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ARTÍCULOS ORIGINALES / *Originals*

MORPHOLOGICAL FEATURES OF HEALING OF EXPERIMENTAL DEFECT OF LONG BONES DIAPHYSIS UNDER THE CONDITIONS OF IMPLANTATION OF BIPHASIC OSTEOPLASTIC MATERIAL

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Abstrat

Purpose: to study the healing process of a defect of the compact bone tissue after the implantation of osteoplastic material «easy-graft CRYSTAL». Methods: the experiment was conducted on 24 Wistar rats. In the middle third of the diaphysis of the femur we created a perforated defect of 2.5 mm diameter in the medullary canal, which was filled with osteoplastic material «easy-graft CRYSTAL». Fragments of the injured bones were studied on the 60th and 120th days by light microscopy with morphometry and scanning electron microscopy. Results: it was found that in the area of the defect of the compact bone tissue «easy-graft CRYSTAL» shows high biocompatibility, osteoconductive properties and provides stability to the volume of the defect due to good integration with the bone tissue of regenerate and absence of signs of resorption of osteoplastic material throughout the period of experiment.

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

Resumen

CARACTERÍSTICAS MORFOLÓGICAS DEL PROCESO DE REPARACIÓN DE UN DEFECTO EXPERIMENTAL EN DIÁFISIS DE HUESOS LARGOS POSTIMPLANTACIÓN DE MATERIAL BIFÁSICO OSTEOPLÁSTICO

Objetivo: estudiar el proceso de reparación de un defecto del tejido óseo compacto después de la implantación de material osteoplástico (easy-graft CRYSTAL). Métodos: el experimento se realizó en 24 ratas Wistar. Se realizó en el tercio medio de la diáfisis del fémur un defecto de 2,5 mm de diámetro en el canal medular que se rellenó de material de osteoplástico (easy-graft CRYSTAL). Los huesos lesionados fueron estudiados en los días 60ª y 120ª mediante microscopía óptica y microscopía electrónica de barrido. Resultados: se encontró que en la zona del defecto del tejido óseo compacto el injerto mostró alta biocompatibilidad y propiedades osteoconductivas y proporcionó estabilidad al volumen del defecto debido a la buena integración con

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el tejido óseo y ausencia de signos de resorción de material osteoplástico durante todo el período experimental.
Palabras clave: ratas, hueso, hidroxiapatita, fosfato tricálcico, reparación.

Introduction

One of the most important problems that orthopaedic physicians face in their practice is bone defects regeneration. The high frequency of occurrence of bone defects dictate the need to find tools that would ensure their full recovery. Transplantation of bone tissue, which is used to treat this condition has a long history and has achieved a considerable success. However, despite this, the used auto- and xenografts still have serious drawbacks. Thus, xenogeneic material has strong antigenic activity, and the restructuring process is much slower compared to other osteoplastic materials. In addition, when bone tissue of animal origins are used, there is a risk of transmission of infectious diseases. In turn, the use of bone autografts is greatly limited in the medical practice because of the additional injuries, failure to use them in case of large bone defects, prolongation of operation time, the possibility of complications (fractures in the donor site, nerve damage, cosmetic defects, and prolapse of bone defects), the rejection of the implant and preservation of the autogenous plastic material. As a result, in recent years there has been a growing interest in calcium phosphate osteoplastic materials to replace bone tissue in the clinical practice. The similarity of their chemical structure with that of bone tissue and inertness to biological tissues makes possible to widely use them to replace the lost bone tissue.[1] In addition, synthetic nature of calcium phosphate material guarantees safety, preventing the risk of infection, and clinical trials consistently demonstrate the exceptional biocompatibility of the materials.[2]

It is known that tricalcium phosphate and hydroxyapatite, which have different resorption in the area of implantation, are most commonly used to replace bone defects.[3] Physicians take into consideration this ability when using implants in each clinical situtation. Thanks to the fact that tricalcium phosphate undergoes resorption faster than hydroxyapatite, it is possible to combine them in a biphasic osteoplastic medication in order to regulate the process of resorption in the area of implantation through the changes of the ratio of its components.[4] Today it is believed that the optimal ratio of hydroxyapatite and tricalcium phosphate as part of biphasic osteoplastic material is 60% to 40%.[5] One of these relatively new substances is «easy-graft CRYSTAL», which was developed in Switzerland by the company Degradable Solutions AG. With the help of the study of β-tricalcium phosphate phase of the «easy-graft» products, it was found that after its implantation into the bone defect it undergoes resorption and replacement by the bone tissue within 6-7 months.[6] At the same time advertising information and scientific and clinical studies indicate that biphasic osteoplastic «easy-graft CRYSTAL» preparation undergoes a partial resorption and mainly due to β-tricalcium phosphate and hydroxyapatite, thus remaining in the area of the defect for a longer period, ensuring prevention of atrophy and preservation of bone tissue volume.[7] However, these conclusions were made from histological studies on the jaws, flat and spongy bones, but there is no information on the impact of «easy-graft CRYSTAL» on healing of compact bone tissue defect.[8] Thus, the purpose of our work was to study the healing process of the experimental defect of the compact bone tissue after the implantation of osteoplastic material «easy-graft CRYSTAL».

Materials and methods

The experiment was performed on 24 white Wistar rats eight months of age with an average weight of 250±10 g. All procedures were accorting to the Commission on

Biomedical Ethics of Sumy State University (Minutes N° 4/13 of 05.18.2015). The study protocol was 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 0.6 mg of acepromazine (2.5 mg per 1 kg body weight of rat), and in 5 minutes 18 mg of ketamine (75 mg per 1 kg of rat weight). After the introduction of anesthesia under aseptic conditions we produced a defect to the medullary canal of 2.5 mm diameter, without rigid fixation, in the middle third of the femoral shaft using a portable drill with a spherical cutter at low speed with cooling. The defect was filled with the osteoplastic material «easy-graft CRYSTAL» (Degradable Solutions AG, Switzerland registration No 2008/03310). The preparation was made in a syringe with granules and a vial with an organic solvent BioLinker®. The granules consist of 40% β-tricalcium phosphate and 60% hydroxyapatite, and BioLinker® – water and N-methyl-2-pyrrolidone (NMP). Each granule of the material (Figure 1) was covered with thin (10 μm) polymer of polylactic and polyglycolic acid (PLGA), which is capable of resorption.[3,5]

Before the injection of the material into the defect the granules were soaked in the BioLinker® solution, which temporarily softened the outer shell of granules and provided their bonding. In this case the material gained a lamellar mass consistency, which was easy to model in the shape of the defect. After injecting the material into the bone defect, it was soaked with blood, which ensured the removal of BioLinker®, and the material began to harden within minutes. Thus, a stable porous mass with microcells inside the granules the size from 1 to 10 μm and macrocells between separate granules was formed.

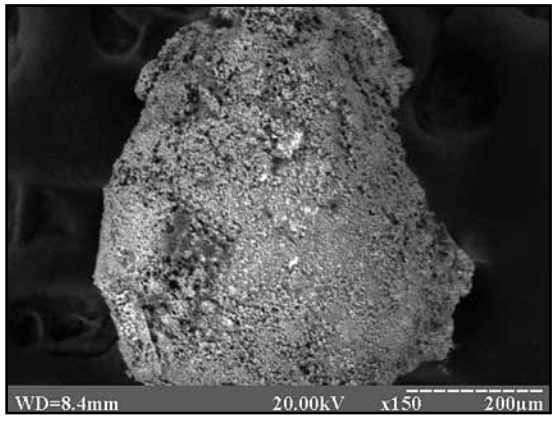

Figure. 1. Granule of osteoplastic material «easy-graft CRYSTAL». Electronic scanning image. 150X.

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 solution of iodine for prevention of septic complications. Ketorolac was injected intramuscularly at a dose of 0.6 mg two times a day for analgesia for 3 days after operation.

Sixty and 120 days after surgery, the animals were taken out of the experiment by decapitation under deep ether anesthesia with further analysis of the injured bones by light (with morphometry) and scanning electron microscopy, which was performed on the electron microscope "REM 106-I". Histological cuts stained with hematoxylin and eosin, were analyzed in light microscope Olympus, and photographed with a digital camera. Morphometric analysis was performed using image processing program "Video-Test" and "Video-Size": we determined in the area of the defect the relative percentage of bone tissue and remnants of osteoplastic material as the ratio of the area of these components (%) to the total area of the site of the defect (100%). In addition, we investigated the structure of the tissue adjacent to the site of implantation in order to establish or refute postoperative complications due to the presence or absence of signs of necrobiosis and necrosis of osteocytes. The resulting digital values were processed statistically calculating the mean value (M) and standard error (m). The significance of differences

between the indicators of the 60th and the 120th days was evaluated using Student t-test with the use of statistical computer program MS Excel XP. The differences were considered significant if $p<0.05$.

Results

On the 60th day of the experiment, the cavity of the defect was filled with bone tissue of the regenerate, which occupied 66.81±1.84% of the whole area of the defect. The latter had lamellar structure and contained a significant number of osteocytes and osteoblasts. The rest of the area of the defect (33.19±1.84%) was occupied by large and small fragments of osteoplastic material, which were fully integrated into the bone tissue of the regenerate. Inside of the remnants of osteoplastic material there were found osteogenic cells with small foci of osteogenesis. The original bone adjacent to the implantation site was characterized by the presence of typical osteocytes.

On the 120th day of the experiment the area of the defect was filled with the bone tissue of the regenerate with remnants of osteoplastic material integrated in its structure. However, compared with the 60th day, the area of the osteoplastic material decreased on 14.52% ($p>0.05$), and the area of the bone tissue of the regenerate on the contrary increased on 7.19% ($p>0.05$) and was 28.37±1.61% in the first and 71.62±1.61% in the second case, the differences did not reach significance. The bone tissue of the regenerate had mature lamellar structure and was characterized by the presence of the formed osteons, osteoblasts and osteocytes. That is, during this period of observation the bone tissue of the regenerate in its structure was approaching to the original bone. In the structure of the latter there were typical osteocytes with the long appendages. Osteoplastic material was represented in the vast majority by large granules of round shape, and there was also found a small amount of small fragments. Inside the granules of osteoplastic material we found osteogenic cells, foci of osteogenesis, and the elements of bone marrow.

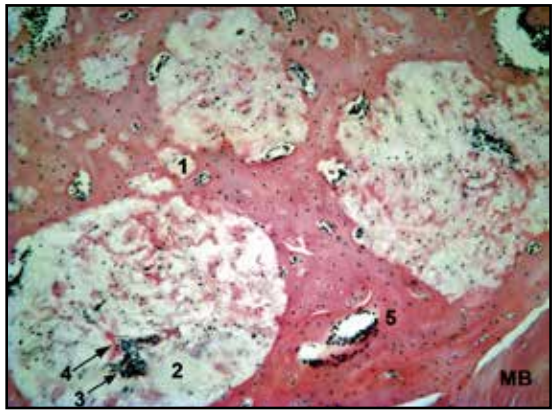

Figure 2. The area of the defect of femur of a rat on the 60th day after the implantation of «easy-graft CRYSTAL». Small fragments (1) and large granules (2) of «easy-graft CRYSTAL» with osteogenic cells (3) and foci of osteogenesis (4), which are integrated into the lamellar bone tissue of the regenerate (5). Adjacent to the site of implantation maternal bone (MB). Haematoxylin & Eosin staining 100X.

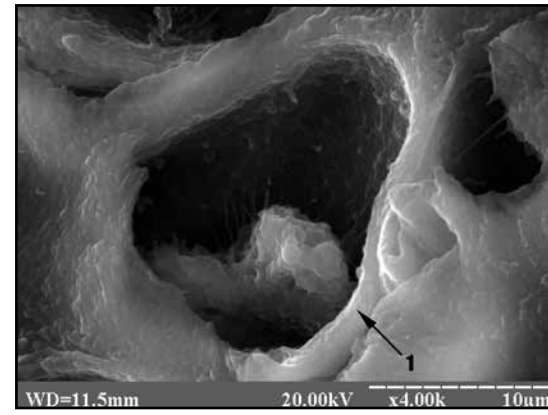

Figure 3. The area of the defect of femur of a rat on the 60th day after the implantation of «easy-graft CRYSTAL». Osteocytes (1) in the lacunae of bone tissue of the regenerate, which is formed directly on the surface of osteoplastic material. Electronic scanning image 4000X.

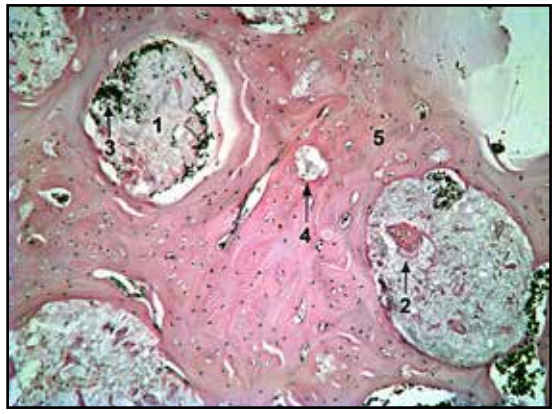

Figure 4. The area of the defect of femur of a rat on the 120th day after the implantation of «easy-graft CRYSTAL». Large granules of osteoplastic material (1) with foci of osteogenesis (2), bone marrow elements (3) and its small fragments (4), which are integrated into lamellar bone tissue of the regenerate (5). Haematoxylin & eosin 100X.

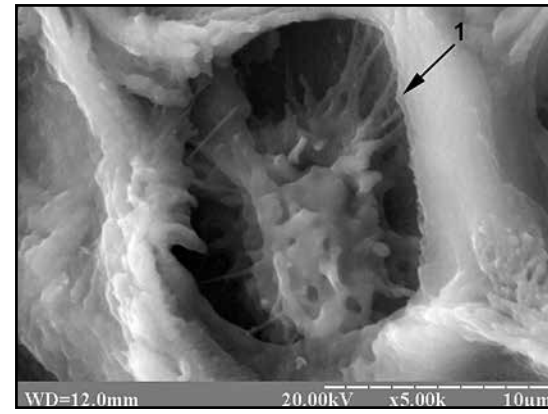

Figure 5. The area of the defect of femur of a rat on the 120th day after the implantation of «easy-graft CRYSTAL». Osteocyte (1) in a lacunae of a newly-formed lamellar bone tissue, which surrounds the remnants of osteoplastic material. Electronic scanning image 5000X.

Discussion

Using microscopic techniques the study has found that the osteoplastic material «easy-graft CRYSTAL» is characterized by high biocompatibility, as evidenced by the absence through the entire duration of the experiment of an inflammatory process in the area of the defect, necrosis, or necrobiosis of osteocytes in the area adjacent to the implantation site of the original bone. These results are consistent with the data of most researchers.[5,6,9] Osteogenic cells showed high tropism for osteoplastic material, as evidenced by their location and formation of small foci of osteogenesis inside the granules of «easy-graft CRYSTAL». The formation and maturation of bone tissue of the regenerate happened directly on the surface of «easy-graft CRYSTAL», indicating the osteoconductive properties of osteoplastic material and its good integration with bone tissue of the regenerate. Schmidlin et al. in their study also observed in the defect of the frontal and parietal bones of rabbits neoplasm of bone tissue of regenerate directly on the surface of the granules of «easy-graft CRYSTAL». However, the percentage of the bone tissue on the 60th and 120th day after the implantation of «easy-graft CRYSTAL» was relatively small (20.16±5.27% and 22.40±5.54%) and most importantly, there was no significant difference between them.[9] According to the authors, one of the factors that could affect this is the low rate of resorption of osteoplastic material. The latter, according to Lindgren et al, may be due to the fact that the biphasic osteoplastic material undergoes resorption through the ß-tricalcium phosphate phase of the drug, whereas hydroxyapatite for a long time does not resolve and keeps its occupied area.[10] In the defect of the compact bone tissue we also observed a slow process of resorption of «easy-graft CRYSTAL» that was evidenced by non significant difference of their areas of osteoplastic material and bone tissue of the regenerate between the 60th (33.19±1.84%

to 66.81±1.84%) and 120th (28.37±1.61% to 71.62±1.61%) day of the experiment. Nevertheless, the bone tissue of the regenerate, which replaced the osteoplastic material, was mature, had lamellar structure, and differed from the maternal bone only by the presence of integrated in its structure remnants of osteoplastic material.

Conclusion

The osteoplastic material «easy-graft CRYSTAL» in the area of the defect of the compact bone tissue shows high biocompatibility and osteoconductive properties and provides stability to the volume of the defect due to good integration with the bone tissue of the regenerate and absence of reliable signs of resorption throughout the period of the experiment.

Conflict of interests The author declare no conflicts interest.

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