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The effect of zinc-containing calcium phosphate coating on the osseointegration of transcutaneous implants for limb prosthetics

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Abstract

Introduction Increasing the integration of transcutaneous implants is an important goal for their application in clinical practice.

The **purpose** of the work was to evaluate the osseointegration of transcutaneous titanium implants with calcium-phosphate coating containing zinc ions.

Materials and methods The studies were performed on 12 male rabbits, who underwent implantation of an original implant into the tibial stump. After implantation, a compression device was installed on the bone, maintaining a load of 3.5 N for 5 weeks. Duration of observation was 26 weeks. The animals were divided into two groups: a control group (n = 6) with an implant without coating and an experimental group (*n* = 6) with a zinc-substituted calcium-phosphate coated implant.

Results The implant fell out in one case in animals from the control group; no cases of implant loss were noted in the experimental group. It was revealed that the weight concentration of Ca and P in all zones of the bone-implant block of the animals in the experimental group significantly exceeded similar indicators in the control group. In the control group, long-term persistence of high levels of C-reactive protein was noted, which was not observed in the experimental group.

Discussion This series of studies has shown that an implant with a zinc-modified calcium-phosphate coating exhibited a more effective integration, in contrast to an uncoated product. The absence of serious adverse reactions to the tested products indicates acceptable tolerability and safety of its use.

Conclusion The implants with a zinc-modified calcium-phosphate coating showed signs of more effective osseointegration compared to the product without additional coating.

Keywords: prosthetics, transcutaneous implant, osseointegration, calcium-phosphate coating

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INTRODUCTION

Transcutaneous osseointegration prosthetics is a developing method of treating patients with limb loss [1, 2]. Currently, along with the two-stage prosthetics technology, a one-stage procedure has been developed [3, 4], which requires new solutions to improve the osseointegration of the implanted part, since the problem of implant stability in a one-stage process is crutial [5]. Previously, we showed that effective osseointegration of transcutaneous implants in a one-stage procedure can be achieved through the use of additional implant fixing devices in combination with the ability of these devices to provide mechanical compression of the implant [6]. Another option for implementing a one-stage accelerated osseointegration protocol is the use of Press-Fit implants [7].

However, such approaches are insufficient for optimal osseointegration of implants in a single-stage procedure. Therefore, there is a need to develop additional technologies that stimulate the integration process of transcutaneous implants, including by modifying the surface of the implanted part [8, 9]. Thus, one of the ways to improve osseointegration of implants is to apply a bioinert oxide or bioactive calcium-phosphate coating to its surface [10]. Among the methods of applying coatings to titanium-based implants, the microarc oxidation method should be highlighted, which allows impregnating additional essential microelements into the coating composition, providing positive effects for osteogenesis. A number of studies have demonstrated a positive effect on osseointegration processes of introducing zinc, strontium and silicon ions into the calcium-phosphate coating [11–13].

The **purpose** of the work was to evaluate the osseointegration of transcutaneous titanium implants with calcium-phosphate coating containing zinc ions.

MATERIAL AND METHODS

Implants In this study, we used implants made of Ti6Al4V alloy for prosthetics of tubular bone stumps [14] (Fig. 1 a). A zinc-containing calcium-phosphate (CP) coating (Zn-CP) was applied to the working surface of the implant using the arc oxidation method (Fig. 1 b). The implants were made of Ti6Al4V powder with an average particle size of 23.5 μm manufactured by Advanced Powders&Coatings Inc. (Canada) using the selective laser melting method on an EOS EOSINT M 280 3D printer (Germany). The coatings were applied to the implants using a Micro-Arc 3.0 semi-industrial system.

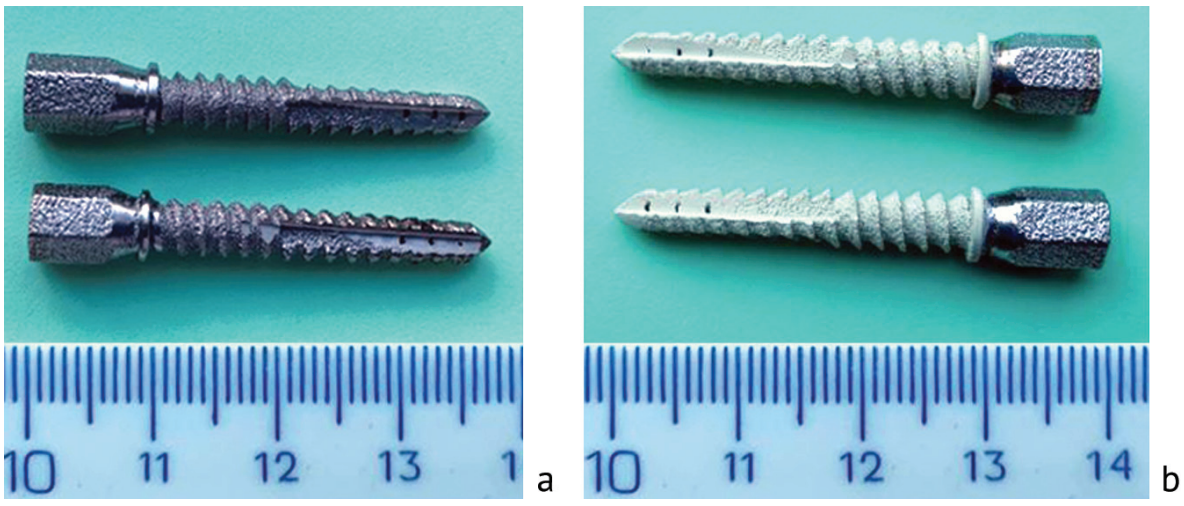

Fig. 1 Photos of implants: *a* without coating (control); *b* Zn-CP coated (experiment)

To apply the coatings, the implants were immersed in a bath with an electrolyte. The coatings were deposited in an electrolyte suspension of the following composition (wt %): H_3PO_4 — 27; $CaCO_3$ — 7; synthetic zinc-substituted hydroxyapatite (Zn-HA) ($Ca_{10}Zn_9(PO_4)_6(OH)_2$) — 5; the rest was distilled

water. Zn-HA in a nanocrystalline state with an average grain size of 30–50 μm was synthesized by a mechanochemical method at the Institute of Solid Body Chemistry and Mechanochemistry SB RAS [15]. The coating application parameters were: anode voltage — 200 V, pulse duration — 100 μs, pulse frequency — 50 Hz, treatment duration — 5 minutes. The coating thickness was determined using a Zubr digital micrometer (measurement accuracy of 1 μm). The coating mass was measured with CAS CAUX-220 analytical scales (measurement accuracy of 1 mg). The surface roughness was determined using a contact Profilometer-296 by the parameter Ra, as the root-mean-square deviation of the profile within the base length. The mass of the coatings formed on the implants was (29.3 ± 2.1) mg, the thickness was (33.5 ± 2.8) μm, and the roughness Ra was (2.3 ± 0.5) μm (Fig. 1 b). The morphology, structure, composition, and properties of the coatings were described in previous studies [11, 13].

All implants were delivered in individual packages, non-sterile. Before use, the implants were sterilized in a dry-heat oven at 180 °C for 1 hour. The maximum shelf life of the products before use was 6 months.

Study design in vivo The experiment was conducted on 12 male chinchilla rabbits aged 8 to 9 months, the average weight of the rabbits was (3.4 ± 0.2) kg. All rabbits underwent resection of the tibia at the border of the upper and middle thirds. After that, the bone marrow canal was processed with a drill; the implant was screwed into the stump of the tibia. The soft tissues were sutured layer by layer with internal sutures; a skin flap was formed in which an opening was made for the exit of a part of the implant to the outside. Then, a retaining compression device [16] with a fluoroplastic prosthesis was installed on the bone. The bone was subjected to a compression load of 3.5 N for 5 weeks. The observation continued 26 weeks. The animals were divided into two groups: group 1 (n = 6) animals had an uncoated product (control); group 2 (n = 6) animals had implants with zinc-substituted calcium-phosphate coating (experimental group). Clinical monitoring was carried out during the entire postoperative period. Attention was paid to the condition of the animals, thermometry, pulse, respiration, local status of the limb, condition of soft tissues, as well as postoperative wounds.

Regulating standards The study was performed in accordance with ISO 10993-1-2021. Medical devices. Biological evaluation of medical devices. Part 1: Risk management evaluation and testing; ISO 10993-6-2021. Medical devices. Biological evaluation of medical devices. Part 6: Local effects studies after implantation.

Ethical standards 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. Prior to the start of the study, approval was obtained from the local ethics committee (protocol No. 1 (71) dated 28.04.2022).

Euthanasia Planned euthanasia was performed after muscle relaxation with a solution of 1 % diphenhydramine (0.02 mg/kg) and 2 % rometar (5 mg/kg), then a lethal dose of barbiturates was administered.

Radiographic, histological and biochemical studies were performed to determine the effectiveness of osseointegration.

Radiographic study A Compact X-ray machine (Italy) was used for radiographic study. X-ray examination of the involved limb was performed in craniocaudal and lateromedial views. Radiography was performed before and after surgery, at 5, 12, 26 weeks after implantation.

Histological study In euthanized animals, a layer-by-layer preparation of the soft tissues of the tibia stump with an intramedullary integrated implant was performed. The material was fixed in a 10 % solution of neutral formalin for at least 10 days. Then the bone-implantation block was sawed longitudinally, leaving the implant in one of the halves.

The bone fragment without the implanted product was demineralized in a mixture of formic and hydrochloric acid solutions, dehydrated in ethyl alcohol. After stages of impregnation in several portions of "liquid" 5 % celloidin, it was poured into thick 40 % celloidin and the blocks were compacted in chloroform.

Sections were prepared using a Reichard sledge microtome (Germany) and stained with hematoxylin and eosin, picro-fuchsin, and Masson's floating section method. Histological preparations were studied using an AxioScope.A1 stereomicroscope; digital images were obtained using an AxioCam ICc 5 digital camera.

The other part of the bone-implant block of the tibial stump was dehydrated, poured into camphene and dried in air until its complete sublimation. The dried preparations were sprayed with Pt in a special sprayer IB-6 (EICO, Japan).

Quantitative determination of the content of Ca and P (W, in weight %) in different areas of bone-implant blocks was performed by the method of energy-dispersive X-ray microanalysis using a BRUKER QUANTAX 200 — XFlash 6/10 spectrometer (Bruker Nano GmbH, Germany), complete with a scanning microscope (SEM) Zeiss EVO MA18 (Carl Zeiss Group, Germany). Analysis of quantitative indicators was carried out in the ESPRIT program (Bruker Nano GmbH, Germany).

Biochemical study included determination of the concentration of total protein, urea, creatinine, glucose, lactate, total calcium, inorganic phosphate, potassium, sodium, chlorides, C-reactive protein (CRP), as well as the activity of alkaline phosphatase (ALP) and tartrate-resistant (bone) isoenzyme of acid phosphatase (TrAP), creatine kinase, and transaminases in the blood serum. The studies were performed on an automatic biochemical analyzer Hitachi/BM 902 (Italy) using Vital Diagnostics and Vector-Best (Russia) reagent kits.

Statistical study The results in the tables are presented depending on the characteristics of the compared samples (normality was assessed using the Shapiro-Wilk criterion) either as the arithmetic mean and standard deviation (Xi ± SD), or as the median, 1–3 quartiles (Me, Q1–Q3). The procedure for statistically assessing the significance of differences in the parameters at the experimental stages with preoperative values was performed using the Wilcoxon W-test. The reliability of differences between the groups at the follow-up time-points was assessed using the nonparametric Mann-Whitney T-test. The minimum significance level (p) was taken to be 0.05.

RESULTS

Clinical monitoring of animals in the postoperative period showed that the condition of rabbits in all groups was satisfactory, there were no unplanned deaths. In the first three days, there was edema in the stump area in all the cases, and a decrease in appetite was noted. The dynamic function of the limb restored in all animals on the 4th–5th day after the operation. No soft tissue inflammation was noted. During the first week after dismantling the special device (6 weeks after implantation), implant loss was noted in one case in an animal of group 1, while there was no implant loss in the experimental group.

X-ray examination showed that in the animals of group 1, there were areas of resorption at the bone/implant junction 5 weeks after implantation, while in the animals of group 2, resoption signs were not detected (Fig. 2).

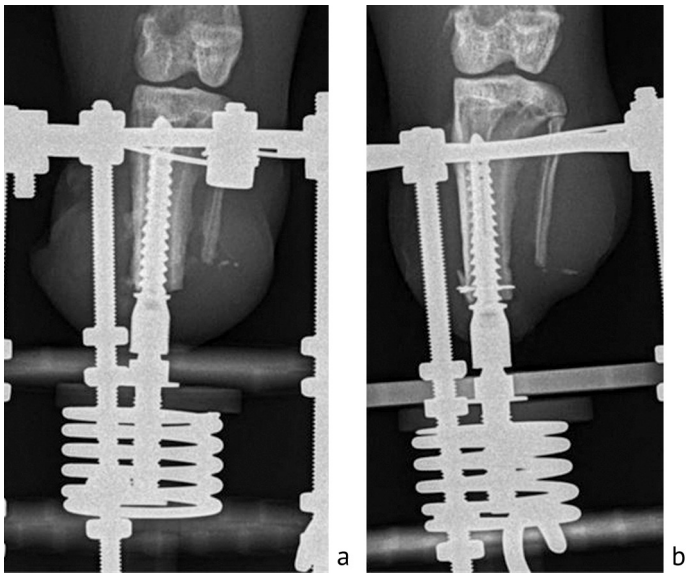

Fig. 2 Radiographs 5 weeks after implantation: *a* group 1; *b* group 2

After 12 weeks of implantation, signs of osseointegration were noted in both groups; resorption was not visualized, and minor periosteal layers were determined in the compaction stage (Fig. 3). At 26 weeks after implantation, complete organotypic reorganization of the bone in the peri-implant zone was noted (Fig. 4). However, in the animals of group 2, bone layers were in the compaction stage, located behind the limiting ring, which indicated an active bone integrative process at the bone-implant border (Fig. 4 b).

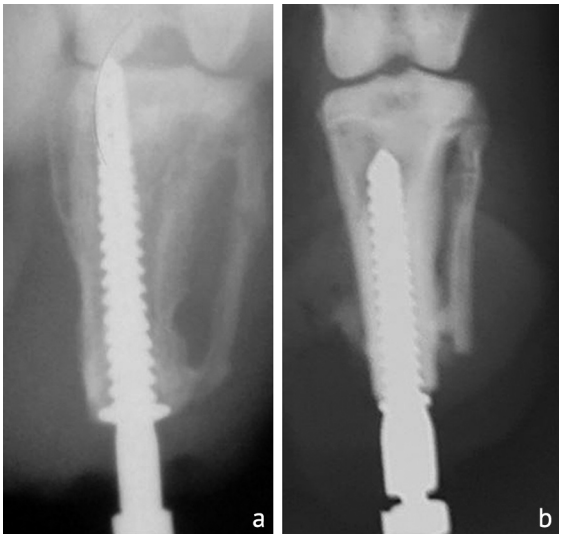

Fig. 3 Radiographs 12 weeks after implantation: *a* group 1; *b* group 2

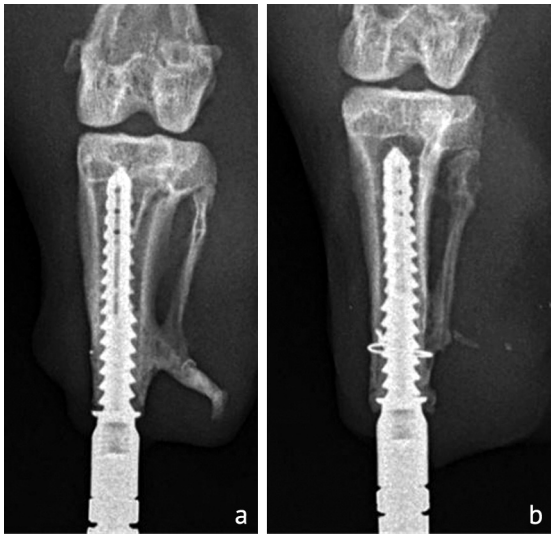

Fig. 2 Radiographs 26 weeks after implantation: *a* group 1; *b* group 2

Thus, in animals of group 2, the X-ray picture at all stages of the experiment revealed the presence of stability and the absence of resorption, what are positive signs of the osseointegration properties of the product.

Histological study In group 1, after 26 weeks of the experiment, a tight contact was observed between the implant surface and the bone tissue, and a single bone-implant block was formed. A continuous compact plate was preserved along the entire length of the bone stump. No pronounced periosteal stratifications were detected. In the distal and middle parts of the tibial stump, bone tissue ingrowth into the thread recesses of the implant was noted (Fig. 5).

Fig. 5 Bone-implant block formed in the animals of the control group by the 26th week of the study: *a* tibia of the rabbit with an intramedullary integrated implant (longitudinal cut); *b* longitudinal histotopographic section of the tibia of the rabbit after removal of the implant. Stained with picrofuchsin; magnification ×1.5

The histostructural characteristics of the tibial stump during the integration of theimplants in the experimental group after 26 weeks of the experiment revealed the preservation of the metaphyseal-epiphyseal part in the bed of the tibial stump and a continuous compact plate, which united with the surface of the implant in the metaepiphyseal region and in the proximal parts of the diaphysis with endosteally formed bone tissue of a medium lamella of trabecular structure. In the distal part, the compact plate tightly adjoined the implant. The presence of bone tissue was detected in the thread recesses of the implant (Fig. 6).

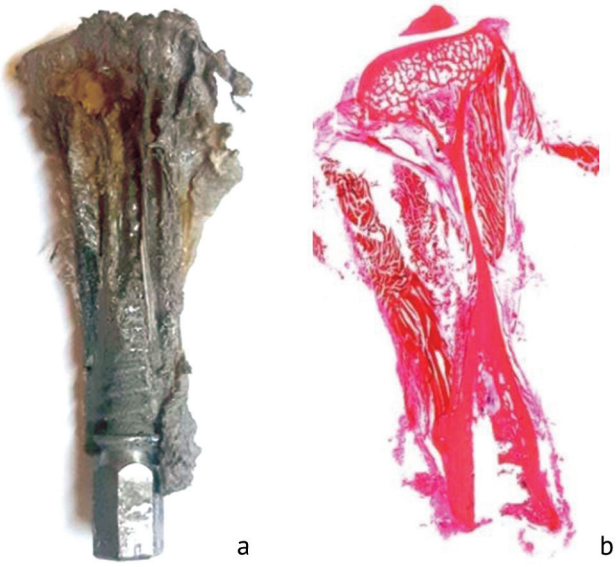

Fig. 6 Bone-implant block formed in the animals of the experimental group by the 26th week of the study: *a* tibia of the rabbit with an intramedullary integrated implant (longitudinal cut); *b* longitudinal histotopographic section of the tibia of the rabbit after removal of the implant. Stained with picrofuchsin; magnification ×1.5

Analysis of quantitative data on the content of osteotropic elements showed that after 26 weeks of the study in the substrate that formed on the surface of the implant in group 2 rabbits, calcium and phosphorus presence was significantly higher in all areas of the implant relative to the animals in the control group (Table 1).

Table 1

W (Ca) и W (P) in different parts of the tibial stump (Xi ± SD)

Study area	Ca, weight %		Phosphorus, weight %	
	Group 1	Group 2	Group 1	Group 2
Proximal	7.3 ± 0.4	15.1 ± 0.7*	3.6 ± 0.1	5.2 ± 0.3*
Middle	9.1 ± 0.3	15.3 ± 0.7*	4.6 ± 0.2	5.8 ± 0.2*
Distal	10.3 ± 0.3	13.2 ± 0.7*	4.5 ± 0.3	5.8 ± 0.4*
Intact animals	19.30 ± 0.91		8.12 ± 0.39	

Note: * — significant differences with group 1 (control) at $p < 0.05$

Thus, the histological study showed that the implant with a calcium-phosphate coating had more pronounced osteointegrative characteristics. In the distal part of the stump, an overgrowth of dense connective tissue was noted on the limiting ring structure and the structures of the implant preceding it, which contributed to the formation of an external barrier and prevented the development of intraosseous infection. A negative point is the imbibition of implant particles in the tissues bordering it.

Laboratory tests Changes in phosphatase activity and calcium and phosphate concentrations in the blood serum of experimental animals are presented in Table 2.

Table 2

Phosphatase activity, calcium and phosphate concentration in the blood serum of rabbits at the time-points (weeks) of the experiment, Me (Q1–Q3)

Week	Group	ALP, u/l	TrAP, u/l	Calcium, mmol/l	Phosphate, mmol/l
0	1	53 (48–63)	26 (22–26)	2.67 (2.61–2.70)	1.22 (1.14–1.34)
	2	54 (32–70)	28 (21–33)	2.69 (2.52–2.75)	1.34 (1.21–1.44)
3	1	42 (38–46)*	41 (35–42)*	2.78 (2.65–2.91)	1.31 (1.20–1.57)
	2	55 (47–63)^	29 (24–40)	2.75 (2.62–2.80)	1.14 (1.10–1.20)*
6	1	57 (49–69)	34 (29–38)*	2.65 (2.54–2.72)	1.34 (1.23–1.42)
	2	60 (53–65)	19 (17–21)*^	2.65 (2.60–2.71)	1.09 (1.07–1.18)*^
12	1	58 (45–66)	20 (16–28)	2.65 (2.57–2.73)	1.43 (1.42–1.45)
	2	46 (45–57)	24 (23–26)	2.51 (2.47–2.59)	1.04 (1.02–1.19)*^
26	1	61 (56–66)	20 (16–29)	2.71 (2.62–2.78)	1.39 (1.26–1.45)
	2	58 (42–63)	23 (22–28)	2.79 (2.55–2.83)	1.03 (0.98–1.14)*^

Note: * — differences with preoperative values (time-point 0), $p < 0.05$; ^ — differences with control group at $p < 0.05$.

It was found that in animals of group 1 (control), the activity of alkaline phosphatase relative to the preoperative level decreased at 3 weeks after implantation. In rabbits of group 2, an increase in the activity of alkaline phosphatase relative to the values before the operation was not noted, but alkaline phosphatase at 3 weeks was higher relative to the control group. The activity of TrAP at 3 and 6 weeks increased in the animals of the control group relative to the initial values. In group 2, a reliable decrease in the activity of TrAP at 6 weeks after implantation was noted both relative to the preoperative level and relative to the control group at that time. The concentration of total calcium in the blood serum of the experiment groups did not change statistically significantly either relative to the preoperative values or between the groups. In group 2, a decrease in the concentration of inorganic phosphate in the blood serum relative to the initial values and relative to the animals of the control group was noted at all stages of the experiment.

Significant differences in the dynamics of C-reactive protein (CRP) were noted (Table 3). Thus, significantly elevated CRP values in the control group (group 1) were observed up to week 26 of the experiment, and up to week 5 in group 2. In group 2, at week 26 of the experiment, a statistically significant decrease in lactate concentration was noted both relative to the initial, preoperative values, and relative to the values of the control group. Other biochemical parameters did not have reliable differences in both groups.

Table 3

Concentration of CRP and lactate in the blood serum of rabbits at the experimental time-points, Me (Q1–Q3)

Parameter	Group	Time-point (weeks) of the experiment				
		0	3	5	12	26
CRP mg/ml	1	0 (0–1)	13* (6–22)	10* (4–17)	2 (0–3)	4* (2–21)
	2	0 (0–1)	6* (2–14)	6* (5–10)	3 (0–4)	0 (0–2)
Lactate, mmol/l	1	9.5 (7.4–10.1)	10.4 (8.3–12.3)	9.0 (7.4–9.9)	8.8 (7.5–11.4)	10.5 (8.9–11.4)
	2	9.1 (7.5–10.8)	9.5 (7.8–10.2)	9.5 (8.1–10.0)	6.4 (4.9–7.8)	4.8*^ (2.7–7.5)

Note: * — differences with preoperative values (time-point 0), $p < 0.05$; ^ — differences with the control group at $p < 0.05$

DISCUSSION

One-stage osseointegration technology of transcutaneous implants is a promising method for solving bone prosthetics problems. A number of studies indicate that the transition to such a procedure helps improve the treatment results of target patients [17, 18]. The development of this version of transcutaneous osseointegration prosthetics technology lies in the direction of how to enhance osseointegration of the implant, ensuring its stability, resistance to infection and sufficient soft-tissue sealing around the outer part of the implant [19, 20].

Based on the available data, within the framework of the development of the methods of osseointegration stimulation, we evaluated a new implant for prosthetics of tubular bone stumps with a modified surface. It was found that the use of the implant with a Zn-containing CP-coating shows signs of improved integration in contrast to the product without such coating. Moreover, the implantation of the CP-coated product did not cause signs of rejection, intoxication (both local and systemic), systemic inflammatory reaction in the animals during the entire observation period. The absence of serious adverse reactions to the tested products allows us to conclude that all the studied implants have acceptable tolerability and safety.

Literature data on the assessment of the possibilities of increasing the integrative properties of implants with Zn-containing CP-coating are rather scarce. There is a work that also stresses the ability of such a coating to improve the osseointegration of metallic transcutaneous implants [21]. Nevertheless, the prospects of using such a coating are obvious, as evidenced by numerous data on improved osseointegration of CP-coated dental implants [22–24]. An additional factor in favor of using CP-coated products is that some studies indicate the ability of such coatings to reduce the inflammatory reaction and infection in the implantation zone [25, 26].

Further improvement of the osteointegrative characteristics of the developed product may be associated with an increase in the number of additional microelements in the composition of the CP-coating [27, 28], the use of implants with rapidly absorbable hydrogels loaded with antibiotics [29]. The possibility of applying biopolymer coatings to metal implants, especially to parts of the implant surface integrated with soft tissues [30], as well as the use of artificial polymers instead of titanium [31, 32] appears interesting.

It is obvious that the results we obtained have limitations in terms of the sample sizes of experimental animals. Extrapolation of the study results to clinical practice is possible, since the experimental model used is close to the clinical application model (single-stage prosthetics), including due to a sufficiently long observation period after implantation.

CONCLUSION

The use of implants with zinc-modified calcium-phosphate coating showed signs of more effective osseointegration compared to the product without additional coating. Such implants with a modified CP surface in the developed design, based on the obtained data on their effectiveness and safety, may be used for the tasks of prosthetics of small bone stumps in a single-stage prosthetics technology.

Conflict of interest *Not declared.*

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