ORIGINAL ARTICLE

Influence of β-tricalcium Phosphate on the Biomechanical Dynamics of Healing of Experimental Defect of the Compact Bone

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Abstract:

Background: One of the important criteria for successful treatment of bone defects with calcium phosphate osteoplastic materials is a full-fledged recovery of the biomechanical characteristics of the injured bone. However, the information regarding the effect of β-tricalcium phosphate on the biomechanical dynamics of the healing of the defect compact bone in the scientific literature is not presented. *Aim and Objectives:* This study was focused on the biomechanical assessment of the healing of experimental defect of the compact bone tissue after the implantation of calcium phosphate osteoplastic material "ChronOS™" (β-tricalcium phosphate block), with identifying the dynamics of the changes of the microhardness and Young's modulus forming the regenerate with the implanted material. *Material and Methods:* In the middle third of the femoral shaft of 48 white Wistar rats eight months of age with the weight of 250 ± 10 g were produced perforated defect to the medullary canal, which in the animals of the experimental group was filled with β-tricalcium phosphate, and in the control animals was left unfilled. Fragments of injured bones examined at 15th, 30th, 60th and 120th day by the method of kinetic identication with the definition of the microhardness and Young's modulus of the site of the defect and adjacent to it maternal bone. *Results:* It was found out that throughout the observation period the site of implantation of osteoplastic material "ChronOS™" by the microhardness and Young's modulus greatly dominated over the area of the defect in animals of the control group, till the 60th day it was inferior to the maternal bone, and on the 120th day of the experiment-equal to its biomechanical indicators. *Conclusion:* Osteoplastic material "ChronOS™" significantly increases the hardness and stiffness of the area of the defect of compact bone tissue and from the biomechanical point of view promotes complete healing during 4 months.

Keywords: Rats, β-Tricalcium Phosphate, Reparative Osteogenesis, Hardness, Young's modulus

Introduction:

Treatment of bone defects is one of the relevant and at the same time not completely solved problem of modern traumatology. For plasty of bone defects, β-tricalcium phosphate and other calcium phosphate osteoplastic materials are very often used because of their similarity in structure to the bone tissue, inertness to biological tissues and ability to induce osteoconductive influence on the reparative osteogenesis [1, 2]. One of the modern drugs based on β-tricalcium phosphate is "ChronOS™", which was named by Pochon J. P. the best material for substitution of bone tissue defects in children [3]. Clinical and experimental studies demonstrate exceptional biocompatibility and the superior performance of osteoplastic material "ChronOS™" and other drugs based on β-tricalcium phosphate during replacement of bone defects in traumatology, spine surgery and dentistry [4, 5, 6]. Moreover, the morphological studies allowed us to obtain data on the formation of the tissues of the regenerate, the nature of their

interaction with β-tricalcium phosphate and dynamics of its resorption [7]. However, we know that in order to comprehensively assess the effectiveness of the influence of β-tricalcium phosphate on healing of bone defects it is extremely important also to measure biomechanical function of the forming regenerate with the osteoplastic material.This is due to the fact that successful treatment of bone defects is considered to be achieved when they have the full restoration not only of the structure but also of the biomechanical characteristics of the damaged bone [8]. Today, in scientific literature there are data about the hardness and rigidity of sites of the implantation of β-tricalcium phosphate, but they are obtained in experiments on the bones of the skull and in one observation period, but the information regarding the influence of "ChronOS™" or other osteoplastic materials based on β-tricalcium phosphate on the biomechanical dynamics of the healing of the defect of compact bone in the scientific literature has not been found [9, 10]. Therefore, the aim of our work was to investigate the biomechanical dynamics of healing of experimental defect of the compact bone tissue after implantation into its cavity of β-tricalcium phosphate ("ChronOS™").

Material 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 № 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) [11]. 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) – control, where the bone defect was left to heal under blood clot;

Group 2 (24 rats) – experimental,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 the macropore size from 100 to 500 microns and micropores to 10 microns (Fig. 1);

Before the implantation the blocks of "ChronOS™" 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.

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 (JSC "Synthesis", Kurgan, Russia) was injected intramuscularly at a dose of 0.6 mg 2 times a day [11].

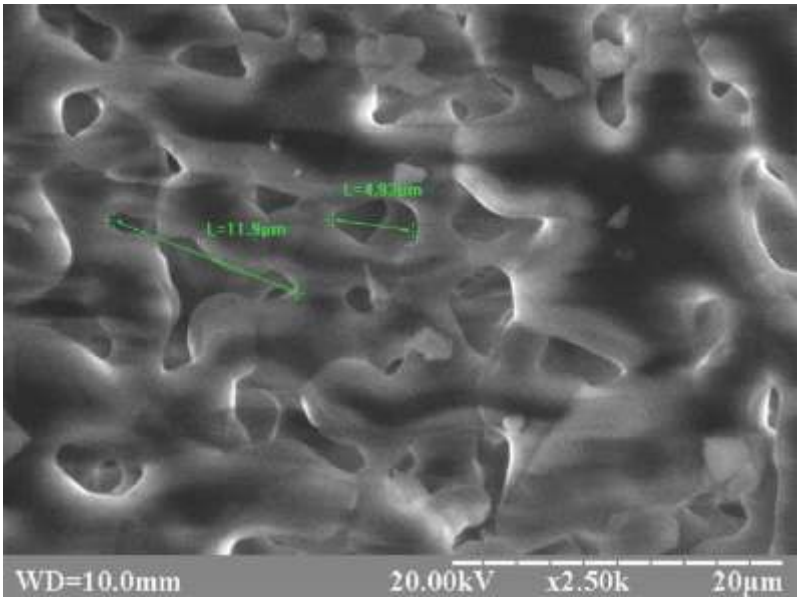

Fig. 1: Microstructure of the Osteoplastic Material "ChronOS™". Visible Micropores of Size from the 4.93 µm to 11.9 µm. Electronic Scanning Image. X 2500.

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 kinetic hentemann on Indentometr "Micron-gamma", which was developed by the Aerospace national aviation University (Ukraine) [12, 13]. The prepared femoral bones were set into a cavity of aconsoleon a par with its polished surface. Next the console with a bone was fixed on two coordinate table Indentometr "Micron-gamma" thus, to ensure the perpendicularity of the axis of the indenter to the area of the defect and adjacent parent bone, in which the measurement of hardness and stiffness was conducted. 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 65^0. The load on the indenter (P) was set 50 cN, and the velocity (V) was 5 g/c. In addition, when carrying out kinetic hentemann there were taken into account the conditions of application 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 [14]. Using Indentometr "Micron-gamma" we had the opportunity to continuously dent the indenter into the area of the defect and adjacent to it parent bone and record the dependence of the depth of his immersion on the strength of indentation. Here, Indentometr "Micron-gamma" showed diagrams of load of the area of the defect with the implanted osteoplastic material "ChronOS™" 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 osteoplastic material (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. 2).

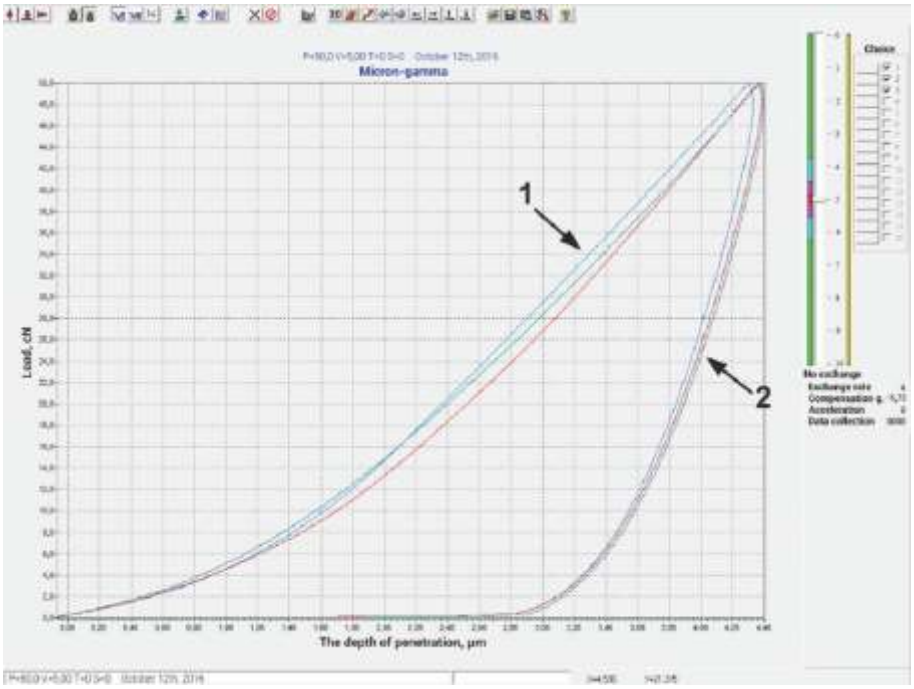

Fig. 2: The Load Diagram of the Site of Implantation of Osteoplastic Material "ChronOS™" by Trihedral Diamond Pyramid of Berkovich on Indentometr "Micron-gamma". The Curve of Loading (1) and Unloading (2).

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: $H_M = \frac{P_m}{A_p}$, де H_M – not restored 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 [14]; 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 [15] and were calculated according to the formula: $\frac{1}{E_r} = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i}$, де E, E_i і ν, ν_i – the elastic moduli and Poisson's ratios for bone and indenter (for the indenterE_i = 1141 GPaandν_i = 0,07; for the bone ν = 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 $p<0.05$ [11].

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 osteoplastic material "ChronOS™" by 712.5 % ($p<0.05$) and 528.12 % ($p<0.05$) were higher than the corresponding figures of the

regenerate of the animals of control series and was 0.845±0.028 GPa, 20.1±0.5 GPa in the first and 0.104±0.005 GPa of 3.2±0.07 GPa in the second case. In addition, non-restored microhardness and Young's modulus of the site of implantation of "ChronOS™" were by 11.05 % ($p<0.05$) and 6.07 % ($p>0.05$), and in the regenerate of control animals by 89.31 % ($p<0.05$) and 86.38 % ($p<0.05$) less than in the maternal bone (0.95±0.026 GPa, 21.4±0.43 GPa and 0.973±0.03 GPa, 23.5±0.53 GPa).

On the 30th day of the experiment the microhardness and Young's modulus of the regenerate of control animals and the site of implantation of osteoplastic material "ChronOS™" compared to the previous period of the experiment increased by 194.23 % ($p<0.05$), 206.25 % ($p<0.05$) and 4.14 % ($p>0.05$), 9.95 % ($p<0.05$) and amounted to 0.306±0.018 GPa, 9.8±0.29 GPa in the first and 0.88±0.027 GPa, 22.1±0.53 GPa – in the second case. At the same time, the microhardness and Young's modulus of the site of implantation of "ChronOS™" exceeded the corresponding parameters of the regenerate in the animals of the control group by 187.58 % ($p<0.05$) and 125.51 % ($p<0.05$). In addition, the studied biomechanical parameters (microhardness and Young's modulus) of the site of implantation of "ChronOS™" were by 7.17 % ($p>0.05$) and 1.78 % ($p>0.05$), and in the regenerate of the control animals by 69.46 % ($p<0.05$) and 59.34 % ($p<0.05$) less than in the maternal bone (0.948±0.029 GPa, 22.5±0.46 GPa and 1.002±0.025 GPa, 24.1±0.41 GPa).

On the 60th day of the experiment, the microhardness and Young's modulus of the regenerate of the control animals in comparison with the previous period of the experiment increased by 94.11 % ($p<0.05$) and 77.55 % ($p<0.05$), and in the site of implantation of osteoplastic material "ChronOS™" on the contrary decreased by 15.56 % ($p<0.05$) and 7.69 % ($p<0.05$) and amounted to 0.594±0.016 GPa, 17.4±0.37 GPa in the first and 0.743±0.019 GPa, 20.4±0.42 GPa – in the second case.However, despite the fact that the microhardness and Young's modulus of the site of implantation of "ChronOS™" decreased compared to the 30-th day of the experiment, they still were by 25.08 % ($p<0.05$) and 17.24 % ($p<0.05$) higher than the similar indicators of the regenerate in the animals of the control group. It should also be noted that the indicators of the microhardness and Young's modulus of the regenerate of the control animals were by 39.45 % ($p<0.05$) and 25.64 % ($p<0.05$), and of the site of implantation of "ChronOS™" by 9.39 % ($p<0.05$) and 7.29 % ($p<0.05$) less than in the adjacent to the site of the defect maternal bone (0.981±0.022 GPa, 23.4±0.48 GPa and 0.82±0.024 GPa, 22.1±0.44 GPa).

On the 120th day of the experiment there was observed the growth of the studied biomechanical parameters in both groups of the experiment.So, compared to the previous period of the experiment, the microhardness by Meyer and Young's modulus of the site of implantation of "ChronOS™" increased by 44.55 % ($p<0.05$) and 20.59 % ($p<0.05$), and of the regenerate of the control animals by 50.84 % ($p<0.05$) and 18.39 % ($p<0.05$), and amounted to 1.074±0.033 GPa 24.6±0.49 GPa in the first and 0.896±0.023 GPa, 20.6±0.39 GPa in the second case.As you can see from the given indicators, the microhardness and Young's modulus of the site of implantation of "ChronOS™" were by 19.86 % ($p<0.05$) and 19.41 % ($p<0.05$) higher than that in the regenerate of the animals of control group.In addition, in the last period of the experiment, the microhardness and Young's modulus of the regenerate in the animals of the control group were by 5.39 % ($p>0.05$) and 16.94 % ($p<0.05$) less than in the maternal bone (0.947±0.031 GPa and 24.8±0.59 GPa), and microhardness and Young's modulus of the site of implantation of "ChronOS™" exceeded the corresponding indicators of the maternal bone (1.027±0.035 GPA, 23.7±0.51 GPa) by 4.58 % ($p>0.05$) and 3.79 % ($p>0.05$).

Table 1: Data of Variation-Statistical Processing of Biomechanical Parameters (Mean ± SD) of the Area of the Defect and Adjacent to it Maternal Bone in Animals of Control and Experimental Groups (N=6)

Day after Surgery	Group Experiments	Biomechanical Parameters and Measurement			
		Microhardness for Meyer (GPa)		Young's modulus (GPa)	
		Area of Defect	Maternal Bone	Area of Defect	Maternal Bone
15th	Control	0.104±0.005*	0.973±0.03°	3.2±0.07*	23.5±0.53°
	Experimental	0.845±0.028	0.95±0.026°	20.1±0.5	21.4±0.43
30th	Control	0.306±0.018*	1.002±0.025°	9.8±0.29*	24.1±0.41°
	Experimental	0.88±0.027	0.948±0.029	22.1±0.53	22.5±0.46
60th	Control	0.594±0.016*	0.981±0.022°	17.4±0.37*	23.4±0.48°
	Experimental	0.743±0.019	0.82±0.024°	20.44±0.42	22.1±0.44°
120th	Control	0.896±0.023*	0.947±0.031	20.6±0.39*	24.8±0.59°
	Experimental	1.074±0.033	1.027±0.035	24.6±0.49	23.7±0.51

*Data of variation-statistical processing of biomechanical parameters (Mean ± SD) of the area of the defect and adjacent to it maternal bone in the animals of control and experimental groups(n=6). Sign * ($p<0.05$) shows significant difference of the microhardness and Young's modulus of the site of the defect in animals of the control group in relation to experimental, and the sign* o *($p<0.05$) shows significant difference between the area of the defect and adjacent to it maternal bone*

Discussion:

The conducted experimental study found that on the 15-30th day of the experiment the site of implantation of "ChronOS™" was characterized by significantly higher values of microhardness and Young's modulus than the regenerate of the animals of the control group and slightly inferior to the similar indicators of the maternal bone. All this suggests that the studied biomechanical parameters of the site of the defect of experimental animals in the initial stages of the experiment were mostly due to the hardness and stiffness of the implanted into its cavity osteoplastic material "ChronOS™". It should also be noted that during the first month of observations, the microhardness and Young's modulus of the site of implantation of "ChronOS™" almost did not change, and on the 60th day of the experiment the studied biomechanical parameters significantly decreased, which may indicate the predominance of the process of resorption of the implant on the maturation of the bone tissue of the regenerate. In contrast with them, in the animals of the control group throughout the duration of the experiment, dynamics of the biomechanical healing of the defect was accompanied by a gradual increase of microhardness and Young's modulus, which may indicate a gradual increase in the number and maturity of the bone tissue of the regenerate. On the

120th day of the experiment in the defect area, which was healing under a blood clot to the site of implantation of osteoplastic material "ChronOS™" compared to the previous period there was observed significant increase of the microhardness and Young's modulus and their approaching to the similar indicators of the maternal bone. At the same time, Al-Hezaimi *et al*. in their study demonstrated that the hardness of the parietal bone of Sprague-Dawley rats on the 10th week after implantation into a cavity of its defect of β-tricalcium phosphate with mesenchymal stem cells was significantly lower (0.058 GPa) and modulus of elasticity (109.11GPa) much higher than in the undamaged bone (0.454 GPa and 13.85 GPa) [9]. Ramalingam *et al*. in turn found that the hardness of the bones of the cranial vault of Wistar albino rats on the 10th week after implantation of β-tricalcium phosphate (0.241±0.006 GPa) was higher and the Young's modulus (4.34±0.06 GPa) on the contrary lower than in the area of the defect, which was healing under a blood clot (0.192±0.004 GPa, 6.76±0,04 GPa) [10]. In our experiment in the area of the defect of compact bone tissue on the 120th day after its application the microhardness and Young's modulus were 0.896±0.023 GPa and 20.6±0.39 GPa, and in the area of implantation of "ChronOS™" – 1.074±0.033 GPa and 24.6±0.49 GPa.That is, the studied biomechanical parameters of the site of implantation of "ChronOS™" were significantly higher than in the regenerate of the animals of the control group. In addition, in the last period of the experiment, the microhardness and Young's modulus of the site of the defect of the control animals approached (0.896±0.023 GPa, 20.6±0.39 GPa), but did not reach the corresponding indicators of the maternal bones (0.947±0.031 GPa, 24.8±0.59 GPa), which according to biomechanical studies can be assessed as not completed reparative osteogenesis.In contrast to them, in the area of implantation of "ChronOS™" in the last observation period the microhardness and Young's modulus (1.074±0.033 GPa and 24.6±0.49 GPa) equaled the corresponding indicators of the maternal bone (1.027±0.035 GPa, 23.7±0.51 GPa), which from a biomechanical point of view, can indicate the restoration of the defect and the replacement of osteoplastic material with well-mineralized bone tissue of the regenerate.

Conclusion:

Thus, osteoplastic material "ChronOS™" significantly increases the hardness and stiffness of the area of the defect of compact bone tissue and from the biomechanical point of view promotes the complete healing in 4 months.

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