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Innovative Implants in the Treatment of Osteoporosis: Materials, Technologies and Prospects for Bone Tissue Regeneration

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Abstract: **The aim of the study:** to investigate the effect of pore size and the presence of a biologically active calcium phosphate coating on the integration process with bone tissue in porous titanium implants manufactured by 3D printing.

Materials and methods. Cylindrical implant samples of three different diameters (100, 200 and 400 μm) were manufactured using electron beam melting technology from titanium powder on an Arcam 3D printer (Sweden). A calcium phosphate coating with a thickness of 20 ± 4 μm was applied to some products using microarc oxidation. The cytotoxicity of the implants was determined in vitro using human skin fibroblast cultures. In vivo samples were implanted into the femurs of 36 rabbits. Animals were divided into 6 groups according to the bone implantation patterns. The prepared samples and peri-implant tissues were studied using scanning electron microscopy and histological methods on days 90 and 180 after implantation.

Results. All the studied samples were found to be non-toxic and had good biocompatibility with bone tissue. No differences were found in the histological structure, vascularity intensity in the early stages, and bone formation in the later stages for coated and uncoated implants with hole diameters of 100 and 200 μm. Samples with hole diameters of 100 and 200 μm were easily removed from the bone tissue; with a hole diameter of 400 μm, a difference was noted between coated and uncoated samples, which was expressed in a stronger osseointegration in favor of calcium phosphate coated samples ($p<0.05$).

Conclusion: The optimal surface properties of the material for replacing bone defects are a pore diameter of 400 microns and the presence of a calcium phosphate coating.

Key points: osseointegration; porous titanium implants; cytotoxicity; bone tissue defect; additive technologies; 3D printing.

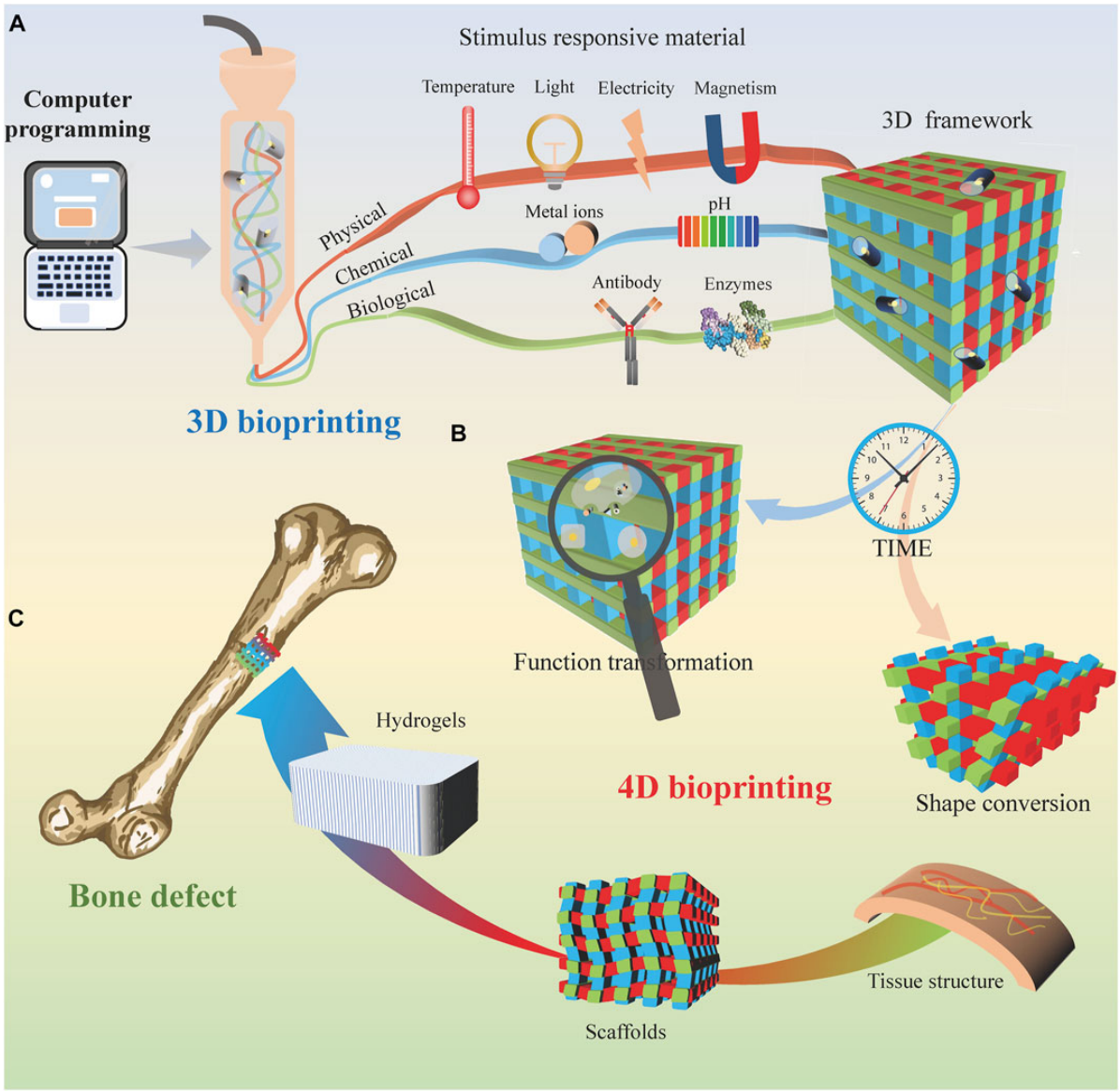
Introduction

The production of joint implants using additive technology allows the formation of individual products of complex shapes that combine high physical-mechanical and medical-biological properties. The development of additive technologies opens up wide opportunities for practicing orthopedists and traumatologists to restore the function of damaged bone tissue [1].

Titanium has high strength and good biocompatibility with tissues during implantation [2, 3]. Individual implants made of titanium and its alloys using 3D printing allow the replacement of

complex bone defects, including the ability to regulate the size and shape of the holes, as well as the overall open porosity, during their production process [2-6].

In experimental replacement of monocortical diaphyseal defects in the femur of animals, the high osteoconductive properties of a calcium phosphate-based 3D porous material and its biodegradability have been noted [7]. However, products made entirely from tricalcium phosphate-based materials do not have sufficient strength properties and are not suitable as joint implants.



It is known that calcium phosphate coatings formed by microarc oxidation (MAO) on the surface of titanium implants significantly improve the osteoinductive properties of products used for extraosseous and transosseous osteosynthesis [8, 9]. However, the widespread use of such implants obtained by 3D printing in clinical practice is limited. It has been found that the process of integration of the implant with bone tissue, as well as its osteoinductive and osteoconductive properties, is largely determined by the pore size and chemical composition of the implant surface [10-13]. Due to the regulation of these parameters, it is possible to control the process of tissue histogenesis. However, questions remain about the influence of pore size and calcium phosphate coating on the ability of titanium implants to integrate with bone tissue. Answers to them will help determine the properties of the material for the manufacture of implants with the most optimal properties, which will allow achieving strong primary and subsequently secondary fixation.

The aim of this work is to investigate the effect of pore size and the presence of a biologically active calcium phosphate coating on porous titanium implants on the integration process with bone tissue.

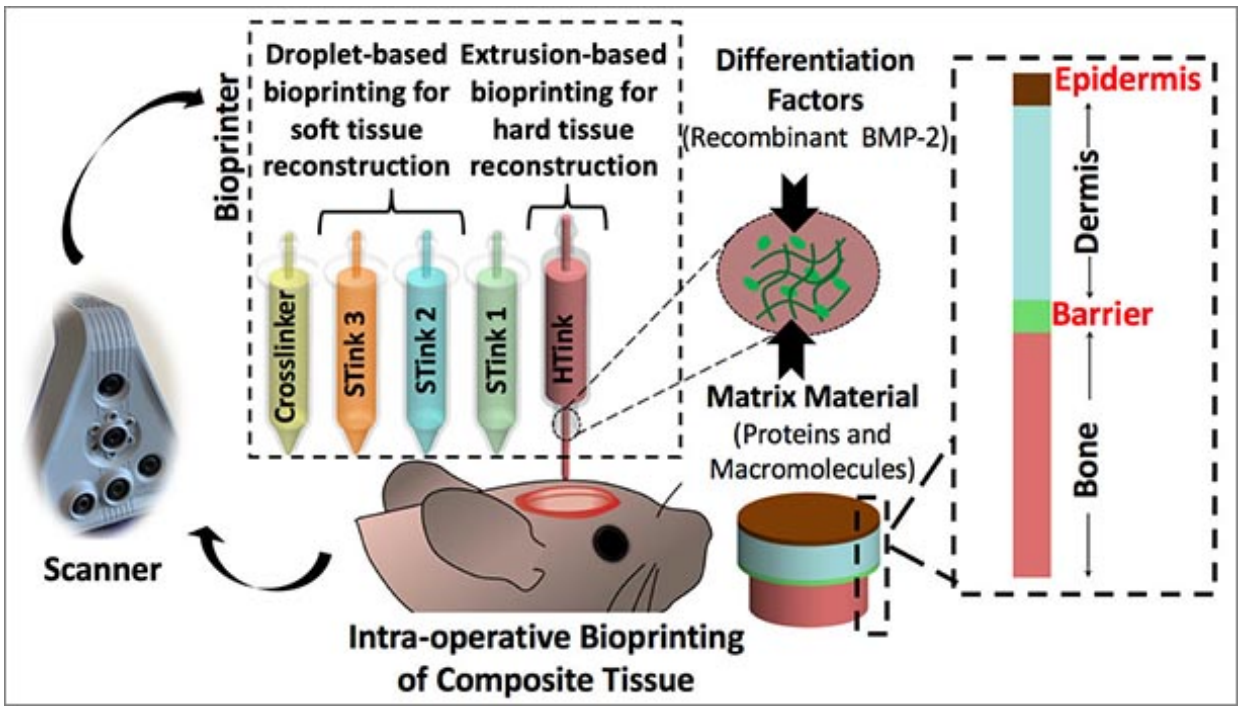
Materials and methods

Cylindrical implant samples of three different diameters (100, 200 and 400 μm) were manufactured using electron beam melting technology from titanium powder on an Arcam 3D printer (Sweden). All samples had a hexagonal structure with their outer layers arranged along the implant axis. To eliminate the scratch effect when fixing the implants, they were created without an aggressive rough coating on the surface. A calcium phosphate coating with a thickness of 20±4 μm was applied to some samples using the MAO method [8] at the pilot plant of the National Research Tomsk Polytechnic University. Subsequently, sterilization was carried out in moist steam at a temperature of 134 ° C for 5 minutes according to GOST R ISO 17665-1, after which the samples were placed in sterile packaging three times. Cytotoxicity was studied using an in vitro model (test culture: human diploid fibroblasts) according to GOST R ISO 10993.5.

In vivo studies were conducted at the Department of Experimental Medicine of the Volga Research Medical University (Nizhny Novgorod) on 36 male chinchilla rabbits aged 6 ± 1 months with a body weight of 4675 ± 258 g by the Ethics Committee of the Volga Research Medical University.

Animals were divided into 6 groups according to the bone implantation patterns (Table 1). The diameter of the base of the patterns was 3.2 mm, and the height was 8 mm.

In accordance with generally accepted ethical standards (Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), as well as the ethical principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2006), all painful manipulations were performed under general and local anesthesia.



After treating the surgical field with antiseptic agents, a skin incision was made in the projection of the lateral condyle of the femur and the soft tissues were separated. Using a 3 mm diameter drill and a drill, a standardized bone defect of a cylindrical shape corresponding to the size of the implant sample was formed perpendicular to the femoral axis. A bone sample was placed in the resulting defect, after which the surgical wound was sanitized and sutured in layers. X-ray monitoring was carried out using a C-arm (mobile X-ray diagnostic surgical unit RTS-612 v. 4.2, Russia). In the postoperative period, dressings were performed and the surgical wound was observed for 10 days. 90 and 180 days after implantation, the animals were removed from the experiment and sections of the femoral bones were collected at the site of direct contact with the implant samples.

Further preparation of the experimental material for the study was carried out using equipment manufactured by Thermo Fisher Scientific (USA). After the femur sections were fixed in 10% formaldehyde and decalcified, the implant samples were carefully removed from the bone tissue and sent to an electron microscope, and the bone tissue was sent for standard histological processing on the Excelsior ES machine. Then, they were embedded in paraffin blocks using the HistoStar embedding station. Serial sections with a thickness of 4-6 microns were taken on a Microm HM 325 microtome. The sections were stained with hematoxylin and eosin using the Gemini AS staining station.

A Leica 2500 microscope (Leica Microsystems, Germany) was used for morphometric processing. Histological examination assessed morphological changes in bone tissue.

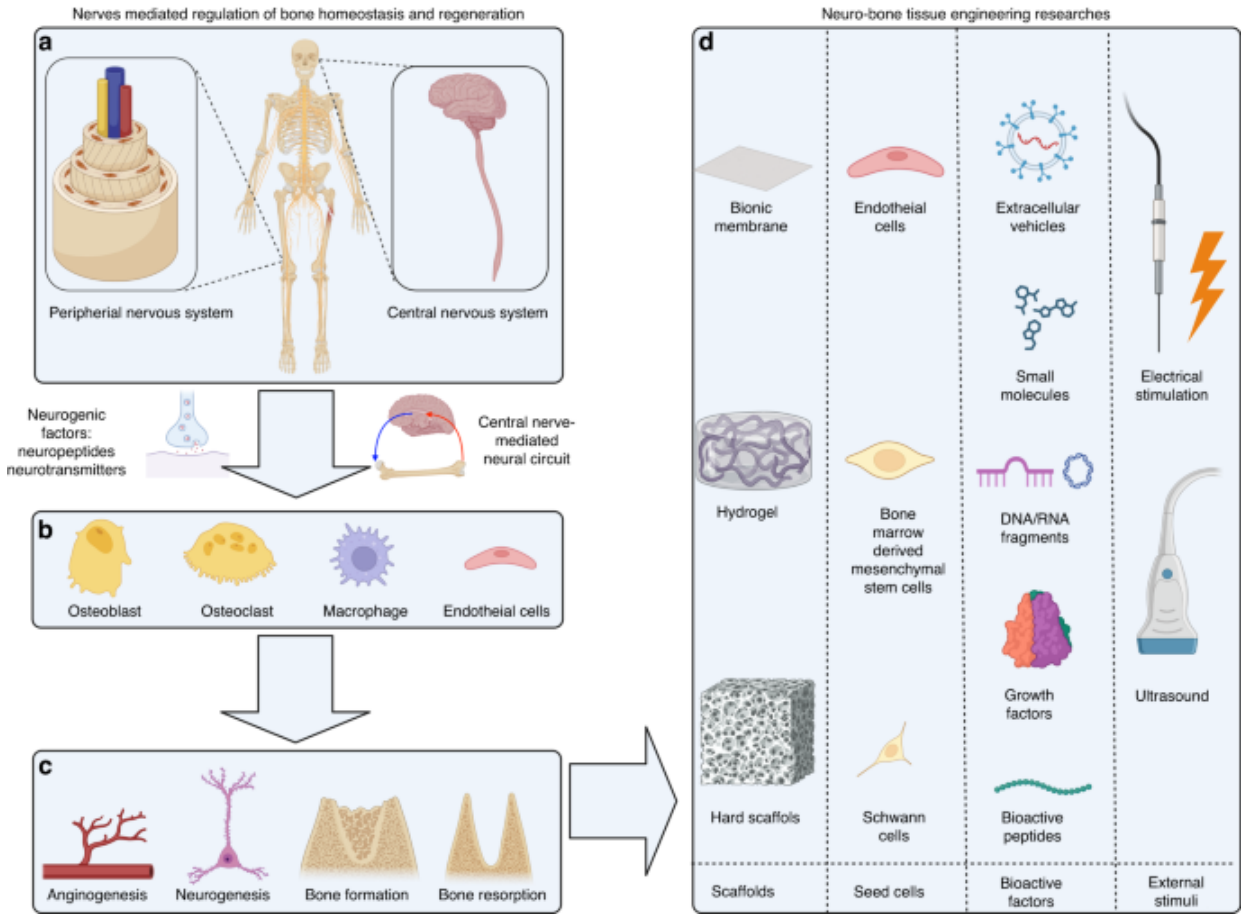
Electron microscopic studies of implant samples were carried out at the Collective Use Center "New Materials and Resource-Saving Technologies" of Nizhny Novgorod State University. NI Lobachevsky was used on a JSM-IT300LV scanning electron microscope (JEOL, Japan) in high vacuum mode and at low probe current values (<0.1 nA) to reduce the effect of the electron beam on the samples under study (electron probe diameter up to 3 nm). Photographic documentation was carried out at magnifications from 27-30 (for general images) to 2500-3700 (for assessing the surface of coated and uncoated implants, as well as pores and individual informative inclusions in the surface structure of the samples under study).

During microscopic examination of implant samples, a semi-quantitative morphometric assessment of bone tissue changes was performed using a four-point rating scale. When assessing the inflammatory reaction, the drug was scored from 0 (no signs of inflammation) to 3 (severe inflammation); when assessing vascularization - from 0 (no signs) to 3 (severe vascularization); when assessing the intensity of osteogenesis - from 0 (no signs of osteogenesis) to 3 (intensely expressed osteogenesis, a large area is occupied by mature bone tissue); when assessing sclerosis - from 0 (no signs) to 3 (severe sclerosis). The depth of penetration of bone tissue into the implant holes was expressed in relative units: 0 - no growth, 1 - weak growth, 2 - moderate growth, 3 - pronounced growth.

Statistical analysis. Statistical data processing was carried out using Microsoft Office Excel and Statistica 6.1 programs. The results of the study were processed using non-parametric statistics: the Mann-Whitney test for paired comparisons and the Kruskal-Wallis test for multiple comparisons were used. Differences between the compared groups were considered statistically significant at $p<0.05$.

Results and discussion

Using an in vitro model, it was determined that the material used to prepare the samples did not have a cytotoxic effect. The results of in vivo studies showed that the majority of experimental animals showed that all parameters studied, including general condition (weight, hair, skin, etc.), hematological and biochemical parameters, were within normal limits at 90 and 180 days after implantation of the samples.



A semi-quantitative morphometric assessment of bone tissue changes was performed during microscopic examination of implant samples, the results of which are presented in Table 2.

Semi-quantitative morphometric assessment of bone tissue changes at different times after implant placement, in points

Histological examination showed that after implantation of the samples, signs of peri-implant tissue inflammation were not found in any case. In groups 5 and 6, mature bone trabeculae were observed along the periphery of the implantation site, the distribution of which was greater than that using products 1-4 ($p<0.05$). Sclerosis was less pronounced when implanting samples 1, 2 and was not expressed at all in samples 5, 6 ($p<0.001$).

During electron microscopic examination of samples taken 90 days after implantation, it was noted that in groups 1 and 2 the holes were filled with bone structures of varying degrees of maturity over the entire area, but the depth of their filling was slightly lower. In groups 3-6. In groups 5, 6, in the implantation area, compared to other groups, mature trabeculae with a clearly defined osteocytic structure characteristic of a mature structure occupied a larger area ($p<0.001$). During the 180-day study period, the area occupied by newly formed mature bone tissue in the holes of the implants increased: in groups 5, 6 the area increase was greater than in implants in groups 1-4 ($p<0.001$). The general appearance of electron microscopic images and the surface of the implant samples is presented in Fig. 2.

In the comparative characterization of implants, it was found that the presence or absence of a coating was not significant for samples with a hole diameter of 100 and 200 μm, since there were no differences in the histological structure, the intensity of vascularization in the early stages and bone formation. later stages were determined. Samples with a hole diameter of 100 and 200 μm were removed from the bone tissue much more easily at all periods of the study, since the bone depth to the implant holes was lower than in samples 5 and 6 ($p<0.001$). A difference was noted between samples 5 and 6 at different periods of the study with a hole diameter of 400 μm, which

was expressed in a stronger osseointegration in favor of sample No. 5 with a coating ($p<0.001$ and $p<0.05$). 90 and 180 days after implantation, respectively).

The opinions of different authors about biodegradable calcium phosphate coatings on titanium implants are the same: the coatings significantly improve their osteoinductive properties and stimulate osteogenesis [6, 8, 9, 14]. SG Kalinichenko et al. [14] studied the effect of biodegradable calcium phosphate and hydroxyapatite coatings applied to titanium implants using plasma electrolytic oxidation on osteogenesis in a closed rat femoral fracture model. It was found that biodegradable implant coatings promote the proliferation and expression of BMP-2, VEGF, and TGF-β2 genes and enhance the regulatory effect of growth factors at different stages of reparative osteogenesis. The authors also concluded that the textured surface of the implant promotes the attachment and retention of osteoblasts under conditions of biomechanical loads. Calcium phosphates are a source of biologically active ions that create a favorable chemical environment for osteogenic cells and ensure the stability of cell proliferation in newly formed bone tissue.

We have shown that the presence of a calcium phosphate coating does not always improve osteogenesis: for implants with pore diameters of 100 and 200 microns, the presence or absence of a coating is not important. In turn, the presence of a coating with a pore diameter of 400 μm promotes more intensive osseointegration.

There are different opinions among researchers about the optimal pore size for improving osseointegration. RM Tixilov et al. [5] studied the bone and soft tissue integration of titanium implants with pores of 100-200 μm. Such products stimulated bone growth to a depth of 2-3 mm and provided good fixation of the implant. The authors consider this pore diameter to be optimal for bone and soft tissue integration.

Another group of researchers [15] found that among the 3D printed porous titanium implants they studied, implants with an actual pore size of approximately 600 μm had a higher osteogenesis rate than the other two groups with 400 and 800 μm.

Some authors conclude that the larger the pore size, the better the osseointegration. D. Zhao et al. [16] found that polylactide scaffolds with large pore sizes showed better results in bone tissue regeneration. A large pore size (2 mm) is more favorable for the exchange of cells and cytokines between the periosteum and cortical bone. In addition, osteogenesis and angiogenesis are two interrelated processes, and the viability of the formed bone tissue depends on the regeneration of new vessels. The authors showed that the vascularization of new bone increases with increasing pore size. However, the mechanical properties of PLA scaffolds gradually decreased with increasing pore diameter. In our case, the use of titanium alloy implants deprives them of such disadvantages as low strength.

In a study by A. Ilea et al. [17], titanium scaffolds with 800 nm pores showed better osseointegration than scaffolds with 1000 nm pores. Similar results were obtained in the work of F. Liu [18] et al. The authors studied the osteogenesis of bone scaffolds prepared by 3D printing from calcium phosphate, with a porosity of 70% and large pore sizes of 0.8; 1.2 and 1.6 mm. The scaffolds were placed in an 8 mm defect created in the calvarium of New Zealand rabbits and studied 4 and 8 weeks after placement. All scaffolds showed excellent mechanical properties and had a better bone formation capacity than controls at 4 and 8 weeks. Among them, the scaffold with a pore size of 800 μm outperformed the others in all parameters of bone formation.

A possible reason for the discrepancy in the results of studies on the optimal pore size for osseointegration is that the authors present the average pore size without taking into account their correlation, the total porosity. In this regard, there is no consensus on the optimal surface properties of materials for the manufacture of bone implants.

Conclusion

Highly porous titanium implants manufactured using additive technologies are promising for reconstructive and revision surgery in replacing bone defects. The studied implant samples are

highly biocompatible and do not have cytotoxic effects. The optimal surface properties of the implant sample material that promote osseointegration are a pore diameter of 400 μm and the presence of a calcium phosphate coating.

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