implant into a single bone-implantation block through the tight connection of their surfaces (Fig. 4).

The intertrabecular spaces were filled with red-yellow and yellow marrow. In the proximal and middle parts of the tibia stump, the compact plate in both groups had a structure close to typical. No signs of inflammation around the implants were detected.

The presence of a tissue component was noted on all implant relief formations (thread ridges and grooves). In these structures under the scanning electron microscopy in combination with microanalysis, calcium and phosphorus were found with calcium predomination in group 2 (Table 2).

In all periods of the experiment,the animals of group 2 demonstrated a higher content of calcium and phosphorus in all zones of the compact plate compared with animals of group 1. In group 2, the calcium content in the newly formed bone tissue on the implant surface at stages of follow-up was statistically significantly higher than that in group 1.

The Ca/P ratio in the newly formed bone tissue on the implant surface in group 1 in 3 weeks after implantation was 1.7 times lower than in group 2 and in 12 weeks — 1.4 times lower ($p<0.05$). This indicated the greater maturity of bone tissue in animals of group 2 at all stages of the experiment.

The phosphatases activity and the level of calcium and phosphate in the blood serum of experimental animals did not have statistically significant intergroup differences in the follow-up dynamics (Table 3).

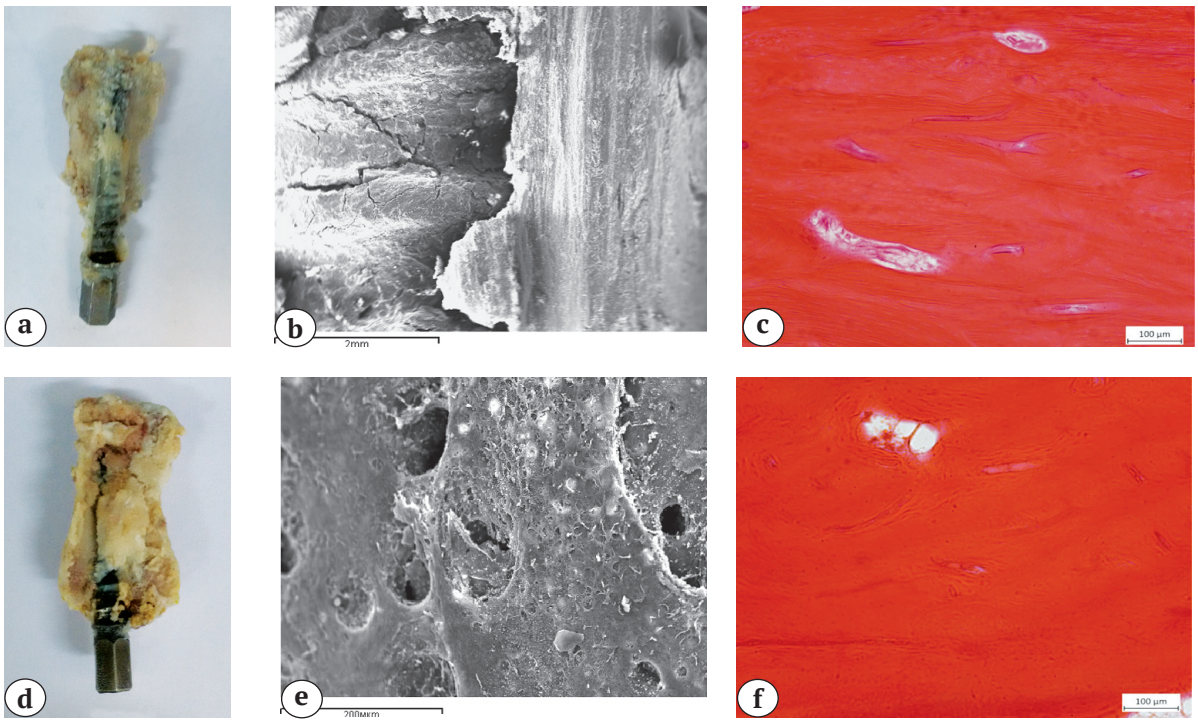

Fig. 4. The bone-implant formation block after 12 weeks implantation:
a, b, c — group 1; d, e, f — group 2; a, d — cuts of the rabbit tibia with an implant;
b, e — bone tissue on the implant surface. Scanning electron microscopy, mag. ×22 (b), ×100 (e);
c, f — the compact plate with extended Haversian channels. Staining: Van Gieson, mag. ×200.

Table 2

The content of osteotropic elements (wt %) in different parts of the compact plate of the tibia stump and in the bone tissue newly formed on the surface and around the implant

Area of interest	Chemical element	Compared groups	3 weeks after placement	12 weeks after placement
Distal part of the compact plate	Calcium	1	10.80±0.48	10.30±0.46
		2	11.50±0.53	14.70±0.51*
	Phosphorus	1	5.14±0.19	4.90±0.21
		2	5.48±0.26	7.05±0.29*
Middle part of the compact plate	Calcium	1	16.50±0.57	16.10±0.71
		2	17.40±0.83*	17.20±0.81
	Phosphorus	1	7.85±0.27	7.66±0.35
		2	8.29±0.36	8.19±0.32
Proximal part of the compact plate	Calcium	1	16.90±0.72	17.10±0.69
		2	18.10±0.73*	17.90±0.82
	Phosphorus	1	8.05±0.29	8.14±0.33
		2	8.61±0.37	8.52±0.41
Newly formed bone tissue on the surface and around the implant	Calcium	1	3.00±0.11	7.88±0.37
		2	5.00±0.15*	10.14±0.42*
	Phosphorus	1	5.11±0.23	8.85±0.43
		2	5.12±0.19	8.23±0.31
The average Ca/P ratio in newly formed bone tissue on the implant surface		1	0.58±0.02	0.89±0.02
		2	0.98±0.03*	1.23±0.04*

* — statistically significant differences in comparison with group 1 at $p<0.05$.

Table 3

Changes in the phosphatases activity, the level of total calcium and inorganic phosphate in the blood serum of experimental rabbits

Stage of the study	Compared groups	Alkaline phosphatase, u/L	Tartrate-resistant acid phosphatase, u/L	Total calcium, mmol/L	Inorganic phosphate, mmol/L
Before surgery	1	53±19	26.8±6.4	3.70±0.19	1.30±0.24
	2	66±11	29.4±7.5	3.70±0.13	1.32±0.11
In 3 weeks	1	33±4*	39.4±5.1*	3.34±0.25*	1.56±0.15*
	2	37±6*	40.4±10.1*	3.51±0.11*	1.40±0.17
In 12 weeks	1	61±10	18.4±6.7	3.65±0.08	1.33±0.05
	2	45±12	22.9±3.1	3.60±0.15	1.34±0.11

* — statistically significant differences in comparison with the preoperative level at $p<0.05$.

Safety

Clinical observation showed that during the first 3 days after implantation there was a temperature increase of 0.3 to 0.5° and edema in the stump area persisted for 3 to 4 days.

There was a decrease in appetite for few days. In 4 rabbits (50.0%) of group 1 and 2 rabbits (16.7%) in group 2, acute suppurative soft tissue inflammation around the implant was observed during 14 days after implantation. Purulent inflammation was eliminated in 7 to 10 days by antibiotics (cefazolin at 0.05 g/kg body weight).

The histological examination revealed that in two cases with chronic inflammation (rabbits of group 1) the compact plate underwent the total osteoporotic changes in 12 weeks after implantation. The implant constructions in such animals were poorly retained inside the bone. Only mosaic areas with signs of tissue component adhesion were found on their surface. In the space between the implant and the compact plate, adipose bone marrow with elements of hematopoiesis and foci of inflammatory infiltration and fibrosis was found.

The laboratory data. Statistically significant intergroup differences were found in some biochemical parameters in animals of the compared groups at 12 weeks after implantation (Table 4). In particular, the concentration of urea, creatinine, CRP and transaminase activity in animals of group 1 was significantly higher than in animals of group 2. Moreover, the concentration of CRP and creatinine in rabbits of group 1 was significantly higher not only relative to the level of group 2 animals, but also relative to the preoperative level.

Discussion

The study demonstrated the ability of the structured implant surface, made by 3D technologies, to adhere the cellular elements and vessels. This led to the formation of low-mineralized reticulofibrous bone tissue on the implant surface after 3 weeks of the experiment. By the 12th week of the study, the results of light and scanning electron microscopy, as well as the results of X-ray electron probe microanalysis, indicated the formation on the surface of the implants of a newly formed bone that was mineralized and sufficiently mature. According to some authors, the formation of bone structures directly on the surface of implants without a connective tissue capsule is possible only under conditions of a structured surface and in the absence of micromobility, i.e. with the firm fixation of the implant in the bed [27, 28]. In the present study, in both groups, this condition was provided by an external fixation apparatus. However, a statistically significant higher mineralization of bone tissue at the stages of the experiment was revealed in the group

Table 4

Changes in blood serum biochemical parameters in rabbits of the compared groups

Stage of the study	Compared groups	Total protein, g/L	Urea, mmol/L	C-reactive protein, mg/L	Creatinine, mmol/L	Glucose, mmol/L	ALT, u/L	AST, u/L
Before surgery	1	67±4	5.8±0.6	0.0	100±14	7.1±1.2	45±13	29±10
	2	70±4	5.5±0.6	0.6±0.5	107±12	7.5±0.5	40±17	26±7
In 3 weeks	1	66±4	4.8±1.0	34.5±7.0*	95±12	7.8±0.8	52±25	34±15
	2	65±6	4.3±0.9	21.7±15.1*	102±6	7.0±0.8	47±19	27±11
In 12 weeks	1	69±2	6.8±0.9	17.7±2.6*	127±10*	7.6±0.3	87±38	55±21
	2	70±5	4.1±0.5*#	7.0±5.2#	92±11#	6.8±0.6	50±10#	21±8#

* — statistically significant differences in comparison with preoperative level at $p<0.05$;
# — statistically significant intergroup differences at $p<0.05$.

with the titanium implant. In our opinion, this occurred due to more roughness of titanium implants, and, consequently, its better adhesive abilities [29, 30, 31, 32, 33, 34]. More pronounced porous changes and outflow of osteotropic elements of the compact plate of the tibial stump were noted in the group with the steel implant. In all stages of the study, the animals of group 2 showed a higher content of calcium and phosphorus in the distal part of the compact plate compared with animals of group 1. The biochemical data also showed a more significant decrease in calcium content and an increase in serum phosphate levels in animals of group 1. Moreover, in 12 weeks after implantation, there was an increase of urea, creatinine and CRP level, transaminase activity in these animals. We associate the development of the mentioned above processes with the lower corrosion resistance of the steel implant to the tissues of the internal environment. This could lead to a greater local and systemic reaction of the body to a foreign construction [35, 36, 37] and could reduce the osseointegration ability compared with the group with titanium implant which revealed the greater corrosion resistance [25]. This also explains the occurrence of an acute inflammatory process in the soft tissues around the steel implant of 33.3% more often than with the use of the titanium implant. In two cases the steel implant even fell out of the implantation bed.

Thus, in choosing a material for printing personalized implants by AT, it is necessary to take into account not only the strength characteristics that contribute to high wear resistance, but also the degree of implant compatibility with biological tissues.

Our study showed a different local and general biological response to products made of biologically tolerant (steel) and biologically inert (titanium) metal alloys. which is confirmed by the results of other studies [7, 38]. This inference is confirmed by the results of other studies [7, 38].

Conclusion

The results of the study allow us to conclude that the effectiveness (implant survival) and safety of the product made of titanium alloy were higher compared with the stainless steel implant. A more pronounced ability for osseointegration with a titanium alloy product is determined by a higher chemical purity, biological inertness and greater roughness. All of these contribute to better adhesion of cells and blood vessels, increased mineralization of bone tissue formed on the surface of the titanium implant, and the absence of chronic inflammation.

Conflict of interest: The authors declare no conflict of interest.

Funding: The study was carried out within the framework of the theme: “The development of methods for managing osseointegration and medical technologies for restoring of amputated limbs functions”. This was the state assignment for the implementation of scientific research and development.

Authors’ contributions

A.A. Emanov — research concept and design, collection of material, data analysis, text preparation and editing.

V.P. Kuznetsov — research concept and design, data analysis, the final version of the article approval.

E.N. Gorbach — collection of material, data analysis and statistical processing, text preparation and editing.

M.V. Stogov — data analysis and statistical processing, text preparation and editing, the final version of the article approval.

E.A. Kireeva — collection of material, data analysis and statistical processing.

E.N. Ovchinnikov — collection of material, data analysis.

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