Genij Ortopedii. 2023;29(5):526-534.

Original article

https://doi.org/10.18019/1028-4427-2023-29-5-526-534

Comparative evaluation of osseointegration of new percutaneous implants made of Ti Grade 4 ultrafine-grained alloy

M.V. Stogov[1]✉, A.A. Emanov[1], V.P. Kuznetsov[1,2], E.N. Gorbach[1], E.A. Kireeva[1], A.V. Korelin[2]

[1] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation
[2] Ural Federal University named after the first President of Russia B.N. Yeltsin, Ekaterinburg, Russia

Corresponding author: Maksim V. Stogov, stogo_off@list.ru

Abstract

Introduction It has been shown that titanium implants with a structured surface provide an increased rate of osseointegration what makes their application quite promising. **The purpose** of this work was to conduct a comparative evaluation of the efficiency of osseointegration of new percutaneous implants for prosthetics made of ultrafine-grained Ti Grade 4 alloy. **Materials and methods** The study was carried out on 12 male rabbits of the Soviet Chinchilla breed. Six rabbits of the control group had implants made of Ti6Al4V powder using selective laser sintering technology that were osseointegrated into the tibia, 6 rabbits of the experimental group had implants made of Ti Grade 4 by equal channel angular pressing. The formation of the "bone-implant" block was examined 26 weeks after the implantation. **Results** Histologically, after 26 weeks of the experiment, porous changes, enlargement of the Haversian canals, and pronounced osteoclastic resorption were not detected in the animals of the experimental group throughout the stump in the compact plate. Around the implant, a bony case repeating the bone shape was formed, represented by lamellar bone tissue. Using X-ray electron probe microanalysis, it was found that in the substrate formed on the surface of the implant in rabbits of the experimental group, there was significantly more calcium in all areas over the implant relative to the animals of the control group. In the control group, relative to the experimental group, an increased level of C-reactive protein in blood serum was retained longer. Complications and significant clinical and laboratory abnormalities were not found in both groups during the entire experiment. **Discussion** Our data are consistent with the results of other experimental studies, which unambiguously noted that titanium implants with a structured surface show increased osseointegration characteristics in comparative studies relative to implants without modification of the structure of the material of the threaded surface. The absence of complications and undesirable reactions of the animal organism also indicates the acceptable safety of the tested products. **Conclusion** Osseointegration of a percutaneous implant that has a mixed nanocrystalline and ultrafine-grained structure was more effective than the reference implant. This makes the use of such implant promising for solving clinical problems in prosthetics.

Keywords: prosthetics, osseointegration, titanium implant, nanocrystalline structure, selective laser fusion, experiment, rabbits, product safety

Acknowledgment The authors express their gratitude to Ruslan Zufarovich Valiev, Doctor of Physical and Mathematical Sciences, Professor, Corresponding Member of the Academy of Sciences of the Republic of Bashkortostan, for providing the Ti Grade 4 bar for research, obtained by equal-channel angular pressing.

For citation: Stogov M.V., Emanov A.A., Kuznetsov V.P., Gorbach E.N., Kireeva E.A., Korelin A.V. Comparative evaluation of osseointegration of new percutaneous implants made of Ti Grade 4 ultrafine-grained alloy. *Genij Ortopedii*. 2023;29(5):526-534. doi: 10.18019/1028-4427-2023-29-5-526-534

INTRODUCTION

The use of titanium alloys in the nanostructured and ultrafine-grained (UFG) states along with their surface modification has been currently a universal strategy for improving the mechanical properties and biocompatibility of medical devices [1, 2]. For orthopaedic practice, studies of the osseointegration of percutaneous implants made of titanium alloys with different chemical composition, structure, and coating are relevant issues [3-6]. It has been shown that osseointegrated titanium implants made of materials with a structured surface provide an increased rate of osseointegration [7, 8]. To a greater extent, this issue has been studied for products used in dentistry and maxillofacial surgery [9-11]. Nevertheless, the studies are a promising direction to improve the survival characteristics of percutaneous implants that have been currently introduced to solve the problems of osteointegral prosthetics [12-14].

Purpose To conduct a comparative assessment of the osteointegration efficiency of new percutaneous implants for prosthetic application that are made from ultrafine-grained Ti Grade 4.

MATERIALS AND METHODS

The study was carried out on 12 male rabbits of the Soviet Chinchilla breed, age 6-10 months, average weight 3.2 ± 0.3 kg. Premedication for the operation was carried out by intramuscular administration of diphenhydramine 1 % 10 mg/kg, atropine 0.01 % 0.05 mg/kg, meditin 0.1 % 1 mg/kg,

zoletil 100 0.025 mg/kg; anesthesia was performed intravenously with propofol 1 % 10 mg/kg. All rabbits underwent osteotomy of the tibia at the border of the upper and middle thirds. Next, the medullary canal was reamed to 4.5 mm and an implant with a diameter of 5 mm (RF patent No. 152558) was screwed into the tibial stump. Animals of the experimental group (n = 6) received titanium implants made of the ultrafine-grained Ti Grade 4 alloy (Fig. 1 a), animals of the control group (n = 6) received titanium implants made of the standard Ti6Al4V alloy (Fig. 1 b). Installation of implants is shown in Figure 1 c. The shin stump was formed by the myoplastic method using one dermofascial flap.

Next, a compression device (RF patent 2631631) with a PTFE prosthesis was attached to the bone (Fig. 2). The bone was subjected to compression load of 3.5 N for 5 weeks after implantation. The choice of the compression value was substantiated by us in a previous study [15].

Implants of the control group were made from Ti6Al4V powder (Advanced Powders & Coatings Inc., Canada) with an average particle size of 23.5 μm and were produced by selective laser sintering (SLS) on an EOS EOSINT M 280 3D printer (Germany) at the Ural Federal University [16]. Implants for the experimental group were made from a bar of ultrafine-grained Ti Grade 4 with a diameter of 10 mm by equal-channel angular pressing at the Nanotech LLC enterprise (Ufa), and were produced on a MANURHIN K'MX 432 longitudinal lathe machine. Mechanical properties of the materials and the arithmetic mean deviation of the threaded surface microprofile of the implants are presented in Table 1. These data on the properties of the material of SLS-implants are consistent with the results of studies presented in the work of Kaplan et al. [17].

Additionally, to assess the structural changes that occur during the turning on lathe of an experimental implant, we studied the microstructure of the implant material after machining using a Zeiss CrossBeam AURIGA scanning electron microscope in the electron backscattered diffraction (EBSD) analysis mode. Figure 3 shows the microsections of the threaded part of the implant, the results of the EBSD analysis of scanning electron microscopy, and the histogram of the grain size distribution of the microstructure, built using the SIAMS 700 software package.

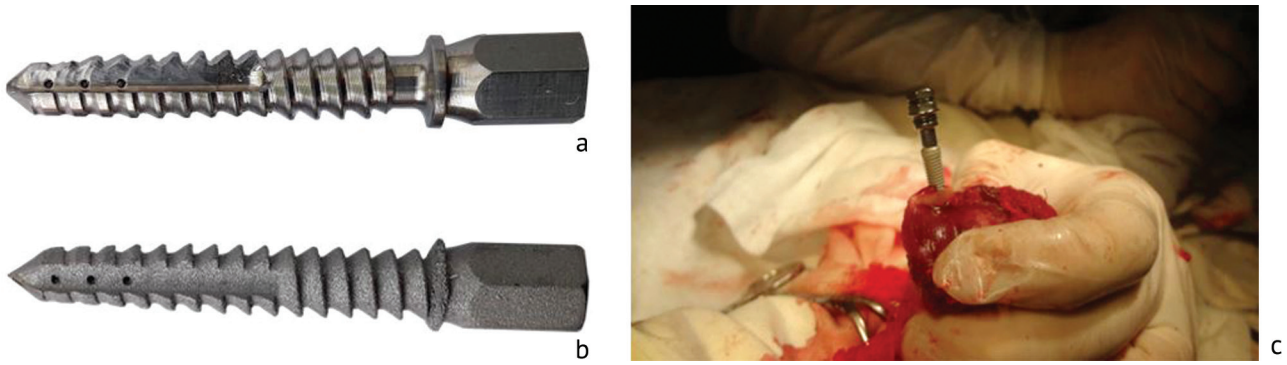

Fig. 1 Implants made of ultrafine-grained Ti Grade 4 alloy after mechanical processing (a) and Ti6Al4V alloy after selective laser sintering (b); installation of the implant in the rabbit tibia stump (c)

Fig. 2 X-ray of the rabbit tibia stump after surgery (a) and photo of the rabbit with the implant fixed in the external fixator featuring compression ability (b)

Table 1

Mechanical properties and mean arithmetic deviation of the threaded surface microprofile of the implants

Implant type	$\sigma_{0,2}$, MPa	σв, МПа	δ, %	Ra, mkm
SLS Ti6Al4V (control)	1045	1200	8	5.59
Mechanical treatment of UFG Ti Grade 4 alloy (experiment)	1071	1240	11	0.95

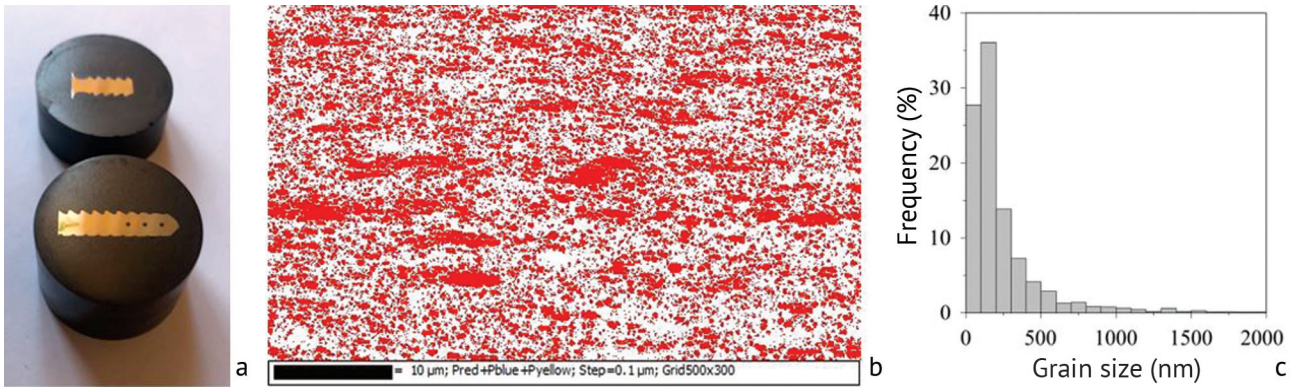

Fig. 3 Photo of microsections of the threaded part of the implant (a); phase-contrast EBSD image of the α-phase of the surface layer of the thread (b); grain size distribution histograms of the implant material after turning (c)

The result of the EBSD analysis showed that the microstructure under the study was a mixed ultrafine-grained and nanocrystalline with separate larger grains up to 2 µm in size. Grains sized from 100 to 300 nm prevailed; their number was about 28 %. There was also a large number of nanocrystallites of the sizes less than 100 nm, the content of which reached 32 %. The presence of such nanocrystallites in the material of the threaded surface of the implant suggests a significant increase in the efficiency of osseointegration.

Animal care In the course of the experiment, the animals were kept in cages, one animal in each, with containers for food and water. Wet cleaning of cells was carried out daily. Food was distributed to the animals once a day, clean drinking water was given without restrictions. Before entering the experiment, the animals were quarantined for 21 days. During the experiment, the animals were observed daily for general condition, respiration, function of the involved limb, as well as the condition of postoperative wounds. The duration of observation of all animals was 26 weeks after implantation. Euthanasia of animals was carried out by introducing lethal doses of barbiturates.

Radiographic study The radiographic complex Compact (Milan, Italy) was used for radiography. The current was 60 mA, the voltage was 57-69 kV, the exposure time was 0.4-0.6 sec. The specific parameters of the operation of the complex depended on the constitution of the animal. Radiography of the rabbit limb was performed in frontal and lateral views. Radiography was performed before and after surgery, on days 21, 42, 84, 105,180 after surgery.

Morphological study The tibia with the implant integrated into it was placed in a 10 % solution of neutral formalin. After 7 days of fixation, the bone was sawn in the longitudinal direction, leaving the implant in one of the halves of the cut, exposing only the surface of the implant; and fixation in formalin continued for another 3-5 days. Next, the half of the tibial stump with the implant in was dehydrated in ethyl alcohol (2 shifts of 70, 80, 96 and 100 degrees of strength), poured into 2.2-dimethyl-3-methylenebicyclo[2,2,1] heptane and dried in an open container until it had completely evaporated. The dried cuts were sprayed with a conductive layer of Pt and Pd and were examined by scanning microscopy using a Zeiss EVO MA18 electron microscope (Carl Zeiss Group, Germany). The distribution of Ca and P in tissues adhered to the surface of integrated implants was performed using a BRUKER QUANTAX 200 – XFlash 6/10 energy dispersive spectrometer (Bruker Nano GmbH, Germany). The work was carried out in the mode of constructing element maps, spectra and obtaining digital data on the content of each osteotropic element.

Fragments of the tibial stump without an implant were decalcified in a mixture of hydrochloric and formic acids, dehydrated, and embedded in paraffin-containing mixtures capable of hardening. Paraffin sections, 6 µm thick, were produced on a Reichard sledge-type microtome (Germany), and after deparaffinization, were stained with hematoxylin and eosin. Next, they were examined using an AxioScope A1 stereomicroscope (Carl Zeiss MicroImaging GmbH, Germany).

Laboratory tests In the dynamics of the experiment, blood samples were harvested before surgery, 3,

5, 12 and 26 weeks after implantation. A complex of biochemical and hematological studies was performed, including total protein, C-reactive protein (CRP), creatinine, urea, total calcium, inorganic phosphate, assessment of the activity of phosphatases (alkaline phosphatase – ALP; bone isoenzyme of acid phosphatase – TrAP) and transaminases (ALT, AST), determination of the content of leukocytes, erythrocytes and platelets.

Hematological studies were performed on a ProCyte Dx automatic analyzer (IDEXX Lab., Netherlands), biochemical studies were performed on a Hitachi/BM 902 automatic analyzer (F. Hoffmann-La Roche Ltd., Italy) using Vector-Best reagent kits (Russia).

Regulation standards The study was carried out in accordance with GOST ISO 10993-1-2021. Medical products. Evaluation of the biological effect of medical devices. Part 1. Evaluation and research in the risk management process; GOST ISO 10993-6-2021. Medical products. Evaluation of the biological effect of medical devices. Part 6. Studies of local response after implantation.

Ethical principles Prior to the start of the study, the approval of the local ethics committee was obtained, protocol No. 1(71) dated 28.04.2022. 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 Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes.

Statistical methods The results in Tables 2 and Table 3 are presented as median, 1-3 quartiles (Me; Q1-Q3). The normality of the samples was determined using the Shapiro-Wilk test. The procedure for statistical assessment of the significance of differences between the parameters at the time of the experiment with preoperative values was performed using the Wilcoxon W-test. The significance of differences between groups was assessed using the Mann-Whitney T-test. The minimum significance level (p) was taken equal to 0.05.

RESULTS

In the post-implantation period, the general condition of the rabbits of both groups was satisfactory. The weight-bearing function of the limb was restored on the 4th to 5th day after the operation and was subsequently maintained in the animals of the experimental and control groups throughout the observation period. In all animals, inflammation and purulent processes of the skin at the site of the implant exit were not detected. Implant prolapse by the time of euthanasia (26 weeks) in animals of both groups was not observed. No serious adverse events were found during the follow-up; suppuration in the stump area and in the peri-implant space was not detected. The pathomorphological study of animals after euthanasia did not find any pathological changes in the internal organs.

In all animals of the experimental group, at 26 weeks after implantation, a complete organotypic restructuring of the bone was radiologically observed (Fig. 4 a). In the rabbits of the control group, there was no implant instability in all the cases; however, in two cases, slight resorption was detected at the "implant-bone" interface (Fig. 4 b).

Histologically, after 26 weeks of the experiment, no porous changes, enlargement of the Haversian canals, or pronounced osteoclastic resorption were detected in the animals of the experimental group throughout the entire length of the tibial stump in the compact plate (Fig. 5). In the proximal part of the stump, the metaphyseal bone was practically unchanged and was presented by large- and medium-cell spongy bone with adipose marrow in the intertrabecular spaces (Fig. 5 b, e). Around the implant, a bone envelope repeating its shape was formed by lamellar bone tissue (Fig. 5c). The bone tissue closely adjoined the implant structures, without gaps and without a connective tissue layer (Fig. 5 a). In the proximal part of the bone-implant block, a few resorption cavities were found in the newly formed bone on the surface of the implant (Fig. 5 d). The bone tissue growing into the inter-thread spaces of the implant was highly mineralized and vascularized (Fig. 5 f).

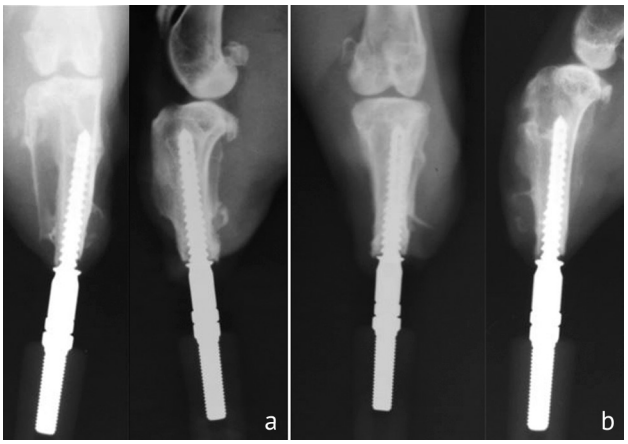

Fig. 4 X-rays of the tibiae 26 weeks after implantation: *a* experimental group; *b* control group

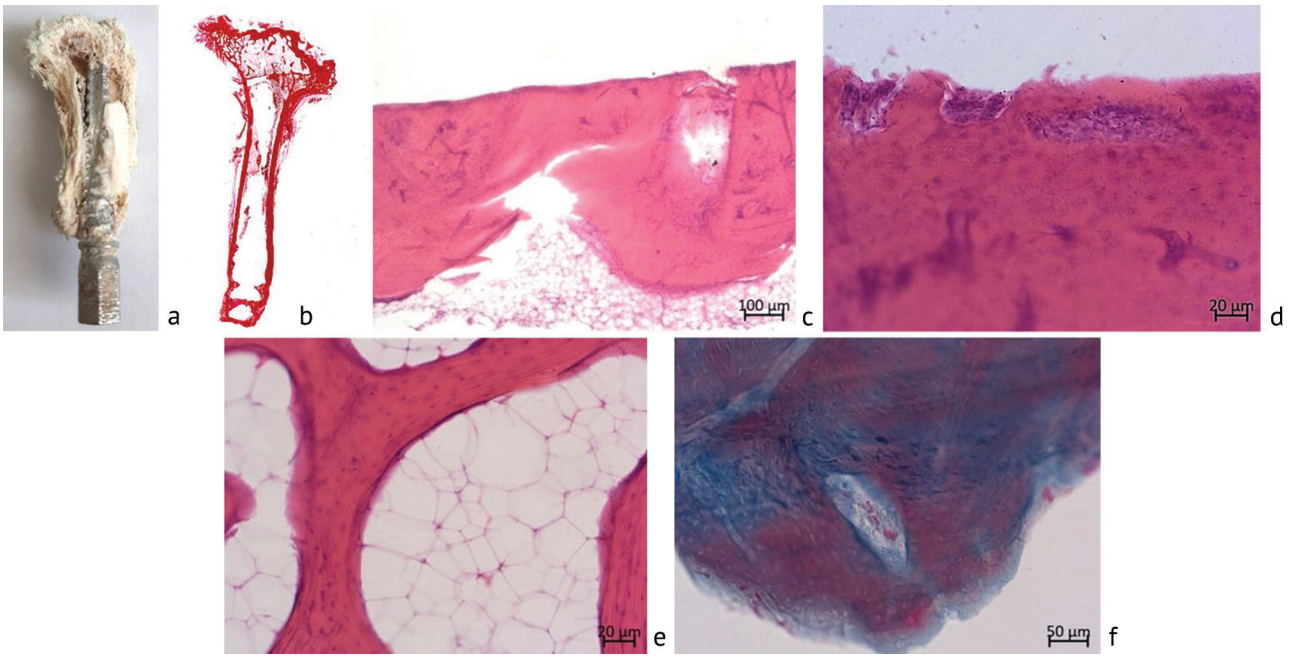

Fig. 5 Proximal area of the tibial stump of the animals of the experimental group: *a* fragment of the tibia of a rabbit with an implant installed; *b* histotopogram of the saw cut of the rabbit tibia after the removal of the implant. Mag. 1.5×; *c* formation of a bony envelope on the surface of the integrated implant. Mag. 50×; *d* resorption cavities on the surface of the bony sheath. Mag. 200×; *e* metaphyseal bone. Mag. 200×; *f* bone tissue in the inter-thread spaces. Mag. 200×. Staining: *b* Van Gieson; *c-e* hematoxylin and eosin; *f* according to Masson

Histologically, in the control group after 26 weeks of the experiment, close contact was observed between the surface of the SLS-implant and the bone tissue, which ensured a strong retention of the implant in the bone bed. By that period, a single bone-implant block had been formed. A continuous compact plate was preserved throughout the bone stump. Pronounced periosteal stratification was not found. In the distal and middle parts of the tibial stump, bone tissue ingrowth into the threaded spaces of the implant was noted (Fig. 6 a, b).

Studies with scanning electron microscopy showed complete integration of bone tissue into the structural surfaces of the implant (inter-thread spaces, holes in the implant structures) throughout the bone-implant contact in the animals of the experimental group (Fig. 7).

In the control group, tight contact of bone tissue and its integration into the surface structures of the implant was also confirmed by the data of energy dispersive analysis in the electronic maps of the distribution of osteotropic elements in the cut structures of the bone-implant block (Fig. 8).

Fig. 6 Formation of the "bone-implant" block in the animals of the control group after 26 weeks of the experiment: *a* cut of the tibia of a rabbit with an implant installed; *b* histotopogram of the saw cut of the rabbit tibia after the removal of the implant. Van Gieson staining; mag. 1.5×

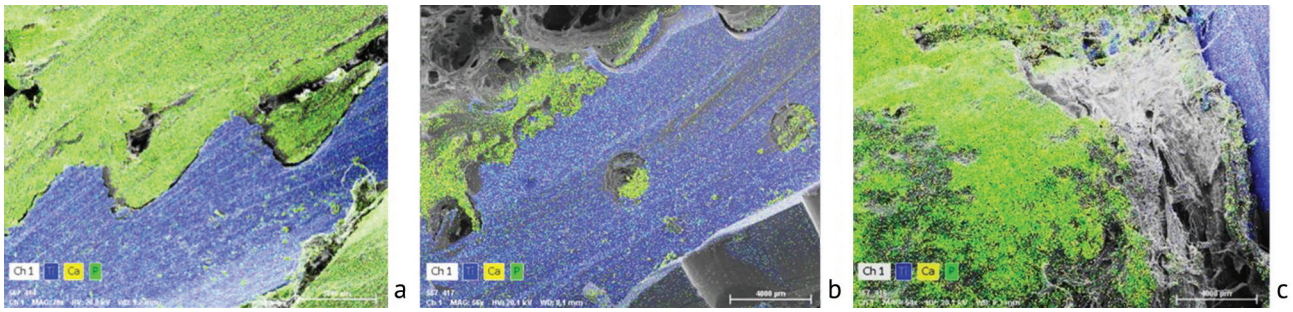

Fig. 7 Distribution maps of osteotropic elements in different parts of the bone-implant block after 26 weeks of the experiment, experimental group: *a* proximal area, *b* middle area, *c* distal area. X-ray electron probe microanalysis. Mag. 68×

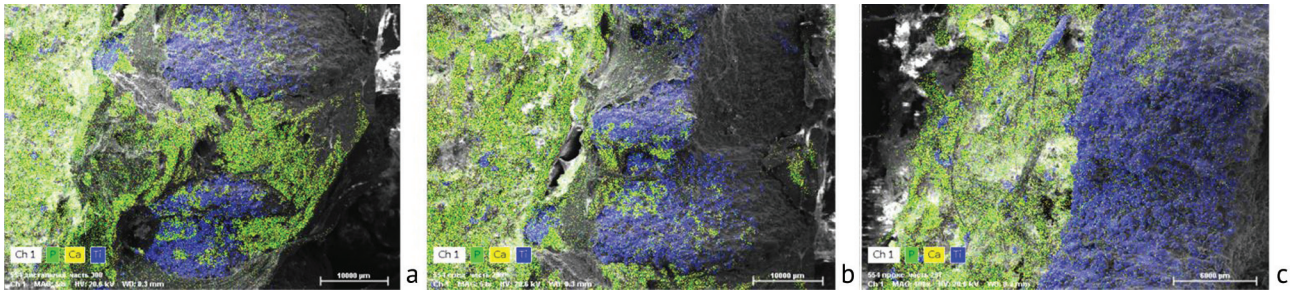

Fig. 8 Distribution maps of osteotropic elements in different parts of the bone-implant block after 26 weeks of the experiment, control group: *a* proximal area, *b* middle area, *c* distal area. X-ray electron probe microanalysis. Mag. 68×

X-ray electron probe microanalysis found that in the substrate formed on the surface of the implant in the rabbits of the experimental group, there was significantly more calcium in all areas of the implant relative to the animals of the control group (Table 2). The content of phosphorus on the surface of the implant in the animals of the experimental group was also significantly higher compared to the control in the proximal and middle regions of the implant.

Differences in the activity of phosphatases in the blood serum of the animals were noted in the dynamics of the experiment (Table 3). Thus, in the experimental group, there was an increase in the activity of alkaline phosphatase 12 weeks after implantation relative to preoperative values. In the control group, the activity of ALP, on the contrary, decreased at the 3rd week, and at the 3rd and 5th weeks the activity of the bone isoenzyme of acid phosphatase increased. Also, an increased level of CRP in blood serum was retained longer in the animals of the control group relative to the experimental group.

Table 2

Ca and P content (weight %) on the implant surface in the different areas of the tibial stump of the rabbits at 26 weeks of the experiment, Me (Q1-Q3)

Area	Calcium, weight %		Phosphorus, weight %	
	Experimental group	Control group	Experimental group	Control group
Proximal	13.6 (13.0-14.1)*	7.3 (6.9-7.6)	4.1 (3.9-4.3)*	3.6 (3.5-3.6)
Middle	15.8 (15.2-16.3)*	9.1 (8.9-9.4)	5.5 (5.1-5.8)*	4.6 (4.4-4.7)
Distal	12.2 (11.7-12.6)*	10.3 (10.0-10.5)	3.9 (3.7-4.2)	4.5 (4.2-4.8)

Notes: * – significant differences relative to the control group at $p < 0.05$

Table 3

Changes in some biochemical parameters of rabbits blood serum in experimental groups on observation time-points (weeks), Me (Q1-Q3)

Parameter	Group	0	3	5	12	26
ALP, u/l	E	51 (50-59)	45 (38-49)	59 (54-61)	67#(62-73)	60 (52-62)
	C	53 (48-63)	42#(38-46)	51 (49-56)	58 (45-66)	61 (56-66)
TrAP, u/l	E	28 (24-30)	30 (28-36)	33 (28-40)	25 (21-29)	19 (18-29)
	C	26 (22-26)	41#(35-42)	34#(29-38)	20 (16-28)	20 (16-29)
CRP, mg/ml	E	0 (0-1)	14#(4-30)	12 (2-20)	5 (0-7)	2 (0-2)
	C	0 (0-1)	13#(6-22)	10#(4-17)	2 (0-3)	4#(2-21)

Note: # – significant differences with preoperative values at $p < 0.05$. E – experimental group; C – control group

Other laboratory parameters (total protein, creatinine, urea, transaminases, total calcium, inorganic phosphate, leukocytes, erythrocytes, platelets) did not change significantly relative to preoperative values in both groups in the course of observation (data not presented). No significant deviations of laboratory parameters were noted in the analysis of individual dynamics for each animal.

DISCUSSION

Our data suggest that the process of osseointegration of a percutaneous implant that has a mixed ultrafine-grained and nanocrystalline structure was better relative to the reference product. This is confirmed by the data of quantitative indicators of X-ray electron probe microanalysis which showed higher calcium content in the newly formed bone tissue on the implant surface throughout the bone-implant block in the animals of the experimental group. The dynamics of alkaline phosphatase activity in rabbits of the experimental group also indicates the activation of osteogenesis in the implantation zone. Changes in laboratory parameters also suggest that the biocompatibility of an implant made of a material with an ultrafine-grained and nanocrystalline structure was higher relative to the SLS implant. First, in the animals of the control group, an osteolytic reaction was noted, as evidenced by an increase in the activity of TrCP and its radiological signs recorded in two animals. Second, the acute phase reaction to implantation in the animals of the control group, based on the dynamics of CRP, was longer than in animals of the experimental group. It should be noted that the absence of pronounced complications and significant clinical and laboratory abnormalities in animals of both groups during the entire experiment also indicates the acceptable safety of the tested products.

Our data are consistent with numerous results of other experimental studies, in which it is unequivocally noted that titanium implants with a structured surface show increased osseointegration characteristics in comparative studies relative to the implants without modification of the structure of the material of the threaded surface [18-24]. However, the experimental models used in those works differ significantly from our model. Thus, in a number of works [18, 21, 22], a dental implant was installed in the bone of the segments of the hind limbs of experimental animals. In other works, dental implants with a structured surface were implanted at the site of application, in the jaw [19, 23, 24]. Nan-Jue Cao et al. [19], using SMAT technology, were able to impart a gradient nanostructured surface (GNS Ti) to a titanium implant. It was established that, compared with coarse-grained titanium CG, the surface of GNS Ti stimulates cell adhesion, proliferation and differentiation and improves osteogenesis and osseointegration.

One of the works [20] reports the advantages of osseointegration of a titanium implant, the surface of which was modified by coating with osteogenic nanofibers, which composition included polycaprolactone, gelatin, hydroxyapatite, dexamethasone, beta-glycerophosphate, and ascorbic acid. In an experiment on rabbits, titanium implants with an osteogenic nanofiber coating showed better results than uncoated controls. Moreover, there were no pathological changes in the regenerated tissue around the implant.

It is worth highlighting the work of Jones et al., who showed the effectiveness of osseointegration of implants with a nanostructured surface both in cancellous bone and cortical bone [27].

We encountered a few works that focus on the advantages in terms of integration of percutaneous implants with a modified surface, and the authors of the works point to an improvement in the integration of these implants with soft tissues [25, 26].

In general, experimental research on the study of osseointegration of modified implants is ongoing. It has been shown, for example, that genetic mechanisms can be involved in the efficiency of biointegration of such implants [28]. It is noted that the introduction of surface modifications of biocompatible metals is the best solution for improving the corrosion resistance characteristics of such products [29]. A promising direction is the incorporation of individual metal ions into the modified surface [30, 31].

Despite numerous experimental studies, clinical experience with the use of titanium implants with spatial or surface ultrafine-grained nanostructures is scarce. The reason for this may be that some aspects of the use of these products in experimental studies are still poorly studied. In this regard, it is worth highlighting the work [32], which notes that the available studies do not cover the effect of modification of the structure of titanium implants, topographic and chemical changes in surfaces after osseointegration, complications of their use, and the survival of products in the long term, especially taking into account the conditions of constant load on the bone. Jayasree et al. point to the fact that the positive effects of osseointegration revealed in most experimental studies may be due to the fact that the implant in animals, as a rule, does not bear mechanical loads, therefore, *in vivo* studies on large animal models with mechanical loading are needed to overcome this gap in the long term [33].

Based on the current state of the topic in general, it can be noted that the advantage of our experiment is that titanium implants with a mixed ultrafine-grained

and nanocrystalline structure were studied on a model that is closer to the model of clinical use, with a fairly long observation period. This makes the use of such implants promising for solving clinical problems of prosthetics. Obviously, the limitations of this study relate to the sample sizes of the experimental animals.

CONCLUSION

Thus, the results of the study on a rabbit model have shown that the use of percutaneous implants made of a Ti Grade 4 alloy with a mixed nanocrystalline and ultrafine-grained structure by mechanical processing under compression loading of 3.5 N for 5 weeks improves the characteristics of osseointegration in comparison with SLS implants made of titanium alloy Ti6Al4V.

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

Financing *The study was carried out within the framework of the topic "Controlled one-stage osseointegration of percutaneous implants with mechanobiological stimulation of bone formation under conditions of an external fixation system" of the state assignment for scientific research and development of the Federal State Budgetary Institution Ilizarov NMRC TO of the Ministry of Health of Russia, as well as with the financial support of the Ministry of Science and Higher Education of the Russian Federation as part of the Development Program of the Ural Federal University named after the first President of Russia B.N. Yeltsin in accordance with the program of strategic academic leadership "Priority-2030".*

Ethical expertise *Prior to the start of the study, the approval of the local ethics committee was obtained, protocol No. 1(71) dated 04/28/2022.*

Informed consen*t Not required.*

REFERENCES

1. Sánchez-Bodón J, Andrade Del Olmo J, Alonso JM, et al. Bioactive Coatings on Titanium: A Review on Hydroxylation, Self-Assembled Monolayers (SAMs) and Surface Modification Strategies. *Polymers* (Basel). 2021;14(1):165. doi: 10.3390/polym1401016
2. Xu J, Zhang J, Shi Y, et al. Surface Modification of Biomedical Ti and Ti Alloys: A Review on Current Advances. *Materials* (Basel). 2022;15(5):1749. doi: 10.3390/ma15051749
3. Jain S, Parashar V. Analytical review on the biocompatibility of surface-treated Ti-alloys for joint replacement applications. *Expert Rev Med Devices*. 2022;19(9):699-719. doi: 10.1080/17434440.2022.2132146
4. Kaur M, Singh K. Review on titanium and titanium based alloys as biomaterials for orthopaedic applications. *Mater Sci Eng C Mater Biol Appl*. 2019;102:844-862. doi: 10.1016/j.msec.2019.04.064
5. Su EP, Justin DF, Pratt CR, et al. Effects of titanium nanotubes on the osseointegration, cell differentiation, mineralisation and antibacterial properties of orthopaedic implant surfaces. *Bone Joint J*. 2018;100-B(1 Supple A):9-16. doi: 10.1302/0301-620X.100B1.BJJ-2017-0551.R1
6. Zhan X, Li S, Cui Y, et al. Comparison of the osteoblastic activity of low elastic modulus Ti-24Nb-4Zr-8Sn alloy and pure titanium modified by physical and chemical methods. *Mater Sci Eng C Mater Biol Appl*. 2020;113:111018. doi: 10.1016/j.msec.2020.111018
7. Chekisheva TN. Nanomaterials and their role in bone tissue regeneration. *Clin Exp Morphology*. 2019;8(4):19-24. (In Russ.) doi: 10.31088/CEM2019.8.4.19-24
8. Souza JCM, Sordi MB, Kanazawa M, et al. Nano-scale modification of titanium implant surfaces to enhance osseointegration. *Acta Biomater*. 2019;94:112-131. doi: 10.1016/j.actbio.2019.05.045
9. Shulyatnikova OA, Rogozhnikov GI, Porozova SE, Rogoznikov AG, Leushina EA. Functional nanostructured materials based on titanium dioxide for use in orthopedic dentistry. *Actual problems in dentistry*. 2020;16(1):171-177. (In Russ.) doi: 10.18481/2077-7566-20-16-1-171-177
10. Wang C, Gao S, Lu R, et al. In Vitro and In Vivo Studies of Hydrogenated Titanium Dioxide Nanotubes with Superhydrophilic Surfaces during Early Osseointegration. *Cells*. 2022;11(21):3417. doi: 10.3390/cells11213417
11. Zhang Y, Gulati K, Li Z, et al. Dental Implant Nano-Engineering: Advances, Limitations and Future Directions. *Nanomaterials* (Basel). 2021;11(10):2489. doi: 10.3390/nano11102489
12. Darter BJ, Syrett ED, Foreman KB, et al. Changes in frontal plane kinematics over 12-months in individuals with the Percutaneous Osseointegrated Prosthesis (POP). *PLoS One*. 2023;18(2):e0281339. doi: 10.1371/journal.pone.0281339
13. Hagberg K, Ghasemi Jahani SA, Omar O, Thomsen P. Osseointegrated prostheses for the rehabilitation of patients with transfemoral amputations: A prospective ten-year cohort study of patient-reported outcomes and complications. *J Orthop Translat*. 2022;38:56-64. doi: 10.1016/j.jot.2022.09.004
14. Sinclair S, Beck JP, Webster J, et al. The First FDA Approved Early Feasibility Study of a Novel Percutaneous Bone Anchored Prosthesis for Transfemoral Amputees: A Prospective 1-year Follow-up Cohort Study. *Arch Phys Med Rehabil*. 2022;103(11):2092-2104. doi: 10.1016/j.apmr.2022.06.008
15. Emanov AA, Gorbach EN, Stogov MV et al. Survival of percutaneous implants under various mechanical loading to the bone. *Genij Ortopedii*. 2018;24(4):500-506. (In Russ.) doi: 10.3390/10.18019/1028-4427-2018-24-4-500-506
16. Kuznetsov VP, Gorgots VG, Anikeev AV, Emanov A.A. Production of new osteointegrated intraosseous implants using the method of additive technologies. *Vestnik Kurganskogo gosudarstvennogo universiteta. Seriya: Tekhnicheskie nauki*. 2017;(2):120-125. (In Russ.)
17. Kaplan MA, Smirnov MA, Kirsankin AA, Sevostyanov MA. Properties of TI-6AL-4V titanium alloys obtained by selective laser melting. *Physics and chemistry of materials treatment*. 2019;(3): 46-57. (In Russ.). doi: 10.30791/0015-3214-2019-3-46-57.
18. Almeida D, Sartoretto SC, Calasans-Maia JA, et al. In vivo osseointegration evaluation of implants coated with nanostructured hydroxyapatite in low density bone. *PLoS One*. 2023;18(2):e0282067. doi: 10.1371/journal.pone.0282067
19. Cao NJ, Zhu YH, Gao F, et al. Gradient nanostructured titanium stimulates cell responses in vitro and enhances osseointegration in vivo. *Ann Transl Med*. 2021;9(7):531. doi: 10.21037/atm-20-7588
20. Das S, Dholam K, Gurav S, et al. Accentuated osseointegration in osteogenic nanofibrous coated titanium implants. *Sci Rep*. 2019;9(1):17638. doi: 10.1038/s41598-019-53884-x
21. Hasegawa M, Saruta J, Hirota M, et al. A Newly Created Meso-, Micro-, and Nano-Scale Rough Titanium Surface Promotes Bone-Implant Integration. *Int J Mol Sci*. 2020;21(3):783. doi: 10.3390/ijms21030783
22. He W, Yin X, Xie L, et al. Enhancing osseointegration of titanium implants through large-grit sandblasting combined with micro-arc oxidation surface modification. *J Mater Sci Mater Med*. 2019;30(6):73. doi: 10.1007/s10856-019-6276-0

23. Hoornaert A, Vidal L, Besnier R, et al. Biocompatibility and osseointegration of nanostructured titanium dental implants in minipigs. *Clin Oral Implants Res*. 2020;31(6):526-535. doi: 10.1111/clr.13589
24. Salou L, Hoornaert A, Stanovici J, et al. Comparative bone tissue integration of nanostructured and microroughened dental implants. *Nanomedicine* (Lond). 2015;10(5):741-51. doi: 10.2217/nnm.14.223
25. Farrell BJ, Prilutsky BI, Ritter JM, et al. Effects of pore size, implantation time, and nano-surface properties on rat skin ingrowth into percutaneous porous titanium implants. *J Biomed Mater Res A*. 2014;102(5):1305-15. doi: 10.1002/jbm.a.34807
26. Van den Borre CE, Zigterman BGR, Mommaerts MY, Braem A. How surface coatings on titanium implants affect keratinized tissue: A systematic review. *J Biomed Mater Res B Appl Biomater*. 2022;110(7):1713-1723. doi: 10.1002/jbm.b.35025
27. Jones CF, Quarrington RD, Tsangari H, et al. A Novel Nanostructured Surface on Titanium Implants Increases Osseointegration in a Sheep Model. *Clin Orthop Relat Res*. 2022;480(11):2232-2250. doi: 10.1097/CORR.0000000000002327
28. Morandini Rodrigues L, Lima Zutin EA, Sartori EM, et al. Nanoscale hybrid implant surfaces and Osterix-mediated osseointegration. *J Biomed Mater Res A*. 2022;110(3):696-707. doi: 10.1002/jbm.a.37323
29. Asri RIM, Harun WSW, Samykano M, et al. Corrosion and surface modification on biocompatible metals: A review. *Mater Sci Eng C Mater Biol Appl*. 2017;77:1261-1274. doi: 10.1016/j.msec.2017.04.102
30. Ding M, Shi J, Wang W, et al. Early osseointegration of micro-arc oxidation coated titanium alloy implants containing Ag: a histomorphometric study. *BMC Oral Health*. 2022;22(1):628. doi: 10.1186/s12903-022-02673-6
31. Li X, Wang M, Zhang W, et al. A Magnesium-Incorporated Nanoporous Titanium Coating for Rapid Osseointegration. *Int J Nanomedicine*. 2020;15:6593-6603. doi: 10.2147/IJN.S255486
32. Gulati K, Scimeca JC, Ivanovski S, Verron E. Double-edged sword: Therapeutic efficacy versus toxicity evaluations of doped titanium implants. *Drug Discov Today*. 2021;26(11):2734-2742. doi: 10.1016/j.drudis.2021.07.004
33. Jayasree A, Ivanovski S, Gulati K. ON or OFF: Triggered therapies from anodized nano-engineered titanium implants. *J Control Release*. 2021;333:521-535. doi: 10.1016/j.jconrel.2021.03.020

The article was submitted 10.07.2023; approved after reviewing 11.08.2023; accepted for publication 25.08.2023.

Information about the authors:

1. Maksim V. Stogov – Doctor of Biological Sciences, Associate Professor, Head of Department, stogo_off@list.ru; https://orcid.org/0000-0001-8516-8571;
2. Andrey A. Emanov – Candidate of Veterinary Sciences, Leading Researcher, a_eman@list.ru; https://orcid.org/0000-0003-2890-3597;
3. Viktor P. Kuznetsov – Doctor of Technical Sciences, Professor, Head of Laboratory, Professor of the Department, wpkuzn@mail.ru; https://orcid.org/0000-0001-8949-6345;
4. Elena N. Gorbach – Candidate of Biological Sciences, Leading Researcher, gorbach.e@mail.ru; https://orcid.org/0000-0002-9516-7481;
5. Elena A. Kireeva – Candidate of Biological Sciences, Senior Researcher; ea_tkachuk@mail.ru; https://orcid.org/0000-0002-1006-5217;
6. Andrey V. Korelin – Candidate of Technical Sciences, Associate Professor, Head of Department, UrFU, a.v.korelin@urfu.ru; https://orcid.org/0000-0003-0650-2805.

Contribution of the authors:

Stogov M.V. – development of methodology, creation of metadata, writing of the initial draft of the work.
Emanov A.A. – conducting experiments, preparing work for publication, visualizing data.
Kuznetsov V.P. – idea, creation of models, preparation of work for publication.
Gorbach E.N. – data collection, preparation of work for publication, data visualization.
Kireeva E.A. – data collection, statistical evaluation.
Korelin A.V. – creation of models, preparation of work for publication.