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Medical Sciences

Regeneration of the Diaphysis of a Long Bone of the Skeleton after the Implantation into its Defect of Osteoplastic Material «Easy-Graft CRYSTAL»

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ABSTRACT. The aim of this work is the microscopic study of the dynamics of the healing of the defect of compact bone tissue after the implantation into its cavity of osteoplastic material «easy-graft CRYSTAL». The experiment was conducted on 24 Wistar rats. In the middle third of the femoral shaft of rats there was reproduced perforated defect in the diameter of 2.5 mm to the medullary canal, which was filled with osteoplastic material «easy-graft CRYSTAL». Fragments of the injured bones were examined on the 15th and 30th day by light microscopy with morphometry and scanning electron microscopy. It was found that the osteoplastic material «easy-graft CRYSTAL» in the area of the compact bone tissue defect shows high biocompatibility, osteoconductive properties and provides stability of the defect volume due to good integration with tissue-specific structures of the regenerate and the absence of reliable signs of resorption throughout the period of the experiment. *© 2016 Bull. Georg. Natl. Acad. Sci.*

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

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 combat this pathology 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, restructuring process is much slower compared to other osteoplastic materials. In addition, when bone tissue of animal originis 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, the possibility of complications (fractures in the donor site, nerve damage, cosmetic defects, prolapse of bone defects), the inability of the workpiece and preservation of 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 in practice preventing the risk of infection, and clinical trials consistently demonstrate exceptional biocompatibility the materials [2].

It is known that to replace bone defects tricalcium phosphate and hydroxyapatite are most commonly used, which have different resorption in the area of implantation [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» producti, it was found that after its implantation into the bone defect it undergoes resorption and replacement by the bone tissue during 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]. Therefore, the aim of our study was to investigate the process of healing of the defect of compact bone tissue after the implantation of osteoplastic material «easy-graft CRYSTAL» using histological, morphometric and electron microscopic methods.

Materials and Methods

The experiment was performed on 24 white Wistar rats eight months of age with a mass of 250±10 g. All procedures were agreed with the Commission on Biomedical Ethics of Sumy State University (Minutes №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 initially 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 the animals in anesthesia under aseptic conditions in the middle third of the femoral shaft using a portable drill with a spherical cutter at low speed with cooling we reproduced the defect to the medullary canal with the diameter of 2.5 mm, without rigid fixation we filled it with the osteoplastic material «easy-graft CRYSTAL» (Degradable Solutions AG, Switzerland registration No 2008/03310). The preparation is produced in the syringe with granules and a vial with an organic solvent BioLinker®.

The granules consist of 40% β-tricalcium phosphate and 60% hydroxyapatite, and BioLinker® – of water and N-methyl-2-pyrrolidone (NMP). Each granule of the material (Fig. 1) is 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® liquid, 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

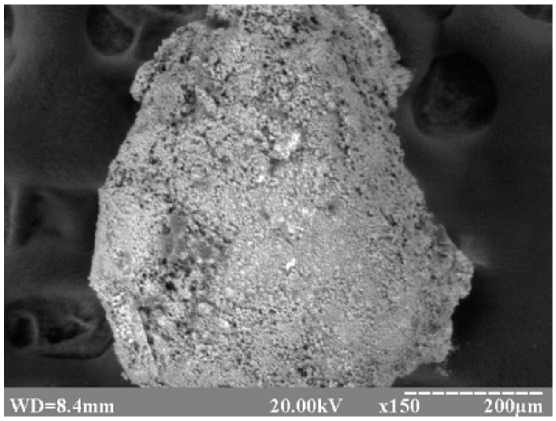

Fig. 1. The granule of osteoplastic material «easy-graft CRYSTAL». Electronic scanning image. X 150.

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.

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. Then, during the next 3 days after operation for prevention of septic complications the after-operation seam was treated with an alcohol solution of iodine, and for analgesia ketorolac was injected intramuscularly at a dose of 0.6 mg 2 times a day.

After 15th and 30th days after surgery the animals were taken out of the experiment by decapitation under deep ether anesthesia with further research of the injured bones by the methods of 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», 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, connective 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 state of the structure of the adjacent to the site of implantation maternal bone in order to establish or refute postoperative complications due to the presence or absence in it 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 comparable indicators was evaluated using Student t-test with the use of statistical computer program MS Excel XP. The differences were considered significant at $p<0.05$.

Results

On the 15th day of the experiment in the area of the defect there were detected solid rounded granules of «easy-graft CRYSTAL» and their small fragments that were integrated into the bone and connective tissue of the regenerate. On the outer surface and inside the granules of «easy-graft CRYSTAL» there was a significant amount of osteogenic cells which formed foci of fibro- and osteogenesis. There were places where between the granules of «easy-graft CRYSTAL» and bone tissue there was located a thin layer of connective tissue as well as places of direct contact of bone tissue with the osteoplastic material (Fig. 2). Bone tissue of the regenerate bone was represented by bone gullies containing in its structure a significant number of osteoblasts, osteocytes and integrated fragments of osteoplastic material (Fig. 3). Bone gullies formed small- and large-loop mesh structures with a total area of 22.92 ± 1.67%. In the inter-gully spaces of the bone tissue there were found the remnants of osteoplastic material and the connective tissue of the regenerate, which occupied 39.23±1.45 % and 37.85±1.52 % of the total area of the defect. Connective tissue was constructed from fibroblasts, collagen fibers, blood vessels and contained in its structure integrated small fragments of osteoplastic material. The maternal bone adjacent to the implantation site of «easy-graft CRYSTAL»

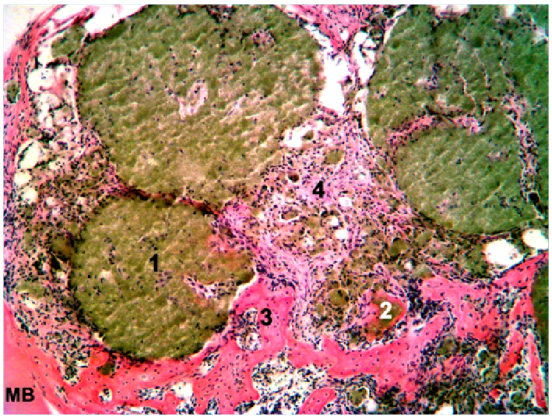

Fig. 2. The area of the defect of the femur of a rat on the 15th day after the implantation of «easy-graft CRYSTAL». The solid granules of osteoplastic material (1) and their small fragments (2) integrated into the bone (3) and connective (4) tissues of the regenerate. Adjacent to the site of implantation maternal bone (MB). Haematoxylin and Eosin staining. X 100.

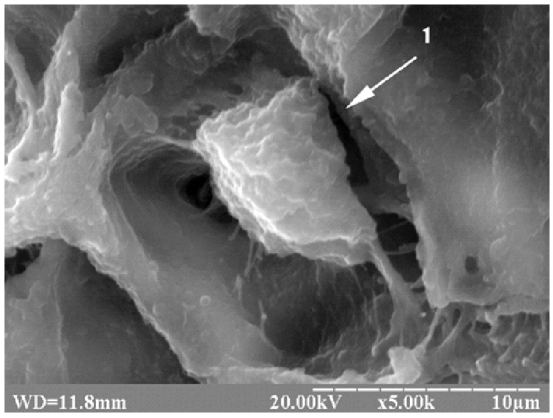

Fig. 3. The area of the defect of the femur of a rat on the 15th day after the implantation of «easy-graft CRYSTAL». Osteoblasts (1) in the newly formed roughly-fibrous bone tissue, which is located directly on the surface of osteoplastic material. Electronic scanning image. X 5000.

was characterized by the presence of typical osteocytes in its composition.

On the 30th day of the experiment in the area of the defect there still remained solid granules and small fragments of osteoplastic material, which were integrated mainly into bone and connective tissue of the regenerate. Bone tissue had rough-fibrous nature, and its gullies with a significant number of primary osteoblasts, osteocytes were formed directly on the surface of granules of «easy-graft CRYSTAL» and integrated their small fragments into own structures. The amount of bone tissue compared with the 15th day of the experiment increased by 49.43% ($p < 0.05$) and mounted to 34.25±1.64% of the total area of the defect. Inside the inter-gully spaces of the bone tissue and directly on the surface of some sections of the granules of osteoplastic material there was located connective tissue. The latter consisted of fibroblasts,

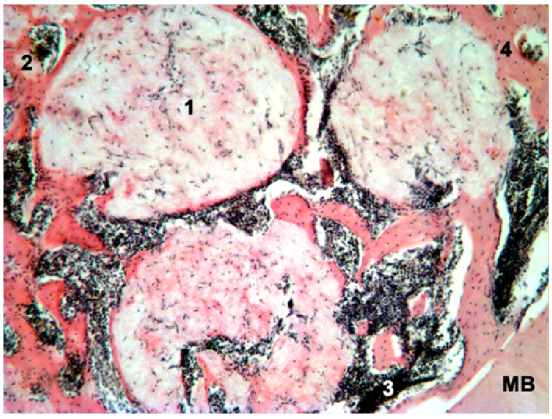

Fig. 4. The area of the defect of the femur of a rat on the 30th day after the implantation of «easy-graft CRYSTAL». The solid granules of osteoplastic material (1) and their small fragments (2) integrated into the bone (3) and connective (4) tissues of the regenerate. Adjacent to the site of implantation maternal bone (MB). Haematoxylin and Eosin staining. X 100.

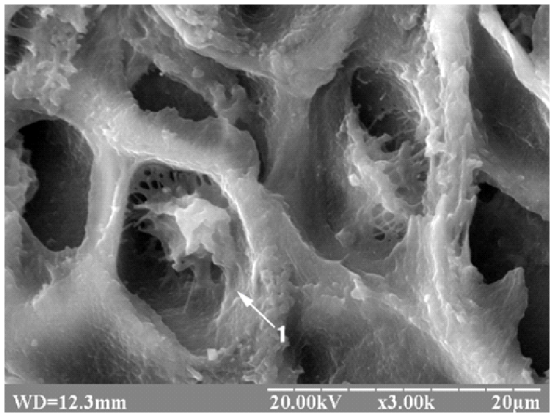

Fig. 5. The area of the defect of the femur of a rat on the 30th day after the implantation of «easy-graft CRYSTAL». Osteocytes (1) in the newly formed bone tissue, which is located directly on the surface of osteoplastic material. Electronic scanning image. X 3000.

collagen fibers, blood vessels and covered 30.51±1.63% of the total area of the defect, which was at 19.39% ($p < 0.05$) less than in the previous period of observation.

The osteoplastic material in the area of the defect was mainly represented by the solid granules of the round shape, inside of which there were determined osteogenic cells with foci of fibro- and osteogenesis. The area of the implanted material compared to the 15th day of the experiment decreased by 10.19% ($p > 0.05$) and was 35.23±1.49%. The adjacent to the area of implantation of «easy-graft CRYSTAL» maternal bone, as in the previous period of the experiment, was characterized by the presence in its composition of typical osteocytes with appendixes.

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 the inflammatory process in the area of the defect and necrosis, necrobiosis of osteocytes in the area adjacent to the implantation site of the maternal bone. These results are consistent with the data of most researchers [5, 6, 9]. In addition, osteogenic cells showed high tropism for osteoplastic material, as evidenced by their location both on the surface and inside the granules of «easy-graft CRYSTAL». On the surface of osteoplastic material between its separate parts and inside the granules of «easy-graft CRYSTAL» osteogenic cells formed foci of fibro- and osteogenesis, indicating osteoconductive properties of osteoplastic material, its good integration with tissue-specific structures of regenerate and the presence in the area of implantation of signs of only desmal osteogenesis. Schmidlin P.R. 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 4th and 16th week 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 [10], 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. In our experiment, «easy-graft CRYSTAL» was also subjected to resorption very slowly as evidenced by the predominance in the defect of diaphysis of the femoral shaft of granules of solid character and not significant difference of their area between the 15th (39.23±1.45%) and 30th (35.23±1.49%) day of the experiment. However, the amount of the bone tissue of the regenerate on the 30th (34.25±1.64%) day increased significantly compared with the 15th (22.92±1.67%) day of the experiment. This was possible due to the reorganization of the connective tissue of regenerate and insignificant resorption of osteoplastic material.

Conclusion

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

სამედიცინო მეცნიერებანი

ჩონჩხის გრძელი ძვლის დიაფიზის რეგენერაცია მის დაზიანებულ ნაწილში ოსტეოპლასტიკური მასალის "Easy-Graft CRYSTAL"-ის იმპლანტაციის შემდეგ

ა. კორენკოვი

სუმის სახელმწიფო უნივერსიტეტი, ადამიანის ანატომიის დეპარტამენტი, სუმი, უკრაინა

(წარმოდგენილია აკადემიის წევრის ფრიდონ თოდუას მიერ)

წინამდებარე ნაშრომის მიზანია კომპაქტური ძვლოვანი ქსოვილის აღდგენის მიკროსკოპული შესწავლა ძვლოვან დეფექტში ოსტეოპლასტიკური მასალის "Easy-Graft CRYSTAL"-ის ჩანერგვის შემდეგ. კვლევა ჩატარდა ვისტარის 24 ვირთაგვაზე. ვირთაგვების ბარძაყის ძვლის შუა მესამედში წარმოიქმნა 2,5 მმ დიამეტრის პერფორირებული დეფექტი ხერხემლის არხისკენ, რომელიც ივსებოდა ოსტეოპლასტიკური მასალით "Easy-Graft CRYSTAL". დაზიანებული ძვლების ფრაგმენტები შემოწმდა მე-15 და 30-ე დღეს სინათლის მიკროსკოპის მორფომეტრიით და სკანირებადი ელექტრონული მიკროსკოპით. აღმოჩნდა, რომ ოსტეოპლასტიკურმა მასალამ "Easy-Graft CRYSTAL"-მა აჩვენა მაღალი ბიოთავსებადობა, ოსტეოკონდუქტიურობის თვისებები და დეფექტის ქსოვილოვან სპეციფიკურ რეგენერირებად სტრუქტურებს შორის ზონაში სტაბილურობა. ასევე დაკვირვების განმავლობაში გამოირიცხა რეზორბციის ნიშნები.

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