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INFLUENCE OF β-TRICALCIUM PHOSPHATE OF DIFFERENT GEOMETRIC SHAPE ON THE MORPHOLOGY OF REGENERATION OF EXPERIMENTAL DEFECT OF COMPACT BONE TISSUE

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Abstract

Purpose: to compare the healing process of a defect of compact bone tissue after the implantation of osteoplastic materials based on β-tricalcium phosphate ("ChronOS™" and "Calc-i-oss®"), which differ by manufacturer, geometrical shape and microscopic structure. Methods: the experiment was performed on 48 white male Wistar rats. In the middle third of the diaphysis of the femur we produced a perforated defect of 2.5 mm diameter in the medullary canal, which in the animals of the first group was filled with the osteoplastic material "ChronOS™" (block, Synthes, Switzerland), and in the animals of the second group with "Calc-i-oss®" (granules, «Degradable Solutions Dental», Switzerland). Fragments of the injured bones were studied on the 60th and 120th day by light microscopy with morphometry and by scanning electron microscopy. Results: it was found that regardless the geometric shape and the microscopic structure, both osteoplastic materials show high biocompatibility, osteoconductive properties, good integration with bone tissue of the regenerate, and that the microscopic structure of β-tricalcium phosphate ("ChronOS™") may significantly affect the microscopic structure of bone tissue of the regenerate, which manifests itself in the specificity of its geometric shape. It was noticed that osteoplastic materials "ChronOS™" and "Calc-i-oss®" almost at the same rate were subjected to resorption and replacement by the bone tissue, the ratio of which was 22.55±1.25 to 77.45±1.25 and 25.72±2.06% to 74.28±2.06% on the 60th day of the experiment, and 17.65±1.09 to 82.35±1.09 and 18.31±1.54% to 81.69±1.54% on the 120th day.

Key words: rats, bone, β-tricalcium phosphate, reparative osteogenesis.

Resumen

INFLUENCIA DEL FOSFATO β-TRICALCICO DE DIFERENTES FORMAS GEOMÉTRICAS EN LA MORFOLOGÍA DE LA REGENERACIÓN DEL DEFECTO EXPERIMENTAL DEL TEJIDO ÓSEO COMPACTO

Objetivo: Comparar el proceso de cicatrización de un defecto del tejido óseo compacto tras la implantación de materiales osteoplásticos a base de fosfato β-tricálcico («ChronOS™» and «Calc-i-oss®») que difieren según

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el fabricante en la forma geométrica y estructura microscópica. El estudio fue realizado en 48 ratas Wistar machos en los cuales se produjo, en el tercio medio de la diáfisis del fémur, un defecto perforado de 2,5 mm de diámetro, el cual fue llenado con el material «ChronOS™» (block, Synthes, Switzerland) en un grupo y con «Calc-i-oss®» (granules, «Degradable Solutions Dental», Switzerland) en el segundo grupo. El sector del defecto fue evaluado en los días 60 y 120 por microscopía óptica y por microscopía electrónica de barrido. Resultados: independientemente de la forma geométrica y la estructura microscópica, ambos materiales osteoplásticos mostraron alta biocompatibilidad, propiedades osteoconductivas y buena integración con el tejido óseo regenerado. La estructura microscópica del fosfato β-tricálcico («ChronOS™») puede afectar significativamente a la estructura microscópica del tejido óseo regenerado, que se manifiesta en su forma geométrica. Adicionalmente, se observó que ambos materiales osteoplásticos «ChronOS™» y «Calc-i-oss®» mostraron valores similares de resorción y reemplazo por tejido óseo, cuya relación al 60º día del experimento fue de 22,55±1,25 a 77,45±1,25 y 25,72±2,06% a 74,28±2,06%, y a los 120 días de 17,65±1,09 a 82,35±1,09 y de 18,31±1,54% a 81,69±1,54% respectivamente.

Palabras clave: ratas, hueso, fosfato β-tricálcico, osteogénesis.

Introduction

Because of the similarity of the chemical structure with the bone tissue and its biological inertness, β-tricalcium phosphate showed excellent characteristics for the replacement of bone defects in traumatology, spinal surgery and dentistry.[1-4] Today, β-tricalcium phosphate, which is produced in the form of granules, blocks or cylinders, is mentioned in numerous promotional publications, which proves its safety and biocompatibility. However, it should be noted that the majority of works devoted to the research of β-tricalcium phosphate were conducted on the bones of the skull and cancellous bones.[5,6,7] We have found no information in the scientific literature about the studies of the comparative influence of various by the manufacturer, geometric shape and microscopic structure β-tricalcium phosphate on the dynamics of healing of compact bone tissue defects. Moreover, the information about such determining properties of β-tricalcium phosphate as dynamics of the biodegradation rate and replacement by bone tissue of the regenerate for certain commercial drugs, is completely absent, whereas for other parameters there is a significant divergence in data (up to one year).[8,9,10] Moreover, these differences are probably influenced by many factors, such as size of defect, bone tissue regeneration potential, the characteristics of the osteoplastic material (manufacturer, geometric shape, total porosity, pore size, design, size, etc.).[11,12] All this indicates the need for a more predictable dynamics of rate of formation of the bone tissue of the regenerate and resorption of osteoplastic materials, which might differ by manufacturer, geometric shapes and microscopic structure, experimental models of bone defects. On the latter in standard conditions and using different research methods it is possible to study and compare with each other osteoplastic materials that optimize reparative osteogenesis. Therefore the aim of our study was to compare the process of healing of an experimental defect of compact bone tissue after the implantation of osteoplastic materials obtained from two different manufacturers.

Materials and methods

The experiment was performed on 48 8-month-old male white Wistar rats, with an av-

erage weight of 250±10 g. All procedures were in agreement with the Commission on Biomedical Ethics of Sumy State University (Minutes N° 4/14 of 06.11.2015). The study protocol was done according to the provisions "European Community Directive of 24 November 1986 on the maintenance and use of laboratory animals for research purposes". Before surgery, animals were injected with 2.5 mg/kg acepromazine intramuscular and 5 minutes later, with 75 mg/kg ketamine intramuscular (Calypsol, Gedeon Richter, Budapest-Hungary). After the induction of anesthesia, a 2.5 mm-diameter defect in the medullary canal was produced under aseptic conditions in the middle third of the femoral diaphysis using a portable drill with a spherical cutter at low speed with cooling. Further, the experimental animals were divided into 2 groups:

Group 1 (n=24) – where the defect without rigid fixation was filled with the osteoplastic material "ChronOS™" (Synthes, Switzerland), which is a pure β-tricalcium phosphate in the form of block with a total porosity of 70%, with macropore size from 100 to 500 μm and micropores to 10 μm (Figure 1).

Group 2 (n=24) – where the defect without rigid fixation was filled with the osteoplastic material "Calc-i-oss®" («Degradable Solutions Dental», Switzerland), which is a synthetic granular material (1-1.6 mm) made of pure β-tricalcium phosphate (β-phase purity of >99%, Ca / P - 1.5) with a total porosity of 50% and the size of micropores is from 1 to 6 μm (Figure 2).

Before the implantation the blocks of "ChronOS™" and the granules of "Calc-i-oss®" were moistened with the rat's own blood (which was taken from the tail vein) to fill pores, remove residual air from the material and ensure the necessary consistency, which would permit easy cutting of the materials by scalpel and thus modelling the shape of the defect.

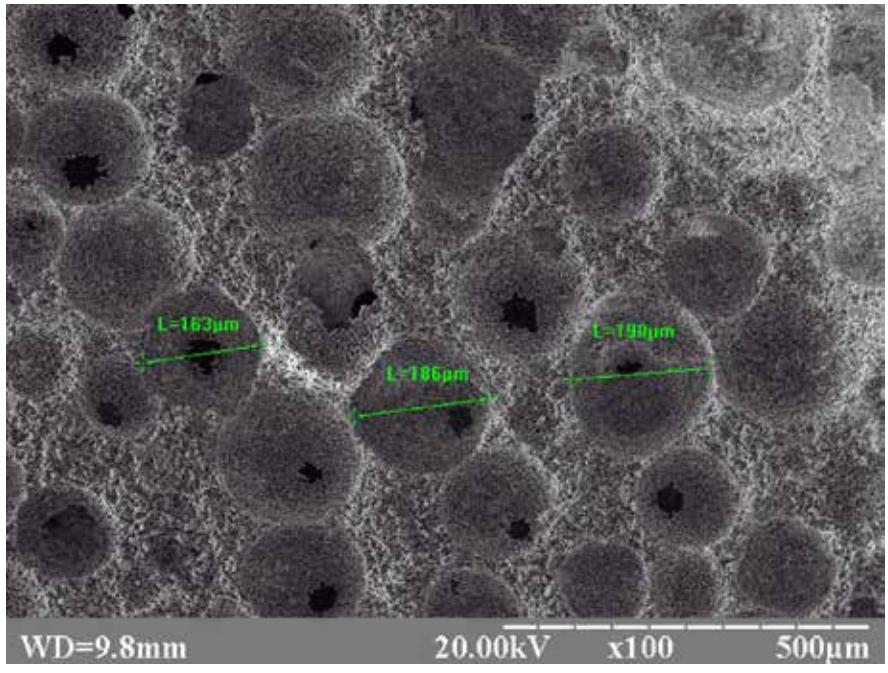

Figure 1. Microstructure of the osteoplastic material "ChronOS™". Visible numerous pores ranging in size from 163 μm to 190 μm. Electronic scanning image 100X.

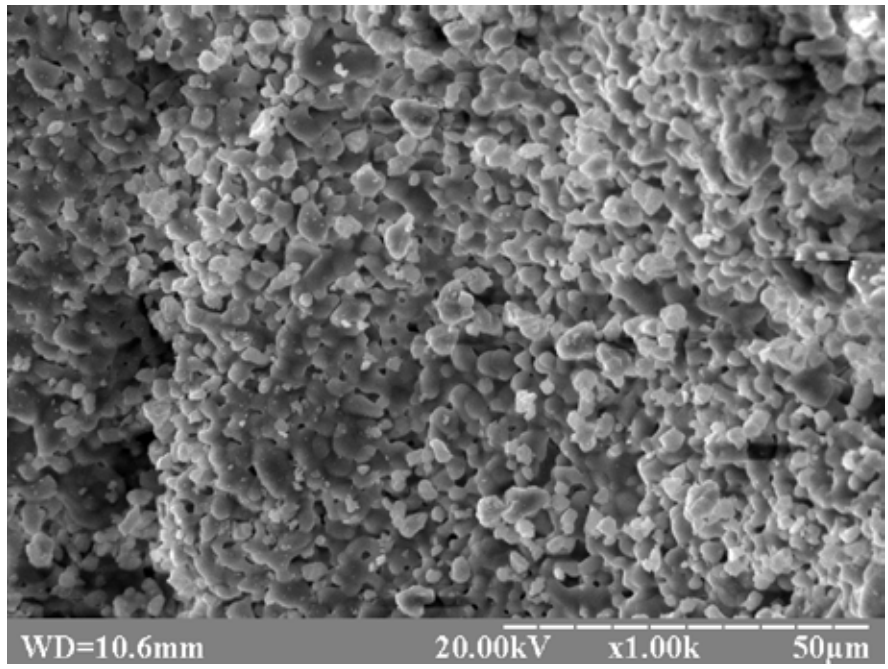

Figure 2. Microstructure of the osteoplastic material "Calc-i-oss®". Electronic scanning image 1000X.

After entering into the bone defect of osteoplastic material the wound was tightly stitched with silk thread through all layers of soft cover, the seam was treated with 3% alcohol iodine solution. Then, during the next 3 days after operation for prevention of septic complications the after-operation seam was treated with an alcohol iodine solution and for analgesia ketorolac (JSC "Synthesis", Kurgan, Russia) was injected intramuscularly at a dose of 0.6 mg twice a day.

Next on the 60th and 120th day after surgery animals were taken out of the experiment by decapitation under deep ether anesthesia, followed by a study of injured bones using light microscopy with morphometry and scanning electron microscopy.

For light microscopy, we extracted the fragments of femoral bones from the site of implantation of osteoplastic material and fixed them in 10% neutral buffered formalin. After washing with water, the bone samples were subjected to decalcification in 5% aqueous solution of Trilon B (Edetic acid), dehydrated in alcohols of increasing concentration and poured into paraffin. Histological sections were made using a Sannomiya microtome "Reichert", stained with hematoxylin-eosin, analyzed at the light microscope «Olympus» and photographed using a digital camera.[13]

Morphometric analysis consisted in identifying the area of bone tissue and remnants of osteoplastic material in the site of the defect, which was performed using the program for image processing "Video-Test" and "Video-Size".

For scanning electron microscopy we extracted the fragments of the femur from implanted osteoplastic material and placed samples in glutaraldehyde holder. Next day the samples were washed in phosphate buffer, fixed in 1% OsO_4 solution and dehydrated

in ethanol of increasing concentrations. Further, the bone fragments were glued to metal tables with electricity conductive adhesive, sprayed with carbon dust in standard vacuum installation of VUP-5 type and examined with an electron microscope "SEM 106-I".

Using light and scanning electron microscopy we established morphological characteristics of bone tissue, the nature of its interaction with the bone material "ChronOS™" and "Calc-i-oss®". In addition, we investigated the state of the structure of adjacent to the site of implantation maternal bone in order to establish or refute postoperative complications due to the presence or absence of signs of necrobiosis and necrosis of osteocytes.[14] The resulting digital values were analyzed statistically by calculating the arithmetic mean (M) and its standard error (m). The significance of differences between the indicators of the animals of the first and second groups, of the 60th and the 120th days was evaluated using Student t-test. The differences were considered significant at $p<0.05$.

Results

In the animals of the first group on the 60th day of the experiment there was located lamellar bone tissue throughout the area of the defect directly in the pockets and on the surface of the osteoplastic material "ChronOS™". The lamellar tissue was presented by separate specific rounded formations that by shape and pores size corresponded to "ChronOS™", were interconnected by "bridges" of bone tissue and contained a significant amount of osteoblasts and osteocytes (Figure 3).

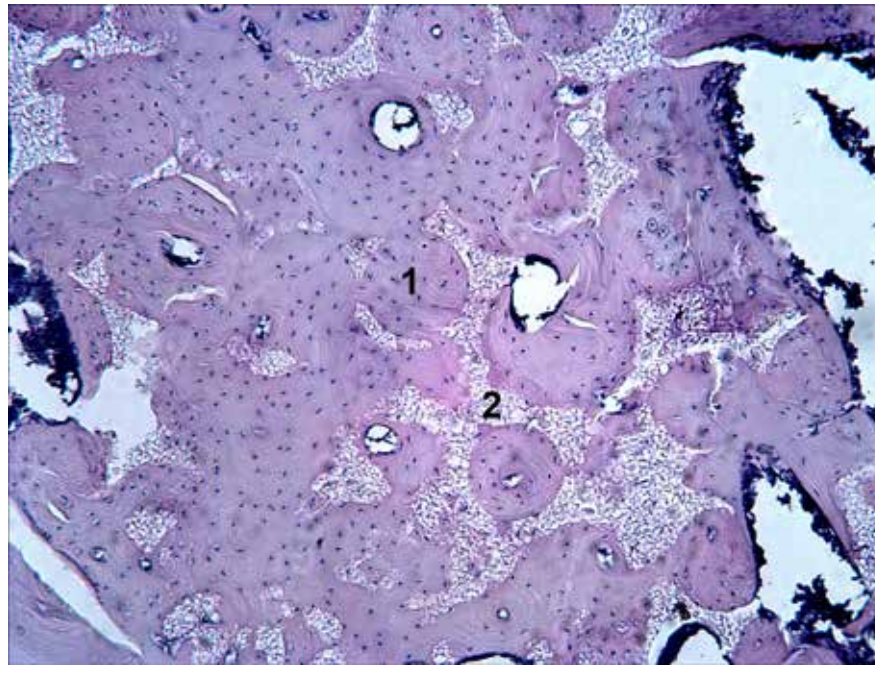

Figure 3. The area of the defect of femur of a rat on the 60th day after implantation of "ChronOS™". Bone tissue of the regenerate, constructed from individual particles of specific rounded shape (1), between which the remnants of osteoplastic material are located (2). Hematoxylin & Eosin 100X.

In the animals of the second group the area of the defect was also filled with lamellar bone tissue. Here, in the peripheral part of the defect the bone tissue lacked "Calc-i-oss®", was solid, and contained even elements of the marrow in the central part and between the individual parts with remnants of osteoplastic material, and in deeper cuts. In the animals of the first group between the separate parts of the bone tissue of the rounded form in the direct connection with them there were also found the remnants of the osteoplastic material, and in peripheral parts of the defect there were areas where the bone tissue was represented by solid margins and immured into its matrix remnants of the osteoplastic material. In the lacunae of bone tissue of the regenerate of the animals of the first and second groups there were well observed osteocytes and osteoblasts with spikes, and inside and on the outer surface of osteoplastic materials osteogenic cells, osteoclasts and sometimes small areas of osteogenic tissue can be seen (Figure 4).

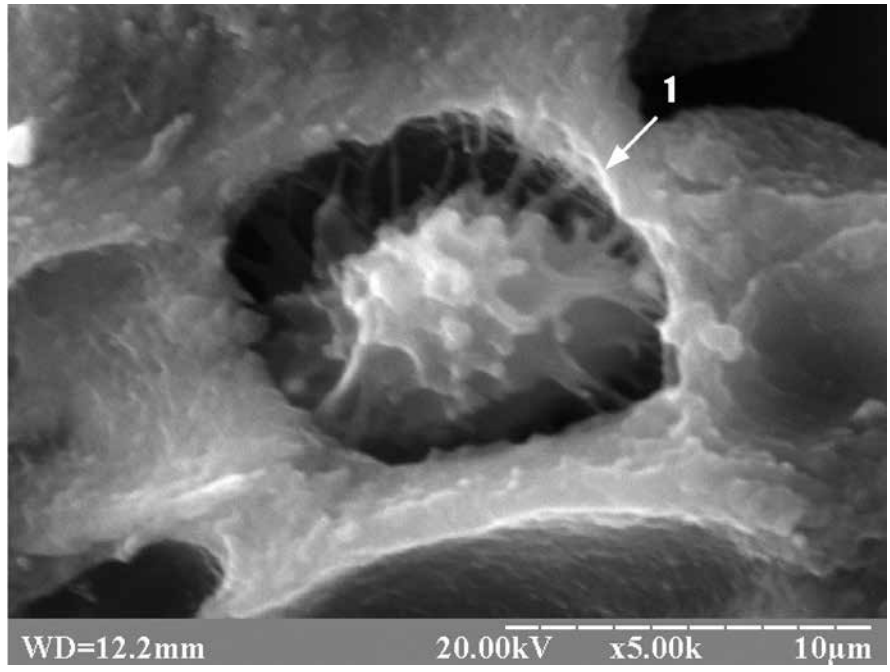

Figure 4. The area of the defect of femur of a rat on the 60th day after implantation of "Calc-i-oss®". Osteocytes (1) with spikes in the lacunae of lamellar bone tissue, which is formed on the surface of the osteoplastic material. Electronic scanning image 5000X.

The relative area of "ChronOS™" and "Calc-i-oss®" within two months of the experiment similarly decreased to 22.55±1.25% and 25.72±2.06% (p>0.05), and was replaced by the bone tissue the relative area of which was 77.45±1.25% in the first and 74.28±2.06% in the second case (p>0.05). In animals of both groups no signs of inflammation were detected in the area of the defect and the maternal bone was characterized by typical osteocytes, which were located in bone lacunae and had long spikes.

On the 120th day of the experiment in the animals of the second group, the area of the defect was filled with the bone tissue of the regenerate, which had lamellar structure and differed from the maternal bone only by the presence of remnants of the osteoplastic material integrated into its structure, and by the elements of the bone marrow (Figure 5).

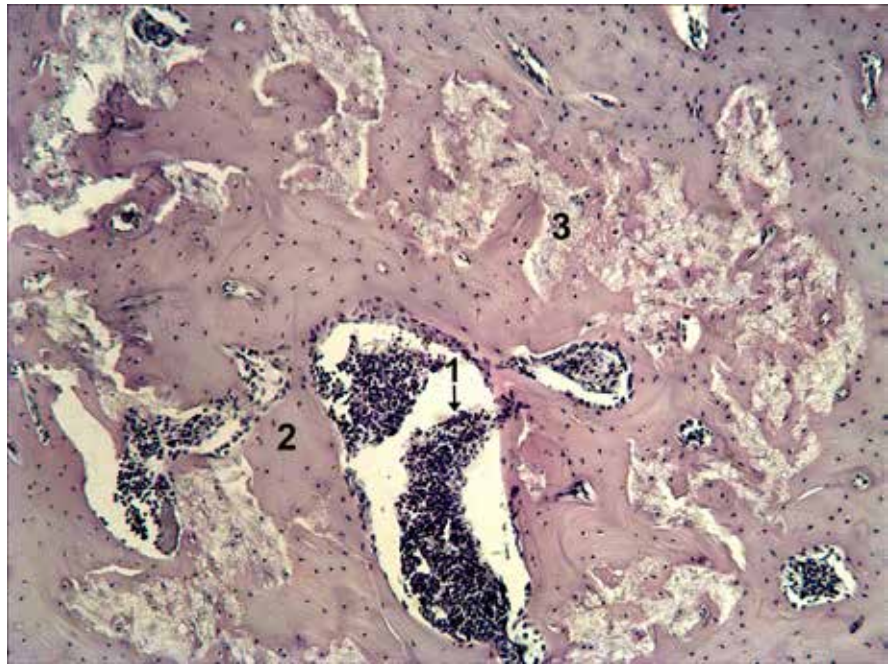

Figure 5. The area of the defect of femur of a rat on the 120th day after implantation of "Calc-i-oss®". Bone marrow (1) and lamellar bone tissue of the regenerate (2) with the integrated into its structures remnants of osteoplastic material (3). Hematoxylin & Eosin 100X.

In the animals of the first group, the area of the defect was also filled with lamellar bone tissue. The latter was formed directly on the surface and in the pores of the osteoplastic material "ChronOS™". Thanks to this, the bone tissue, as on the 60th day of the experiment, looked like separate parts of specific round shape (corresponding to the pores of the implant), which were closely in contact with each other and contained typical osteoblasts, osteocytes, and sometimes osteoclasts. Osteoblasts had an oval shape and numerous short spikes, which allow the interaction with each other and the differentiated osteocytes (Figure 6). Osteocytes, with a size of approximately 10-15 μm were located in the bone lacunae and had longer spikes than osteoblasts, and osteoclasts on the electronic scanning image had ellipsoid shape with the flattened basis with the size of approximately 100 μm. Furthermore, in the animals of the first group rounded areas of lamellar bone tissue merged together in continuous fields were seen, but in the thickness of their matrix the remnants of the osteoplastic material were still observed.

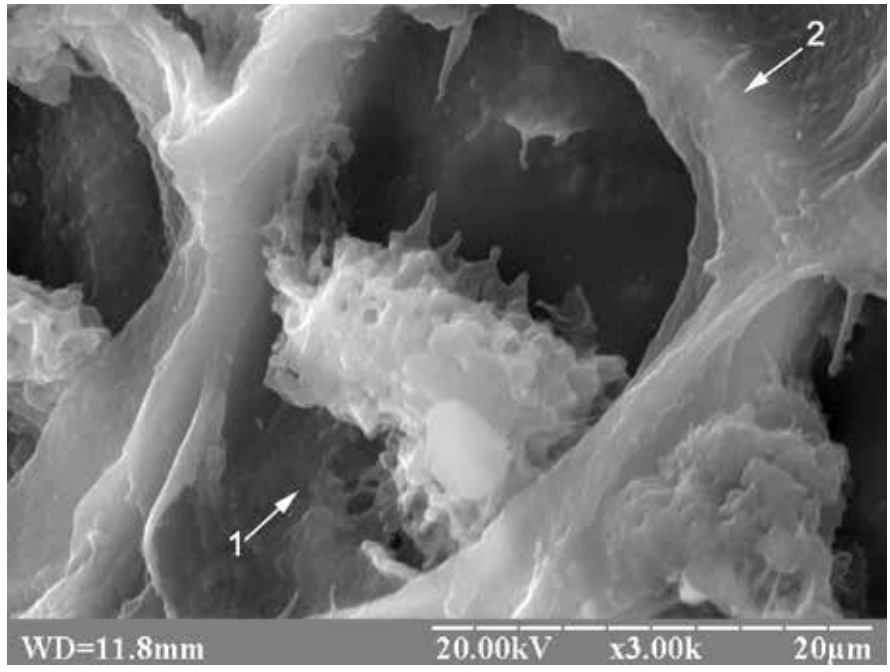

Figure 6. The area of the defect of femur of a rat on the 120th day after implantation of "ChronOS™". Osteoblast (1) in the lacunae of the bone tissue (2), which was formed in the pore of the osteoplastic material. Electronic scanning image 3000X.

The area of the bone tissue of the regenerate in the animals of the first and the second groups compared to the 60th day increased by 6.32% ($p<0.05$) and 9.97%, and the area of the osteoplastic material on the contrary, decreased by 21.73% ($p<0.05$) and 28.81% ($p<0.05$) and was 82.35±1.09 and 81.69±1.54% in the first and 17.65±1.09% and 18.31±1.54% in the second case. Inside and on the outer surface of the osteoplastic materials osteogenic cells, which formed foci of osteogenesis were found. In the area of implantation of "ChronOS™" and "Calc-i-oss®" no signs of inflammation were seen, and the maternal bone, as in the previous term of experiment, had a normal structure.

Discussion

The conducted study with the help of light and scanning electron microscopy showed that the dynamics of the healing of the defect of the femoral shaft diaphysis had both similarities and differences depending on origin of the implanted calcium phosphate osteoplastic material.

To common features we can refer those that osteoplastic materials "ChronOS™" and "Calc-i-oss®" during the whole period of the experiment demonstrated their high biocompatibility, as evidenced by the absence the inflammatory process in the site of the defect, of necrosis and necrobiosis of osteocytes in the composition of the maternal bone. These results are consistent with the data of most researchers.[8,9] Furthermore, in all the periods of observation high tropism of osteogenic cells to osteoplastic materials was found, as evidenced by their location and formation of foci of osteogenesis, both on the outer surface

and inside "ChronOS™" and "Calc-i-oss®". It should be noted that the established fact is also the evidence of good integration of bone tissue of the regenerate with osteoplastic materials and manifestation of their osteoconductive properties. This is connected with the fact that one of the definitions of osteoconduction is the ability of osteogenic cells to use osteoplastic material as a platform for attaching and generating on the surface and in its cavities of the new bone tissue.[15]

One of the most important properties of calcium phosphate osteoplastic materials is their ability to be resorbed and replaced by the bone tissue of the regenerate. In the literature there are works on the impact of β-tricalcium phosphate on healing of the bone defects. But such studies were conducted on the bones of the skull and cancellous bone, and the results of these studies either lack the data of morphometric parameters or have significant divergence. So, Jensen SC, et al established almost complete disappearance of β-tricalcium phosphate granules from mandibular angle defect of mini-pigs on the 60th day, where their number remained 2.5% (from 0 to 5.1%), and on the 180th day 0.8% (from 0 to 2.5%).[16] At the same time Gotterbarm T. et al found 4.35% of remnants of β-tricalcium phosphate granules even in a year after their implantation with collagen into the defect of epiphysis of the tubular bone of mini pigs.[17] Osteoplastic material "ChronOS™", according to the advertising information can completely undergo resorption. However, the period for which this occurs, is from 6 to 18 months. In addition, the research of T. Stoll have shown that if you implant "ChronOS™" with bone marrow into the defect of the tibia of sheep, in 6 weeks its balance would amount 43.10%, and if with blood - 53.10%. Here, the area of the defect was filled with bone (16.50% and 4.10%) and connective tissue (40.40 and 42.80%).[12]

During conducting the research of compact bone tissue defect we also observed gradual resorption of implanted material in the cavity containing the osteoplastic materials "ChronOS™" and "Calc-i-oss®" and their replacement by bone tissue of the regenerate. However, in our experiment, the ratio of the amount of the implant and the bone tissue of the regenerate on the 60th day of the experiment was 22.55±1.25% to 77.45±1.25% in the area of implantation of "ChronOS™" and 25.72±2.06% to 74.28±2.06% in the area of implantation of "Calc-i-oss®", and on the 120th day 17.65±1.09% to 82.35±1.09% and 18.31±1.54% to 81.69±1.54%, respectively. And here, the given quantitative indicators had no authentic difference. Although, on the 60th day of the experiment a slight predominance in the rate of resorption of the osteoplastic material (by 14.05%, $p>0.05$) and formation of the bone tissue of the regenerate (by 4.09%, $p>0.05$) was found in the area of implantation of "ChronOS™". On the 120th day of the experiment none of osteoplastic materials underwent complete resorption, though all of their remnants were fully integrated into lamellar bone tissue of the regenerate. In this case, the area that was occupied by "ChronOS™" (17.65±1.09%) and "Calc-i-oss®» (18.31±1.54%) and bone tissue of the regenerate (82.35±1.09 and 81.69±1.54%) in the animals of both groups was almost the same.

A vivid distinction between the studied osteoplastic materials became the geometric shape of the bone tissue of the regenerate, which was formed in the area of implantation of "ChronOS™" and "Calc-i-oss®". Thus, in the area of implantation of "Calc-i-oss®" the bone tissue had no significant features and differed from the maternal bone only by the presence of integrated into its structures remnants of the osteoplastic material. Unlike "Calc-i-oss®", in the area of implantation of "ChronOS™" the geometric shape of the bone tissue had a fundamental difference. In all periods of surveillance the bone tissue of the regenerate was mainly represented by specific individual and interrelated rounded

formations, which in shape and size were direct reflections of the pockets of osteoplastic material "ChronOS™". This fact indicates that the pores of the implant served as the conductor for the vessels, cellular elements and bone tissue, which in turn is the proof of the osteoconductive impact of the osteoplastic material "ChronOS™" on the reparative osteogenesis. Here, it should be noted that the described and vividly demonstrated structure of the cells of the bone tissue did not depend on which of the calcium-phosphate osteoplastic material was implanted into the defect of the femoral shaft diaphysis.

Conclusion

Regardless of the geometric shape and microscopic structure of β-tricalcium phosphate the osteoplastic materials based on it ("ChronOS™" and "Calc-i-oss®") exhibit high biocompatibility, osteoconductive properties, good integration with bone tissue of the regenerate and almost the same speed of resorption and replacement by the bone tissue. However, the microscopic structure of β-tricalcium phosphate ("ChronOS™") might significantly affect the microscopic structure of bone tissue of the regenerate, which manifests itself in the specificity of its geometric shape.

Conflict of interests The author declares no conflicts interest.

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