

Influence of biocomposite calcium phosphate materials on the biomechanical dynamics of healing of experimental defect of the compact bone

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SOUHRN

Korenkov A.: **Vliv biokompozitních kalciofosfátových materiálů na biomechanickou dynamiku hojení experimentálních defektů kompaktní kosti**

Cíl: Studie měla za cíl stanovit vliv biokompozitních kalciofosfátových materiálů různého složení a od různých výrobců na biomechanickou dynamiku hojení experimentálních defektů kompaktní tkáně.

Materiál a metody: Experiment byl proveden na 48 potkanech kmene Wistar. Ve střední části diafýzy femuru byl vytvořen perforační defekt o průměru 2,5 mm zasahující do dřevné dutiny. U zvířat v první skupině byl defekt vyplněn biokompozitním kalciofosfátovým materiálem Collapan (hydroxyapatit/kolagen/antibiotikum) a u zvířat druhé skupiny byl použit easy-graft™ CRYSTAL (hydroxyapatit/ beta trikalcium fosfát). Části poškozené kosti byly vyšetřeny po 15, 30, 60 a 120 dnech dynamickou mikroindentací ke stanovení mikrotvrdomosti a Youngova modulu v místě implantace kalciofosfátového materiálu a okolní původní kosti.

Výsledky: Během experimentu se prokázalo, že mikrotvrdomost a Youngův modul v místech implantace materiálů Collapan a easy-graft™ CRYSTAL se postupně blíží parametrům původní kosti. Po 30 a 60 dnech byly mikrotvrdomost a Youngův modul v místě implantace materiálu Collapan vyšší než v místě implantace easy-graft™ CRYSTAL. Po 120 dnech byly hodnoty pro oba materiály stejné a převyšovaly parametry původní kosti.

Závěry: Z biomechanického hlediska biokompozitní kalciofosfátové materiály Collapan a easy-graft™ CRYSTAL přispívají k úplnému hojení kompaktní tkáně během 4 měsíců.

Klíčová slova: reparační osteogeneze, hydroxyapatit, beta trikalcium fosfát, kolagen, mikrotvrdomost, Youngův modul

SUMMARY

Korenkov A.: **Influence of biocomposite calcium phosphate materials on the biomechanical dynamics of healing of experimental defects in compact bone**

Objective: This study was aimed at establishing the influence of different by composition and manufacturer biocomposite calcium phosphate materials on the biomechanical dynamics of healing of experimental defect of the compact bone tissue.

Materials and Methods: The experiment was carried out on 48 white Wistar rats. In the middle third of the diaphysis of the femur there was reproduced the perforated defect with a diameter of 2.5 mm to medullar channel, which in the animals of the first group was filled with biocomposite calcium phosphate material „Collapan“ (hydroxyapatite/collagen/antibiotic), and in the animals of the second group with „Easy-graft™CRYSTAL“ (hydroxyapatite/ β -tricalcium phosphate). The fragments of injured bones were examined at the 15th, 30th, 60th and 120th day by the method of dynamic microindentation with determining of the microhardness and Young's modulus of the site of implantation of calcium phosphate material and the adjacent to it maternal bone.

Results: It is established that throughout the duration of the experiment, the microhardness and Young's modulus of the site of implantation of „Collapan“ and „Easy-graft™CRYSTAL“ were gradually approaching the same indicators of the maternal bone. 30th and 60th day of the experiment, the area of implantation of „Collapan“ by microhardness and Young's modulus exceeded the area of implantation of „Easy-graft™CRYSTAL“, and on the 120th day they did not just equal among themselves, but also exceeded the corresponding indicators of the maternal bone.

Conclusions: The biocomposite calcium phosphate materials „Collapan“ and „Easy-graft™CRYSTAL“ from a biomechanical point of view, contribute to complete healing of the defect of the compact bone tissue in 4 months.

Keywords: reparative osteogenesis, hydroxyapatite, β -tricalcium phosphate, collagen, microhardness, Young's modulus

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Introduction

Treatment of bone defects is one of the important and at the same time, still unresolved problem of modern traumatology. Biocomposite calcium phosphate materials through a similar chemical structure with the bone tissue, inertness to biological tissues, the ability to osseointegration, gradual biodegradation are widely used in everyday clinical practice for replacement of the lost bone tissue [1,2,3]. One of the most famous components of biocomposite calcium phosphate materials is hydroxyapatite, which Professor Kaufman in 1997 at the International Congress of oral implantology in Germany announced as „the best medical material of all time“ [4]. To create biocomposite drugs hydroxyapatite is most often combined with β -tricalcium phosphate, or with organic natural and synthetic polymers (collagen, polycaprolactone, etc.). Due to this biocomposite materials acquire properties which were absent in its individual components. It is known that β -tricalcium phosphate undergoes reabsorption faster than hydroxyapatite, and therefore due to the changes of their ratio one biocomposite preparation has the opportunity to regulate the process of resorption of calcium phosphate material in the area of implantation [5,6,7,8,9]. It was found that such biocomposite material undergoes partial resorption and primarily due to the β -tricalcium phosphate and hydroxyapatite remains at the site of the defect for a longer period, ensuring the prevention of atrophy and preservation of bone tissue volume [10,11]. Using biocomposite materials in which the hydroxyapatite is combined with collagen, the opportunity to fill in the bone defect deficiency not only of mineral but also of organic component of bone tissue is created [12]. Moreover, the given combinations of constituents of biocomposite materials are capable of osteoconductive influence on the reparative osteogenesis and positively influence the restoration of the structure of the injured bone [13,14,15,16,17]. Here it should also be noted that the treatment of bone defects is considered successful not only with the full restoration of the structure but also of the biomechanical characteristics of the damaged bone [18]. However, the existing scientific literature data on hardness and stiffness of the sections of the implantation of calcium phosphate materials in the vast majority were obtained in experiments on the bones of the skull and in one observation

period, but information concerning the comparative influence of different by composition and by manufacturer biocomposite drugs on the dynamics of the biomechanical healing of the defect of compact bone tissue in the scientific literature was not found by us. It should also be noted that information about the effect of some commercial biocomposite materials in terms of hardness and stiffness of the formed regenerate in the scientific literature is completely absent, and for other drugs the established biomechanical parameters have a significant divergence [19,20,21,22]. While, it is clear that the existing divergence of the biomechanical parameters is probably affected by many factors. For example, the size of the defect in the regenerative potential of bone tissue, the characteristics of the calcium phosphate material (manufacturer, composition, geometric shape, total porosity, the pore size, the design, size, etc.) [12]. All this suggests that for a more predictable influence of different by composition and other characteristics biocomposite materials on the biomechanical dynamics of the healing of bone defects their experimental models are required. On the latter in standard conditions and using different research methods the biomaterials, which optimize reparative osteogenesis can be studied and compared with each other. Therefore, the aim of our work was to determine the influence of different by composition and by manufacturer biocomposite calcium phosphate materials on the biomechanical dynamics of healing of experimental defect of the compact bone tissue.

Materials and Methods

The experiment was performed on 48 white Wistar rats eight months of age with the weight of 250 ± 10 g. All procedures were agreed with the Commission on Biomedical Ethics of Sumy State University (Minutes No. 2/2 of 26. 02. 2016). 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 initially injected with 2.5 mg/kg of acepromazine intramuscular and in 5 minutes 75 mg/kg of ketamine intramuscular (Calypsol, Gedeon Richter, Budapest-Hungary). After the induction of the animals in anesthesia, a defect of the medullary canal with diameter of 2.5 mm was reproduced

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 (24 rats) – defect was filled with the biomaterial „Collapan“ (Intermedapatyt, Russia registration number 2011/10304 FES) (Fig. 1). The latter is an artificial granular biocomposite material consisting of nanostructured hydroxyapatite, of collagen type I from skin of cattle and antibiotics (lincomycin, gentamicin, etc.) [15].

Group 2 (24 rats) – defect was filled with the biomaterial „Easy-graft™CRYSTAL“ (Degradable Solu-

tions AG, Switzerland registration number 2008/03310 FES). 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. 2) is covered with thin (10 μ m) polymer of polylactic and polyglycolic acid (PLGA), which is capable of resorption [6,17].

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 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 μ m and macrocells between separate granules was formed.

After entering into the bone defect of calcium phosphate 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 (JSC „Synthesis“, Kurgan, Russia) was injected intramuscularly at a dose of 0.6 mg 2 times a day.

Next on the 15th, 30th, 60th and 120th day after surgery animals were taken out of the experiment by decapitation under deep ether anesthesia with subsequent study of injured bones by the method of dynamic microindentation on Indentometer „Micron-gamma“, which was developed by the Aerospace national aviation University (Ukraine) [23,24]. The surface of prepared bones was polished down gradually with 250 nm diamond paste slurry. After that the femurs were mounted on self-polymerizing resin (VariDur 10; Buehler, Lake Bluff, IL, USA) and subjected to a fine special silicon carbide grinding paper polishing (EcoMet 250 Grinder Polisher; Buehler, Lake Bluff, IL, USA), dried, and treated with absolute alcohol to decontaminate the surface. Then the samples were fixed on twocoordinate table Indentometer „Micron-gamma“ and using the optical microscope there were identified the site of implantation of calcium phosphate material and adjacent to it maternal bone, in which the measuring of hardness and stiffness was carried out. As the indenter, there was used a triangular diamond Berkovich pyramid with base in the form of an equilateral triangle and an apex angle between opposite faces 65°. The load on the indenter (P) was set 50 cN, and the velocity (V) was 5 g/c. In addition, when carrying out dynamic microindentation there were taken into account the conditions of application

Figure 1

The granule of the biomaterial Collapan (3.54 μ m of size) Electronic scanning image. X 30 magnification

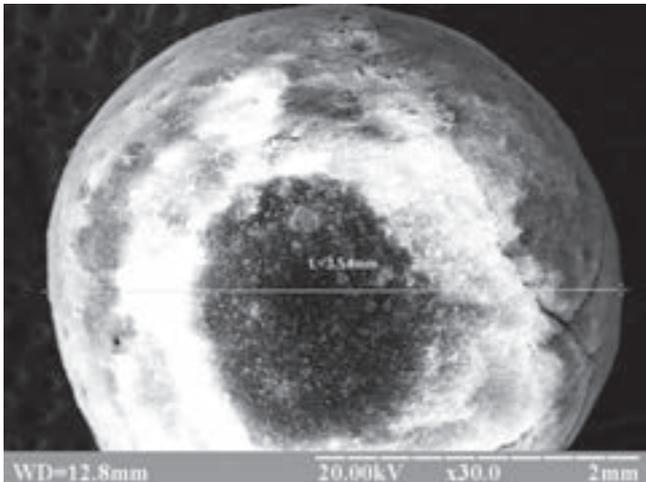
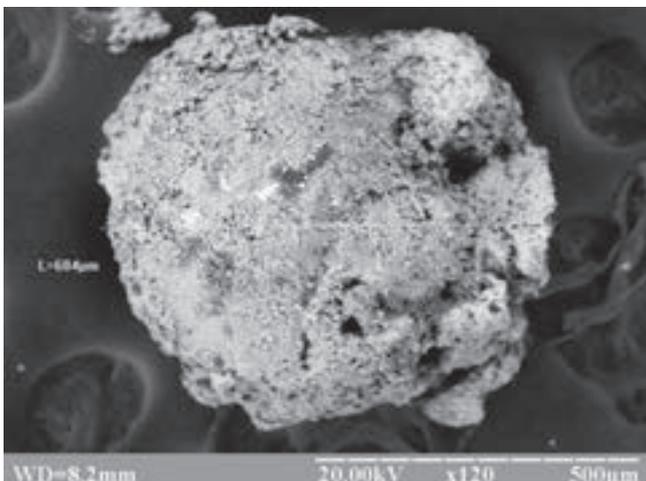


Figure 2

The granule of the biomaterial „Easy-graft™CRYSTAL“ with size of 684 μ m. Electronic scanning image. X 120 magnification



of the prints – the distance from the edge of the bone to the imprint of $> 2.5 d$ (d – is the diagonal length of the imprint), and the distance between adjacent prints $> 3 d$ [25].

Using Indentometer „Micron-gamma“ we had the opportunity to continuously push the indenter into the site of implantation of calcium phosphate material and the adjacent to it parent bone and record the dependence of the depth of its immersion on the strength of pressing. At the same time the Indentometer „Micron-gamma“ showed a chart of the load of the site of the defect with implanted calcium phosphate material and the adjacent to it parent bone, which consisted of a loading curve and unloading curve. The diagram displayed the work, which was spent by the indenter at overcoming the resistance of the bone, regenerate and biomaterial (the area above the branch of load), as well as work of elastic forces, which was carried out on the recovery of their shape after indentation (the area under the unloading branch) (Fig. 3).

After registration of the diagram of load in the software „Micron-gamma“ the hardness by Meyer, GPa and modulus of elasticity (by Young), GPa were automatically calculated. In this case, the calculation of the hardness was performed according to the formula:

$$H_M = \frac{P_m}{A_p}$$

where H_M – microhardness at the Meyer,

$$\frac{N}{mm^2}$$

which represents the average pressure on the surface of the imprint and by quantity most accurately reflects the physical essence of hardness [25]; P_m – the maximum load on the indenter, N ; A_p – the projection of the contact area, mm^2 . Determining of the elastic modulus by the load diagram was based on the method of Oliver WC and Pharr GM [26] and were calculated according to the formula:

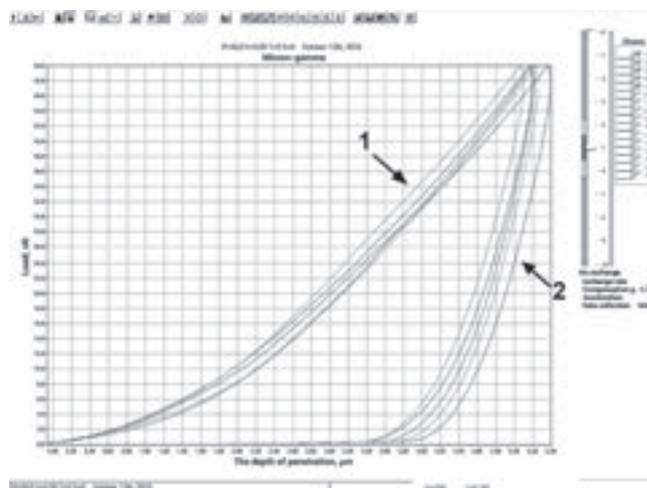
$$\frac{1}{E_r} = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i}$$

where E , E_i i ν , ν_i – the elastic moduli and Poisson's ratios for bone and indenter (for the indenter $E_i = 1141$ GPa and $\nu_i = 0.07$; for the bone $\nu = 0.3$); E_r – reduced contact modulus of elasticity, GPa.

The resulting digital values were treated 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 was evaluated using Student t-test with the use of statistical computer program MS Excel XP. The differences were considered significant at $p < 0.05$.

Figure 3

The load diagram of the site of implantation of biocomposite materials by trihedral diamond pyramid of Berkovich on Indentometer „Micron-gamma“. Load curve (1) and unload curve (2).



Results

On the 15th day of the experiment by the method of kinetic hentemann it was found that the microhardness and Young's modulus of the site of implantation of biomaterial „Collapan“ by 11.04 % ($p > 0.05$) and 5.62 % ($p > 0.05$) were higher than in the site of implantation of „Easy-graft™CRYSTAL“ and was 0.573 ± 0.022 GPa, 16.9 ± 0.41 GPa in the first and 0.516 ± 0.019 GPa, 16 ± 0.36 GPa in the second case. In addition, unrestored microhardness and Young's modulus of the site of implantation of „Collapan“ was by 39.04 % ($p < 0.05$) and 23.18 % ($p < 0.05$), and in the area of implantation of „Easy-graft™CRYSTAL“ by 48.24 % ($p < 0.05$) and 35.22 % ($p < 0.05$) lower than in the adjacent maternal bone (0.94 ± 0.029 GPa, 22 ± 0.45 GPa and 0.997 ± 0.033 GPa, 24.7 ± 0.52 GPa).

30th day of the experiment, the microhardness and Young's modulus of the site of implantation of biomaterial „Collapan“ and „Easy-graft™CRYSTAL“ compared to the previous period of the experiment increased by 37.69 % ($p < 0.05$), 31.36 % ($p < 0.05$) and 32.55 % ($p < 0.05$), 23.75 % ($p < 0.05$) and was 0.789 ± 0.024 GPa, 22.2 ± 0.39 GPa in the first and 0.684 ± 0.023 GPa, 19.8 ± 0.47 GPa in the second case. At the same time, the microhardness and Young's modulus of the site of implantation of „Collapan“ exceeded the similar indicators of the site of implantation of „Easy-graft™CRYSTAL“ by 15.35 % ($p < 0.05$) and 12.12 % ($p < 0.05$). In addition, the studied biomechanical parameters (microhardness and Young's modulus) of the site of implantation of „Collapan“ was by 21.95 % ($p < 0.05$) and 10.48 % ($p < 0.05$), and the

Table
Data of variation-statistical processing of biomechanical parameters (Mean \pm SD) of the site of implantation of biocomposite material and the adjacent to it maternal bone (n = 6)

Day after surgery	Group experiment	Biomechanical parameters and measurement			
		Microhardness for Meyer (GPa)		Young's modulus (GPa)	
		The area of the defect	Maternal bone	The area of the defect	Maternal bone
15	Group 1	0.573 \pm 0.022	0.94 \pm 0.029**	16.9 \pm 0.41	22 \pm 0.45**
	Group 2	0.516 \pm 0.019	0.997 \pm 0.033**	16 \pm 0.36	24.7 \pm 0.52**
30	Group 1	0.789 \pm 0.024*	1.011 \pm 0.031**	22.2 \pm 0.39*	24.8 \pm 0.57**
	Group 2	0.684 \pm 0.023	1.165 \pm 0.038**	19.8 \pm 0.47	23.8 \pm 0.55**
60	Group 1	1.019 \pm 0.025*	1.092 \pm 0.018**	23.3 \pm 0.27*	24.9 \pm 0.43**
	Group 2	0.875 \pm 0.021	0.945 \pm 0.017**	22.3 \pm 0.36	23.9 \pm 0.29**
120	Group 1	1.083 \pm 0.036	0.989 \pm 0.035	25.2 \pm 0.51	21.6 \pm 0.47**
	Group 2	1.185 \pm 0.039	1.045 \pm 0.032**	25.9 \pm 0.49	24.5 \pm 0.54

Note. Asterisk * ($p < 0.05$) shows significant difference of the microhardness and Young's modulus of the site of the defect in animals of the 1st group in relation to the 2nd, and the sign ** ($p < 0.05$) shows significant difference between the area of the defect and adjacent to it parent bone

site of implantation of „Easy-graft™CRYSTAL“ by 41.29 % ($p < 0.05$) and 16.81 % ($p < 0.05$) lower than in the maternal bone (1.011 \pm 0.031 GPa, 24.8 \pm 0.57 GPa and 1.165 \pm 0.038 GPa, 23.8 \pm 0.55 GPa).

60th day of the experiment, the microhardness and Young's modulus of the site of implantation of bio-material „Collapan“ compared to the previous period of the experiment increased by 29.15 % ($p < 0.05$) and 4.95 % ($p < 0.05$), and in the area of implantation of „Easy-graft™CRYSTAL“ by 27.92 % ($p < 0.05$) and 12.62 % ($p < 0.05$) and amounted to 1.019 \pm 0.025 GPa, and 23.3 \pm 0.27 GPa in the first and 0.875 \pm 0.021 GPa, 22.3 \pm 0.36 GPa in the second case. The microhardness and Young's modulus of the site of implantation of „Collapan“ were by 14.13 % ($p < 0.05$) and 4.29 % ($p < 0.05$) higher than the similar indicators of the site of implantation of „Easy-graft™CRYSTAL“. It should also be noted that the studied biomechanical parameters (microhardness and Young's modulus) of the site of implantation „Collapan“ was by 6.68 % ($p < 0.05$) and 6.42 % ($p < 0.05$), and in the area of implantation of „Easy-graft™CRYSTAL“ by 7.4 % ($p < 0.05$) and 6.69 % ($p < 0.05$) lower than in the maternal bone (1.092 \pm 0.018, 24.9 \pm 0.43 GPa and 0.945 \pm 0.017 GPa, 23.9 \pm 0.29 GPa).

120th day of the experiment – in the site of implantation of „Collapan“ compared to the previous period, the microhardness by Meyer and Young's modulus increased by only 6.28 % ($p > 0.05$) and 8.15 %

($p < 0.05$), and in the area of implantation of „Easy-graft™CRYSTAL“ by 35.42 % ($p < 0.05$) and 16.14 % ($p < 0.05$) and were 1.083 \pm 0.036 GPa, 25.2 \pm 0.51 GPa in the first and 1.185 \pm 0.039 GPa, 25.9 \pm 0.49 GPa in the second case. As seen from the given indicators, on the 120th day, in contrast to other periods of the experiment, the microhardness and Young's modulus of the site of implantation of „Collapan“ were already by 8.6 % ($p > 0.05$) and 2.7 % ($p > 0.05$) lower than in the site of implantation of „Easy-graft™CRYSTAL“. In addition, on the 120th day of the experiment, the microhardness and Young's modulus of the site of implantation of „Collapan“ and „Easy-graft™CRYSTAL“ exceeded by 9.5 % ($p > 0.05$), 16.67 % ($p < 0.05$) and 13.39 % ($p < 0.05$), 5.71 % ($p > 0.05$) the corresponding indicators of the maternal bone (0.989 \pm 0.035 GPa, 21.6 \pm 0.47 GPa and 1.045 \pm 0.032 GPa, 24.5 \pm 0.54 GPa).

Discussion

The conducted experimental study found that on the 15th day of the experiment, the microhardness and Young's modulus of the site of implantation of „Collapan“ and „Easy-graft™CRYSTAL“ had no significant difference, were much lower than corresponding indicators of the maternal bone, indicating almost the same low mineralization tissue structures of the regenerate and relatively minor biomechanical character-

ristics of the implanted biomaterials. Since the 30th day of the experiment and further on the microhardness and Young's modulus of the site of implantation of „Collapan“ and „Easy-graft™CRYSTAL“ were gradually approaching the similar indicators of the maternal bone, which from a biomechanical point of view may indicate the increase in the number and maturity of the bone tissue of the regenerate. Simultaneously on the 30th and 60th day of the experiment the microhardness and Young's modulus of the site of implantation of „Collapan“ exceeded the similar indicators of the site of implantation of „Easy-graft™CRYSTAL“, and on the 120th day they were equal among themselves. In addition, on the 60th day of the experiment the studied biomechanical parameters of the site of implantation of „Collapan“ and „Easy-graft™CRYSTAL“ approached the similar parameters of the maternal bone. However, full recovery of the defect of the cortical layer of bone has not yet occurred as evidenced by a slight advantage of the microhardness and Young's modulus of the maternal bone over the sites of implantation of „Collapan“ and „Easy-graft™CRYSTAL“. At the same time the study of Du C. et. al. indicates that the restored microhardness of the site of the defect of the diaphysis of the femur of New Zealand white adult rabbits even 12 weeks after implantation into its cavity of biocomposite material (hydroxyapatite/collagen) was almost two times lower (0.85 ± 0.102 GPa) than in the adjacent to it maternal bone (1.58 ± 0.101 GPa) [20]. In turn, the biomechanical parameters of the sites of defects of cranial bone that have been identified by various authors after implantation of biocomposite materials differ considerably between themselves. Thus, according to Al-Qutub et. al. microhardness and Young's modulus of the maternal bones of Wistar albino rats on the 10th week after implantation in a cavity of a defect of biphasic calcium phosphate material (hydroxyapatite/ β -tricalcium phosphate/bone morphogenetic protein 2) is 0.637 ± 0.006 and 21.49 ± 0.095 GPa [21]. However, Liao HT et. al. found that the rate of microhardness in the area of the defect of the temporal bone of swine (pigs) in 6 months after implantation of biocomposite calcium phosphate material (Polycaprolactone/hydroxyapatite/stem cells from the bone marrow) was much smaller (0.02 GPa) [22]. In our study, on the 120th day of the experiment, the microhardness and Young's modulus of the site of implantation of biomaterial „Collapan“ was 1.083 ± 0.036 GPa and 25.2 ± 0.51 GPa and of „Easy-graft™CRYSTAL“ – 1.185 ± 0.039 GPa and 25.9 ± 0.49 GPa. Besides, during the last period of the experiment, biomechanical indicators of the site of implantation of „Collapan“ and „Easy-graft™CRYSTAL“ did not just equal among themselves, but also exceeded the corresponding indicators of the maternal bone, which from a biomechanical point of view can be assessed as complete restoration of the integrity of the bone.

Conclusion

Thus, biocomposite material „Collapan“ and „Easy-graft™CRYSTAL“ from a biomechanical point of view, contribute to complete healing of the defect of the compact bone tissue in 4 months.

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