



Osseointegration of new percutaneous Press-fit implants coated with zinc-containing calcium phosphate in the limb stump: experimental study

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Abstract

Introduction The use of transcaneous implants for the attachment of limb prostheses is a rapidly developing modern technology for restoring function of lost limbs, particularly relevant for lower limb prosthesis applications. The primary challenge in developing this technology is the need to significantly improve implant survival, which depends on its design and the presence of biocoatings.

The **purpose** of the work is to experimentally evaluate the survival rate of original Press-Fit-type percutaneous implants produced without coating the surface of the osseointegrated part and with an applied zinc-containing calcium phosphate coating during one-stage osseointegration.

Materials and Methods The study was performed on 10 mongrel male dogs aged 1.5 to 2 years and weighing 19.2 ± 1.3 kg. Animals in Group 1 ($n = 5$) received an uncoated implant, while animals in Group 2 ($n = 5$) received an implant coated with a zinc-containing calcium phosphate. Osseointegration was assessed using a combination of clinical, radiographic, and laboratory studies. Quantitative analysis of the results was performed using statistical methods.

Results Twenty-six weeks after implantation, all animals had implant stability. A single bone-implantation unit with a continuous and sufficiently dense compact plate was formed. Animals in Group 1 demonstrated organotypic bone remodeling and compaction of periosteal layers. By that time, animals in Group 2 featured completion of organotypic remodeling around the implant that indicated signs of more pronounced osteogenesis in the implant-bone system. The zinc-containing calcium phosphate coating promoted the formation of denser and more mineralized structures on the implant surface and prevented calcium efflux from the compact plate of the bone stump.

Discussion The studies conducted and clinical observations demonstrated the absence of significant local or systemic disorders in the experimental animals during the implant osseointegration period, indicating good tolerability. The zinc-containing calcium phosphate coating provided excellent biocompatibility and stimulated osteogenesis. The obtained results are consistent with data presented in the domestic and international scientific literature and expand the evidence base of the effectiveness of Press-Fit titanium SLM implants with zinc-containing calcium phosphate coating for osseointegration prosthetics.

Conclusion The data obtained during the experiment show fairly good results of osseointegration of Press-Fit implants without coating the surface of the osseointegrated part and with an applied zinc-containing calcium phosphate coating. They did not show pronounced restrictions on their use. However, from the point of view of minimizing the risks, preference should be given to product with a zinc-containing calcium phosphate coating of the osseointegrated part.

Keywords: osseointegration, prosthetics, titanium implant, Press-Fit, calcium phosphate coating

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INTRODUCTION

The use of transcutaneous implants for the attachment of limb prostheses is a rapidly developing modern technology for restoring the functions of lost limbs [1–6], and is particularly relevant in the case of lower limb bone prosthetics [7, 8]. The main problem in the development of this technology is the need to significantly improve the implant survival characteristics, which depend on its design and biocoatings [9–13]. In terms of acceptable design of osseointegrated implants for long bone prosthetics, preference is increasingly being given to Press-Fit systems [14–19]. Additional factors that contribute to improving the quality of osseointegration of titanium Press-Fit implants include options for additional fixation and compression loading on the bone [20], as well as the application of bioactive and antibacterial coatings, in particular zinc-containing calcium phosphate coatings, to the osseointegrated part [21–22].

The **purpose** of the work is to experimentally evaluate the survival rate of original Press-Fit-type transcutaneous implants produced without coating the surface of the osseointegrated part and with an applied zinc-containing calcium phosphate coating during one-stage osseointegration.

MATERIALS AND METHODS

The study was performed on 10 mongrel male dogs aged 1.5 to 2 years and weighing 19.2 ± 1.3 kg. Animals in Group 1 ($n = 5$) received an uncoated implant, while animals in Group 2 ($n = 5$) received an implant coated with a zinc-containing calcium phosphate.

The experimental animals were kept in the vivarium of the Ilizarov National Medical Research Center of Traumatology and Orthopedics. The dogs were housed in individual cages with equipped food and water containers, and hay was used as bedding. The cages and boxes were wet-cleaned daily. The animals were fed once daily and had access to clean drinking water ad libitum. The animals were quarantined for 30 days before the experiment. To assess the efficacy and safety of the experimental devices, the observation period after implantation was 26 weeks.

Prior to the study, approval was obtained from the institutional ethics committee (protocol dated 29.11.2024, No. 1(76)). 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 Experimental and other Scientific Purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22.09.2010 on the protection of animals used for scientific purposes.

Study design

The implants were manufactured using SLM printing on a DMP Flex 350 3D printer (USA) using Russian-produced PTN-8 titanium powder with a particle size of 15–45 μm . The design is described in Russian patents for utility models No. 194912 and 207637 [23, 24]. Photos of the implants are shown in Figure 1.

To ensure implantation in the tubular bone of the limb stump, the implant's insertion section is designed as a truncated cone with teeth on the stepped entry and calibration sections. The teeth on the entry section gradually decrease in diameter toward the implant tip by a value of $D_i - D_{i-1} = 0.1$ mm (Fig. 2 a).

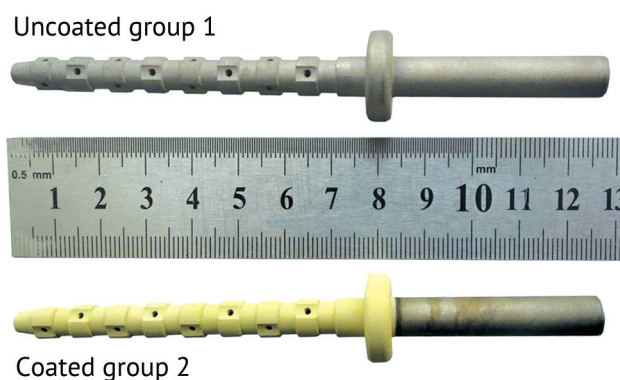


Fig. 1 External appearance of Press-Fit implants (uncoated group 1 and coated group 2)

The step-type entry section has teeth with cutting edges and grooves for the sheared bone chips, which act as autologous material (Fig. 2 b, c). The cutting teeth of the entry section contain openings connected to a through-channel inside the implant for drug delivery. The through-channel is closed with a plug.

The cutting edges on the teeth of the entry and calibration sections are inclined at an angle of 5° (Fig. 2 c). The arrangement of the edges around the teeth is shown in Fig. 2 d. Flat areas on the teeth ensure the delivery of medications in the bone-implant system.

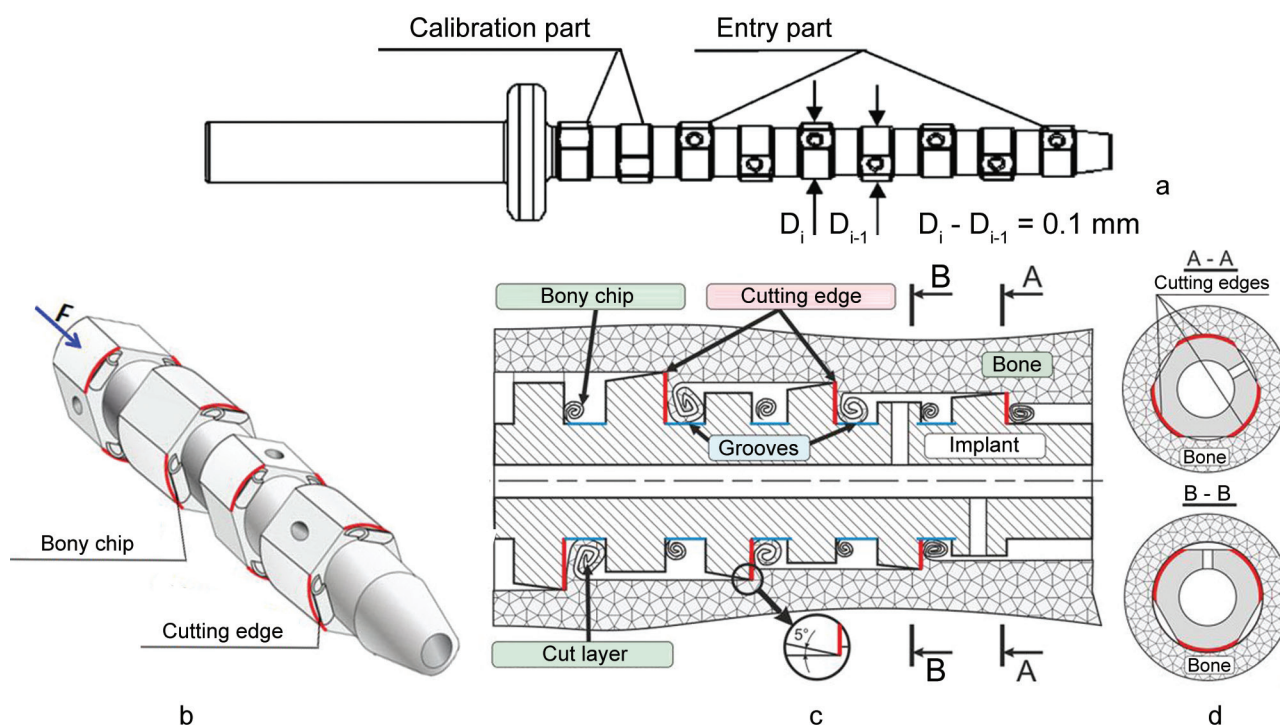


Fig. 2 Press-Fit implant for prosthetics of tubular bone stumps: (a) schematic representation; (b) three-dimensional image of the entry part; (c) longitudinal section of the entry part; (d) cross-sections of the entry part

Coating application

To obtain group 2 implants, the surface of the osseointegrated part of the products was modified by applying a zinc-containing calcium phosphate coating. The coatings were applied using microarc oxidation (MAO), which allows for the deposition of a homogeneous coating on the surface of samples of complex geometric shapes of any size. The semi-industrial Micro-Arc 3.0 MAO unit (IPMS SB RAS, Tomsk) includes a high-voltage pulsed current source, an electrolytic water-cooled titanium bath (cathode), titanium sample holders (anode), and a computer for controlling the MAO process [21, 25, 26]. The suspension composition and electrophysical parameters of coating application are presented in the work of Stogov et al. [21]. Zinc-substituted hydroxyapatite included in the suspension was obtained by mechanochemical synthesis [27]. The coating on the implants had a thickness of $30\text{--}35\text{ }\mu\text{m}$ and a surface roughness of $R_a = 2.0\text{--}2.5\text{ }\mu\text{m}$. In our previous studies [28, 29], the microstructure, phase and chemical compositions, physicochemical, and mechanical properties of the coatings were described in detail. The duration of sterilization treatment in a dry-heat oven was 60 min at a temperature of 180°C .

Insertion of the implant into limb stump

A study of osseointegration characteristics, as well as an assessment of the safety profile and determination of limitations associated with the product on a post-amputated limb, was conducted experimentally on two groups of mongrel dogs. The surgeries were performed in two stages under sterile conditions under intravenous anesthesia with propofol 10 mg/ml and preliminary premedication.

The first stage involved shaping a limb stump. A skin incision was made in the middle third of the tibia on the medial surface. The soft tissues were dissected, and an osteotomy was performed on the tibia using an oscillating saw at the junction of the upper and middle thirds. After the stump was formed, the soft tissues were sutured layer by layer.

The second stage of the experiment was conducted 10 weeks later. During the second operation, a skin flap was created in the stump area, adapted to the bone edges. The medullary canal was then drilled to accommodate the implant diameter, in our case up to 7 mm. The implant was driven into the hole with precisely aimed short hammer blows (Fig. 3).



Fig. 3 Photo of the procedure for installing a Press-Fit implant into a tubular bone

To expose the external portion of the implant, an opening was created in the skin flap, after which the stump was modeled and the soft tissues were sutured layer by layer. To prevent loosening and to apply a compressive load of 20 N to the prosthetic bone, the implant was secured with a special device for the first five weeks after surgery (Fig. 4 b) [30].

Assessment of osteointegration

To assess the effectiveness (survival) and safety of the implants, a series of clinical, radiological, histological and laboratory studies were performed.

Clinical examination of animals (physical examination, local status, condition of soft tissues and postoperative wounds) was carried out throughout the entire experiment term.

A Compact X-ray system (Milan, Italy) was used for radiography. The current was 60 mA, the voltage was 57–69 kV, and the exposure time was 0.4–0.6 s. Specific operating parameters depended on the animal's constitution.

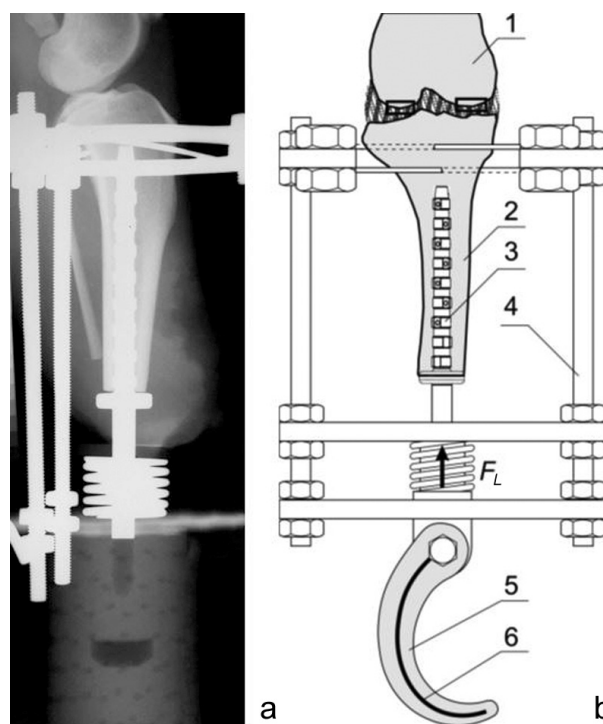


Fig. 4 Radiograph of the dog's lower leg after implant placement (a) and the fixation and compression loading device (b): 1 – femur; 2 – tibia; 3 – implant; 4 – assembled fixation and compression loading device; 5 – silicone prosthesis; 6 – stiffness spring, F_L – compression load

Radiographs of the limb were taken in the frontal and lateral views. Radiographs were taken before and after surgery, every seven day for the first five weeks after surgery, and then once every 14 days of the experiment.

Histological study

The weight fraction of Ca and P (in wt.%) in different areas of the tibial stump with an integrated implant was determined using a Zeiss EVO MA18 scanning electron microscope (Carl Zeiss Group, Germany) and a BRUKER QUANTAX 200 - XFlash 6/10 energy-dispersive spectrometer (Bruker Nano GmbH, Germany) mounted on the microscope base. The operating accelerating voltage was 20 kV. Data collection and analysis were performed using ESPRIT software (Bruker Nano GmbH, Germany).

Biochemical study

Levels of total protein, urea, creatinine, total calcium, inorganic phosphate, C-reactive protein (CRP), as well as the activity of alkaline (ALP) and tartrate-resistant (bone) isoenzyme of acid (TrAP) phosphatase, transaminases (AST, ALT) in the blood serum of experimental animals was determined on an automatic biochemical analyzer Hitachi/BM 902 (F. Hoffmann-La Roche Ltd., Italy) using reagent kits from Vital Diagnostics and BioSystems (Russia).

Statistical methods

The results in the tables are presented as the median and quartiles 1–3 (Me, Q1–Q3), the median and minimum and maximum values (Me, min–max), and the arithmetic mean and standard deviation ($X \pm SD$). The statistical evaluation of the significance of differences in quantitative indicators at the experiment time points compared to preoperative values was performed using the Wilcoxon signed-rank test for related samples. The significance of intergroup differences was assessed using the Mann – Whitney t-test. The Barnard test was used for frequency indicators; differences were considered significant at $p < 0.05$.

RESULTS

The clinical condition of all animals after implantation was satisfactory; the implants remained stable. A week after surgery, despite intermittent lameness, all dogs began using the operated limb. By the fourth week of the experiment, dogs in both groups showed only slight residual lameness, which completely resolved by the sixth week of the study.

One animal in Group 1 experienced inflammation in the area of the compression device pins in the early postoperative period. The inflammation resolved within a week treated with an antiseptic solution and anti-inflammatory ointment. It should be noted that all animals in that group experienced hyperemia in the area of skin-implant contact in the early postoperative period, which also resolved within seven days with the use of a heparin-based decongestant ointment.

In Group 2, one dog had a suture rupture, but the wound healed by primary intention. No inflammatory changes were observed in the early postoperative period in animals in this group.

Six weeks after implant placement, the compression device was removed. The implant was stable in animals from both experimental groups; however, dogs in Group 1 showed some signs of bone resorption and minor periosteal stratification in the distal bone (Fig. 5 a). In contrast, in Group 2, stratification appeared near the "stop ring", indicating active endosteal bone formation in the implantation area (Fig. 5 b).

Twenty-six weeks after the implantation, implants were stable in all animals. In animals of group 1, organotypic bone restructuring and compaction of periosteal layers were noted (Fig. 6 a). In animals of group 2, the organotypic reorganization of the bone around the implant was already completed (Fig. 6 b).

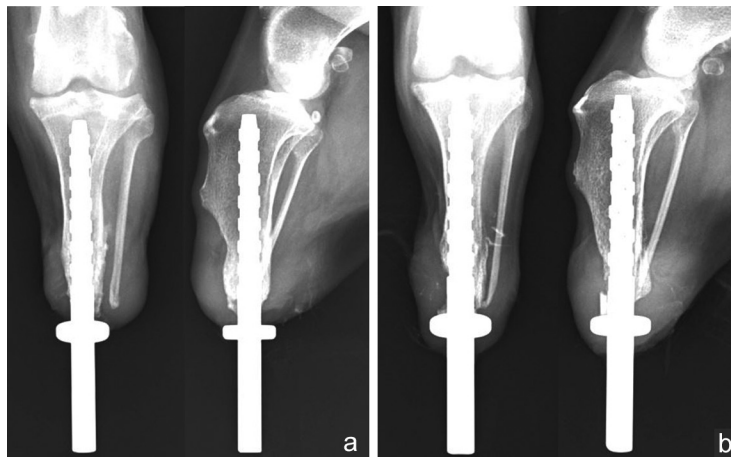


Fig. 5 Fragments of radiographs of the lower leg of dogs from group 1 (a) and group 2 (b) 42 days after surgery

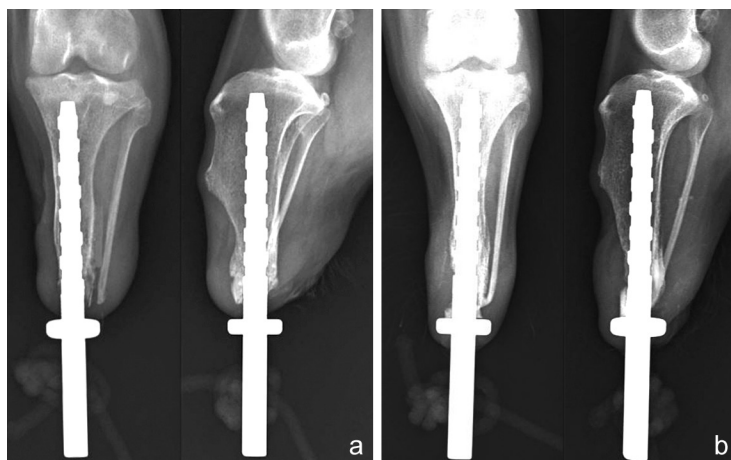


Fig. 6 Fragments of radiographs of the lower leg of dogs from group 1 (a) and group 2 (b) 180 days after surgery

The radiographic images indicate that signs of more pronounced osteogenesis in the "implant-bone" system were observed in the animals of group 2.

By the end of the experiment (26 weeks), a single bone-implant block was formed in both groups (Fig. 7), with the implant fully integrated into the bone tissue. Cancellous bone tissue, predominantly fine-meshed and, in some areas, medium-meshed, was formed along the entire perimeter of the implant surface in both groups. Throughout the entire bone-implant block, the compact plate was continuous and sufficiently dense.

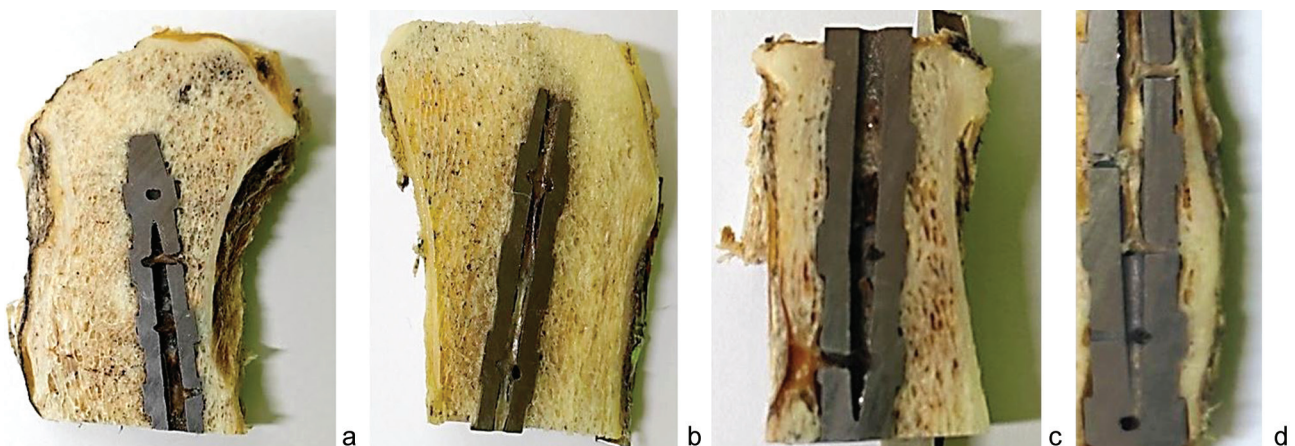


Fig. 7 Photos demonstrate the formation of the bone-implant block 26 weeks after implantation: (a) saw cut through the proximal part of the tibia of a dog from group 1 (without coating); (b) saw cut through the proximal part of the tibia of a dog from group 2 (with coating); (c) saw cut through the diaphysis of the tibial stump of a dog from group 1 (without coating); (d) saw cut through the diaphysis of the tibia of a dog from group 2 (with coating). Magnification: 1.5×

Energy-dispersive analysis revealed that the bone tissue calcium content in all areas of the bone-implant blocks of animals in Group 1 was significantly lower than in Group 2 (Table 1), and the phosphorus content in Group 1 was significantly higher than in Group 2. The exceptions were the areas of the compact plate in the distal part and the cancellous bone formed around the implant in the middle part of the tibial stump, where the phosphorus content in both groups was identical and did not differ significantly. In both groups, in the compact plate zone, the concentration of the determined elements was slightly higher in the distal part. A lower content of osteotropic elements was noted in the proximal part of the bone stump. Endosteally formed bone tissue that was localized around the implant in both groups contained lower amounts of calcium and phosphorus compared to the compact plate in each of the analyzed zones of both groups.

Table 1

Content of Ca and P in different parts of bone-implant block 26 weeks after implantation

Group	Element	Content of Ca and P (wt.%), X ± SD					
		Distal part		Middle part		Proximal part	
		Compact plate	Round the implant	Compact plate	Round the implant	Compact plate	Round the implant
1	Ca	18.4 ± 0.14	8.37 ± 0.23	16.16 ± 0.97	8.99 ± 0.71	14.12 ± 0.43	6.16 ± 0.13
	P	9.90 ± 0.31	4.98 ± 0.42	9.02 ± 0.23	4.87 ± 0.04	6.98 ± 0.25	3.04 ± 0.04
2	Ca	19.9 ± 0.84*	9.60 ± 0.17*	18.1 ± 0.67*	9.98 ± 0.91	16.97 ± 0.83*	8.61 ± 0.11*
	P	9.10 ± 0.85	3.99 ± 0.12*	8.89 ± 0.09*	4.78 ± 0.07	6.36 ± 0.17*	3.63 ± 0.07*

Note: * significance of difference at $p < 0.05$ compared to group1

The dynamics of changes in ALP activity revealed an increase relative to preoperative values in the animals of group 1 at the third and sixth weeks after implantation, and in group 2 at the sixth and 12th weeks (Table 2). Moreover, at the sixth and 12th weeks, ALP activity in the animals of group 2 was higher relative to the animals of group 1. TrAP activity in group 1 was higher than the preoperative level at the sixth and 26th weeks after implantation, while in group 2 it was reduced at the third week and increased at the 26th weeks, while the values were significantly higher relative to the values of group 1 at those time points. A single decrease in the level of total calcium in the blood serum of group 1 dogs was noted at the third week after implantation. Such changes indicate a more significant activation of osteogenesis in the animals of group 2 relative to group 1.

Table 2

Changes in phosphatase activity and concentrations of total calcium and inorganic phosphate in the blood serum of dogs after implantation

Week	Group	Activity and concentrations, Me (Q1–Q3)			
		ALP, units/l	TrAP, units/l	Calcium, mmol/l	Phosphate, mmol/L
0	1	36 (29–42)	4.85 (4.78–4.93)	2.60 (2.57–2.63)	1.20 (1.09–1.30)
	2	37 (33–41)	4.40 (4.22–4.95)	2.58 (2.44–2.61)	1.25 (1.12–1.42)
3	1	62* (59–65)	4.50 (3.90–5.10)	2.50* (2.46–2.54)	1.30 (1.23–1.37)
	2	38 (33–60)	3.40*(2.03–3.60)	2.58 (2.44–2.62)	1.45 (1.23–1.47)
6	1	54*(50–58)	5.85*(5.73–5.98)	2.64 (2.62–2.67)	1.29 (1.22–1.36)
	2	64*(60–76)	3.70 (2.23–5.08)	2.59 (2.54–2.64)	1.13 (1.09–1.27)
12	1	38 (30–45)	4.50 (4.12–5.12)	2.61 (2.50–2.64)	1.28 (1.21–1.38)
	2	67*(58–93)	3.85(3.45–4.43)	2.60 (2.56–2.65)	1.24 (1.24–1.29)
26	1	43 (36–61)	8.30*(7.05–9.55)	2.68 (2.53–2.70)	1.43 (1.32–1.55)
	2	39 (26–53)	5.90*(5.15–6.10)	2.54 (2.52–2.58)	1.24 (1.17–1.31)

Notes: * — differences with preoperative (period 0) values at $p < 0.05$; **bold** — differences in the indicator with group 1 at $p < 0.05$.

No differences were observed in the dynamics of changes in total protein, urea, creatinine, or transaminase (ALT, AST) levels between the animals in the studied groups (data not shown). However, in the animals in Group 1, CRP levels were significantly elevated relative to preoperative values at three weeks post-implantation, which was not observed in any animals in Group 2.

Thus, based on the histological study, it can be concluded that both uncoated and zinc-containing calcium phosphate-coated Press-Fit threaded implants exhibited acceptable osseointegration characteristics. The coated Press-Fit implant exhibited greater osseointegration, as confirmed by quantitative characteristics obtained during the histological study. The formation of denser, more mineralized structures on the surface of the bioactive-coated implant prevented calcium efflux from the compact plate of the bone stump. Furthermore, the zinc-containing calcium phosphate coating promoted the formation of a denser bone envelope around the implant in the distal portion of the stump, which is beneficial for implant stabilization in the bone at the implant-abutment junction.

The laboratory findings show the absence of any significant pathological changes in parameters characterizing the body's systemic response to implantation (including the response of visceral organs, such as the liver and kidneys). The individual changes noted, such as an increase in CRP, can be attributed to expected changes associated with the body's response to implantation, moreover those changes were transient.

In general, a complex of histological and laboratory studies, as well as clinical examination, allow us to note the absence of significant local and systemic disorders in the animals of both groups during the period of implant osseointegration, which indicates their good tolerability. At the same time, zinc-containing calcium phosphate coated products have a more beneficial safety profile compared to uncoated products.

DISCUSSION

Currently, there are opinions about the advantages of using percutaneous implants, such as Press-Fit, for solving prosthetic problems, in particular, the implementation of a one-stage integration which reduces the rate of complications] and increases the survival rate of the implant [32]. Literature data [33] and the findings of our study show the absence of any pronounced adverse effects during implantation, including reactions of visceral organs (liver, kidneys, etc.) after Press-Fit type implant application.

The improvement of this type of implants for prosthetics is being pursued in several directions: improvement of the design of the product and materials it is made of [34, 35], including an attempt to personalize the implant [36]; modification of the surface of the integrated part, including the application of additional coatings that facilitate integration [37–39]; control of the load on the implant [40, 41]. These principles were also implemented by us in the study, demonstrating the advantages of using a zinc-containing calcium phosphate coating under the conditions of compressive load on the implant due to an increase in the efficiency of osseointegration and the safety profile, what is consistent with the literature data [42].

Thus, the results of the study are confirmed by the data reported in the domestic and foreign scientific literature and expand the evidence base for the effectiveness of the use of titanium SLM Press-Fit implants with a zinc-containing calcium phosphate coating in osseointegration prosthetics.

Study limitations Osseointegration of the implants in this study was tested on a small number of animals. The lack of long-term results necessitates additional comparative studies of the long-term stability of implant integration with this type of coating. Furthermore, growing experience with the use of osseointegration technology for prosthetic applications demonstrates the need to develop specialized instrumentation for implant removal (infection, reinstallation, wear) [43].

CONCLUSION

The data obtained in this study demonstrate that both products are able to sufficiently integrate into the bone stump and have no significant limitations for their intended use. Given the approach that requires minimizing the risks associated with the use of implantable medical devices, the zinc-containing calcium phosphate coated product may be preferred as it offers certain advantages in terms of osseointegration efficiency and safety.

Conflict of interests Not declared

Funding source This work was conducted as part of the state assignment to the Ilizarov National Medical Research Center of Traumatology and Orthopedics "Development of new Press-Fit implants that ensure the formation and activation of bone matrix particles to improve the survival rate and reliability of prosthetics". The production of coatings on medical devices and the study of their characteristics were carried out within the framework of the state assignment of the Institute of Strength Physics and Materials Science SB RAS (project No FWRW-2026-0004).

Ethical statement 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 Experimental and other Scientific Purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Approval for the study was obtained from the institutional ethics committee (protocol dated 29.11.2024 No. 1(176)).

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