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Porous calcium phosphate ceramic granules and their behaviour in differently loaded areas of skeleton

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Abstract Two kinds of calcium phosphate ceramic (CPC) granules of high porosity (50 ± 5%) and improved (for such materials) compressive strength (10–25 MPa) consisted of hydroxyapatite (PHA) and a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) in 60 HA/40 β-TCP composition (PCPC) were developed. A comparative study of in vivo behavior of the materials implanted into an almost unloaded (greater trochanter of femur) and loaded (distal methaphysis of femur) zones in the skeleton of rabbits was performed. Significant activating influence of loading on the processes of new bone formation and reconstruction in macropores of both materials during all periods of implantation (up to 6 months) was observed. The role of relevant cells in the processes in the unsoluble PHA and the degradable PCPC (in which the processes was observed to intensify due to dissolution of the material) was studied and is discussed. Great disturbance in pore structure of the BCPC was revealed in more late periods of implantation. After 6 months, presence of large composite fragments located in intertrabecula spaces of greater trochanter was a characteristic feature of the PCPC crushing. The developed CPC materials seems to have good perspective for using in bone defect plasty in some loaded areas of the skeleton.

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1 Introduction

Calcium phosphate ceramic (CPC) materials based on hydroxyapatite (HA) have been widely used for medical purposes. At first, HA itself was the focus of attention. However, it soon turned out that, beside excellent functional properties, HA had some inherent drawbacks such as poor dissolution in vivo and brittleness. Wishing intensify the dissolution, highly porous HA and macroporous biphasic calcium phosphates (i.e. HA plus beta-tricalcium phosphate, β-TCP, in various proportions) mainly as ceramics were developed. These materials actually manifested better dissolution, however, their strength characteristics were worse than those for pure dense HA [1, 2].

Much effort for strengthening the CPC materials was made [1]. In particular, based on peculiarities revealed during densification of a nanocrystalline HA powder [3], a few possibilities for strengthening HA ceramics were proposed and realized [4]. As a result, nonstoichiometric HA granules were developed and, after a positive animal laboratory test [5], successfully employed in orthopaedic surgery [6].

Improved strength characteristics allowed to use the granules for plasty of bone defects in moderately loaded areas of skeleton. More than 8-year follow-up has displayed that the emergence of ceramics biodegradation roentgenological signs depended on the chemical composition, structure and porosity degree of the material used and—what should be emphasized—on the extent of functional load. An effect of rather fast bone-ceramics

integration stimulated by the loading in early rehabilitation was clearly observed. For instance, the influence of the distributed functional load on the rate of ceramics biodegradation was seen in case of acetabular cavity defects plasty when revising hip endoprosthesis [6].

Though the observations have attested to very successful results of the operations, the material replacement by bone was slow. The aim of this study was to develop a variety of porous biphasic CPC granules of improved both mechanical and dissolution characteristics for application in orthopaedic surgery and to study the peculiarities of bone-ceramics integration in the granules implanted into differently loaded areas of animal skeleton.

2 Materials and methods

2.1 Processing the materials and measurements

Conventional methods were used in the materials science part of the study: scanning electron microscopy (SEM; REMMA-200 microscope, Sumy, Ukraine), X-ray diffraction (XRD; DRON-3 diffractometer, USSR; Cu K_α radiation), infrared spectroscopy (IR; Specord-2x model, Hungary), and an Instron-type laboratory machine - for the compression mechanical tests.

Two kinds of materials were used for implantation. One material was highly porous HA ceramic granules (denoted hereafter as PHA) developed earlier and already approbated clinically [6]. Since the PHA was found to exhibit an effect of faster biodegradation in bone defects under loading, it was essential to study its interaction with cells in both unloaded and loaded zones of skeleton in order to clarify the nature of the effect. The other one was a kind of highly porous biphasic calcium phosphate ceramic granules (hereafter as PBCP) with 60HA/40 β-TCP ratio developed by us and combining enhanced dissolution (as compared with the PHA) and the compressive strength that allowed to use them for bone defects plasty in areas under loading.

The PBCP were processed of a nanocrystalline nonstoichiometric HA powder. The powder was synthesized by a known method [7] with some modification in final stage in order to avoid agglomeration of initial crystallites [3]. The given proportion in the components of the reaction resulted in the molar ratio of Ca/P $\cong$ 1.5 $\pm$ 0.03 in a powder obtained. As earlier [6], Ca/P ratios of the samples were determined by standard EDTA titration for Ca and phosphomolybdate techniques for PO_4. The estimated error in a Ca/PO_4 value was $\pm$2%.

The processing of the green granules in essence did not differ from that developed earlier [6]: cellular polystyrene as a foamer, preparation of an elastic paste of the foamer and the powder, obtaining rounded (often spherical) green granules using a two parallel-plane discs rotated device and preliminary drying. However, in contrast to the processing route of the PHA [6], the green granules for the PBCP were not subjected to preliminary calcinations in humid atmosphere and were sintered in air (annealing at 1,200 °C for 2 h and slow cooling then). As a result, porous granules of 4–6 mm in diameter were manufactured.

Value, structure and degree of "transparency" (interconnection) of the porosity were studied both by Archimedes method and microscopically using SEM techniques. In the first case, the granules were put into a vacuum chamber, and the air was evacuated up to the pressure of 10^{-1} Pa; then, the granules were thrown off into the liquid (an epoxy solution and stained distilled water, having different viscosity, were used) in vacuum, and air was let into the chamber. Complete filling of the pores was revealed even in case of epoxy solution having higher porosity. This was clearly seen in SEM pictures of the cross section of granules removed of the chamber and dried in air at 120 °C. Polymerized epoxy substance was found in pores throughout the bulk. Penetration of the epoxy solution into the whole bulk could happen only in case of interconnected porosity. Using such cross-sections, the architecture of the pore system was found out; the best results were obtained operating in secondary-electrons regime.

The details of strength testing of granules were described earlier [6].

2.2 Animal testing the PHA and PBCP granules

2.2.1 Implants

The PHA and PBCP granules of a rounded form and about 5 mm diameter were used. The characteristics of the powder used for their processing corresponded to ASTM F1185–88.

2.2.2 Animals

The experiments were carried out on 24 white rabbits aged 2 months and having 2140 $\pm$ 150 g of body weight.

The rabbits were sacrificed by means of thiopental overdose in 14 days, 1, 3 and 6 months after the implantation of ceramic samples.

Research protocols were approved by Bioethics Committee of Sytenko Institute of Spine and Joint Pathology in accordance with the rules of European convention for the protection of vertebrate animals used for experimental and other scientific purposes [8].

2.2.3 Surgical procedures

Operations were carried out under general thiopental anesthesia. A bone defect in the form of a cavity having 5 mm diameter and 6 mm depth was modeled in lateral section of distal metaphysis and in greater trochanter of femur. The modeling was performed by a dental drill. The choice of implantation zones was caused by the different loading conditions in these areas. More loaded zone was the distal metaphysis of femur and less loaded one was the greater trochanter of femur. In defects, one granule was placed in close contact with bone. The wound was washed with antiseptic solutions and stitched layer by layer. No additional immobilization was performed. After the operation, the animals were under regular observation.

2.2.4 Histological research

Fragments of rabbits' femur having areas of PHA and PBCP implantation were fixed in 10% neutral formalin, decalcified in 5% nitric acid and embedded into celloidin. Serial sections were cut at the central portion of the defect alone frontal plane by "Reichert" microtome. Histological sections were stained with haematoxylin and eosin, toluidine blue (pH = 2.5) and were analyzed using a light microscope (Carl Zeiss, Jena). Collagen fibers in the ceramic pores were studied in polarized light of Polmy-A microscope after the staining of the sections with picrosirius red [9]. Photos were taken with a Canon digital camera.

2.2.5 Histomorphometrical studies

Relative volume (%) of bone tissue (compared with total pores volume) in PHA inner macropores was identified in accordance with the principles of counting of points available on the studied object. During implantation of the PBCP granules the relative volume (%) of ceramic particles, bone and soft tissue (connective tissue, blood vessels, bone marrow) was accounted on comparison with the total volume of defect. Following parameters were investigated:

ROI (1): the percentage of bone area in total pores space [(bone area/total pore space) × 100%] (Table 1);
ROI (2): the percentage of bone area in total implant area [(bone area/total implant area) × 100%] (Table 2);
ROI (3): the percentage of PBCP area in total implant area [(PBCP area/total implant area) × 100%] (Table 2);
ROI (4): the percentage of soft tissue area in total implant area [(soft tissue area/total implant area) × 100%] (Table 2);

Table 1 Volume (%) of bone formed in inner macropores of PHA samples implanted into distal metaphysis and greater trochanter of rabbits' femur ($n = 15$)

Study duration	Distal metaphysis	Greater trochanter
14 days	8.82 ± 0.77	0
1 month	19.64 ± 1.42*	11.79 ± 1.74*,**
3 months	80,70 ± 2,11*	67.39 ± 4.44*,***

*$P < 0.001$ vs. previously study duration; **$P < 0.001$ vs. area implantation of distal metaphysis; ***$P < 0.05$ vs. area implantation of distal metaphysis

A BIOLAM microscope (usually at 80 magnification) and a square net ocular insert with 289 points (both produced by "LOMO", Russia) were used [10]. Digital data were processed using the methods of variation statistics and STATISTICA 5.11 application for Windows.

3 Results

3.1 Characterisation of materials

The PHA of improved mechanical characteristics were processed of a nanocrystalline HA powder of the Ca/P = 1.65 ± 0.03 [6]. The lowering in Ca/P ratio from the almost stoichiometric value of 1.65–1.5 caused little change in the extent of particle sizes in the final powder. The particle size distribution curve had two main peaks near 70 nm and 130 nm for the powder of Ca/P ≅ 1.65 [6]. SEM pictures of the powder with Ca/P ≅ 1.5 also showed crystallites of few tenth nanometers in size (Fig. 1a). Consequently, it was expected that (as when using the nanodispersive Ca/P ≅ 1,65 powder [6]) strength properties in granules of the powder should improve owing to increase in the density of their framework.

IR spectroscopic and XRD analyses showed that within the sensitivity limits of the methods (approximately 3 wt.% and 0.5 wt.%, respectively) the powder was a nanocrystalline substance based on HA with some carbonate contamination (Figs. 2a and 3a).

XRD pictures of the processed granules consisted of two systems of maxima attributed to HA and β-TCP (Fig. 2b). Using a calibration dependence of "intensity versus concentration" obtained earlier for mechanical mixtures of pure HA and β-TCP in various proportions (according to known requirements [11]) showed that the intensity ratio of the strongest diffraction peaks of (110) HA and (112) β-TCP in Figure 2b corresponded to near 60HA/40 β-TCP composition in the granules.

Though from IR analysis could not be drawn information on the relative amount of phases in a sample, IR spectra of the granules also confirmed a BCP material: the

Table 2 Volume (%) of bone, PBCP and connective tissue at the area implantation of distal metaphysis and greater trochanter of rabbits' femur ($n = 15$)

Study duration	Distal metaphysic			Greater trochanter		
	Bone	PBCP	Soft tissue	Bone	PBCP	Connective tissue
7 days	1.76 ± 0.35	65.29 ± 1.77	6.23 ± 1.29	1.79 ± 0.2**	65.41 ± 1.42	10.93 ± 2.11
1 month	44.34 ± 2.35*	39.91 ± 2.14*	15.75 ± 0.88*	29.0 ± 2.63*,**	47.56 ± 2.5*,***	16.68 ± 3.02
3 months	47.58 ± 1.23	23.49 ± 1.7*	28.93 ± 1.48*	40.49 ± 2.91*,***	38.87 ± 3.22****	20.34 ± 1.04**
6 months	42.3 ± 3.35	3.1 ± 1.04*	54.6 ± 4.39*	38.86 ± 1.46**	14.45 ± 1.38*,**	47.36 ± 1.86*

* $P < 0.001$ vs. previously study duration; ** $P < 0.001$ vs. area implantation of distal metaphysis *** $P < 0.05$ vs. area implantation of distal metaphysis **** $P < 0.05$ vs. area implantation of distal metaphysis

absorption bands in Fig. 3b corresponded to HA and β-TCP.

The granules had two kinds of pores: micropores and quasispherical macropores. The micropores formed a network of interconnected channels with a transverse size (primarily) of a few microns (Fig. 1b). The extent of microporosity was within 20–30% and was evaluated using granules prepared under the same conditions but without adding the porosizer. The macroporores of 300–650 μm in diameter (Fig. 1c) were mainly formed as a result of decomposition and burnup of the foaming agent. The volume of the macroporosity could be increased up to 45% and was given by the amount of introduced foamer. However, the total porosity was usually around 50% (±5%), i.e. similar to that in the natural bone.

The extent of compressive strength of the PBCP developed in this study was compared with that for other HA-based materials (Fig. 4). The left points in the drop-down curve corresponded to a dense ceramics produced by usual uniaxial molding of a HA powder with Ca/P = 1.65 at 20 MPa and subsequent thermal treatment of the powder compact. The (micro) porosity of the dense HA ceramics was about 7%, and the crushing strength was between 230 and 300 MPa. The group of points in the center of the curve reflected the compression strength of around 80 MPa for the "dense" HA granules (i.e. having micropores only), and the right point group of 20–30 MPa for the porous HA granules [6] denoted in this study as the PHA. The strength values of the developed PBCP were within the last group of points. It meant that they had similar porosity and strength characteristics to those of the porous HA (PHA) granules already approbated clinically. Consequently, the PBCP could be also used for bone defects plasty in both unloaded and loaded sites in the skeleton. Owing to the fact, that the two materials, PBCP and PHA, practically had the same porosity and strength characteristics but would differ in solubility, the first step in clarifying the nature of the effect of loading on the biodegradation was to study the morphological features of their integration to bone after implantation into differently loaded osseous defects and the role of the material dissolution in this process.

3.2 Behaviour of animals

Animals were mobile and the operated extremities were loaded to full extent in all research stages. No complications were observed. Compact connection tissue was located above implantation zone.

3.3 Implantation of the PHA granules

Fourteen days: Distal metaphysis. PHA samples in defects closely contacted the bone tissue. New bone trabeculas having high-density osteocytes and osteoblasts on the surface prevailed in outer macropores (Fig. 5a). In inner macropores, fibrous tissue was observed while the formation of coarse woven bone was seen only in minor areas (Table 1). Bunches of collagen fibers in these areas were seen in polarized light. The fibers were oriented along the surface of pore and possessed bright refraction (Fig. 5b). Numerous osteoclasts were seen in pores adjacent directly to ceramic material (Fig. 5c). In micropores, tissue liquid and single undifferentiated cells with rounded nuclei were observed.

Proximal metaphysis (greater trochanter). In contrast to distal metaphysis, fibrous tissue with irregular collagen fibers of weak refraction was seen in major part of inner and outer pores. Coarse woven bone was identified only in single outer pores. Single osteoclasts were situated on ceramic surface in such pores. Some inner macropores were filled with chondroid like in distal metaphysis (Fig. 5d). Tissue liquid was identified in micropores.

1 month. Outer pores of the PHA implanted into *distal metaphysis* were filled with lamellar bone connected to the host bone. Bone and fibrous tissues were seen in inner macropores. Comparing with the previous period (14 days), the relative volume of bone tissue increased by 122.6% (Table 1).

In *proximal metaphysis* (*greater trochanter*) the granule was surrounded by newly formed bone. However, in contrast to the distal part, coarse woven bone was seen in some pores.

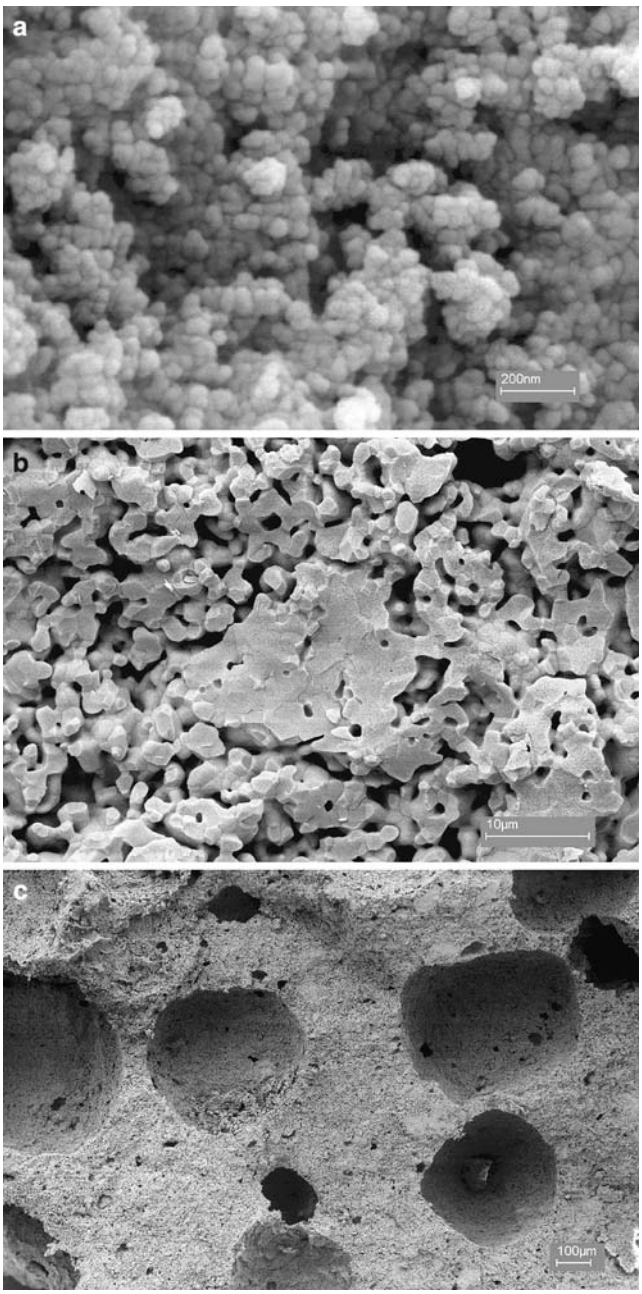

Fig. 1 SEM micrographs of: (**a**) the nanodispersive starting HA powder of Ca/P ≅ 1.5; (**b**) micropores and (**c**) macropores in the PBCP granules (60 HA/40 β-TCP)

In inner macropores fibrous tissues were prevailed (Fig. 5e). The relative volume of bone was increased in comparison with that of 14 days but was 40% less than in PHA pores of distal metaphysis (Table 1). Micropores were filled with loose connective tissue.

In *3 months*, the samples in *distal metaphysis and greater trochanter* were closely surrounded by bone which filled all the outer pores (Fig. 5f). In inner pores of PHA implanted into *distal metaphysis*, the bone volume was

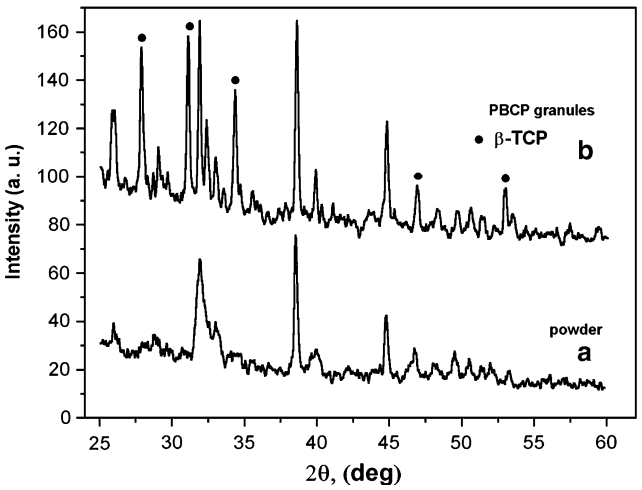

Fig. 2 Diffractograms of (**a**) the starting powder and (**b**) the PBCP granules (60 HA/40 β-TCP)

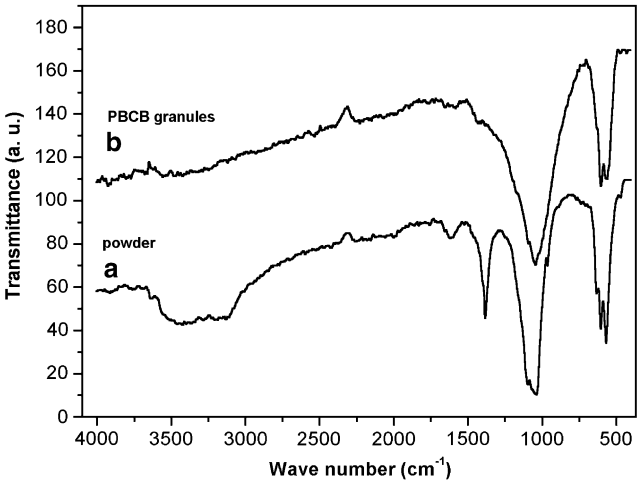

Fig. 3 IR spectra of (**a**) the starting powder and (**b**) the PBCP granules (60 HA/40 β-TCP)

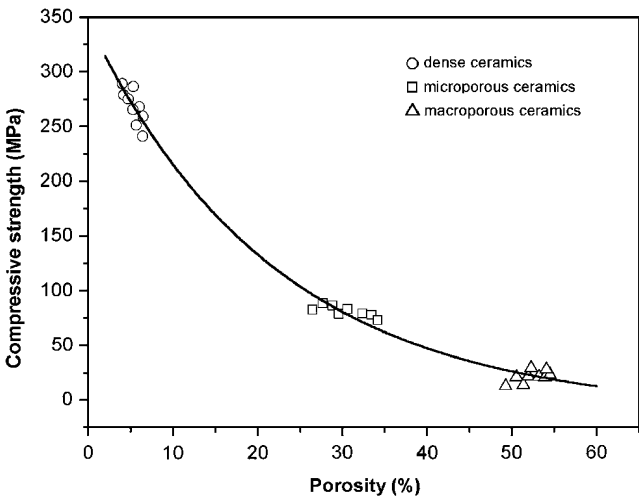

Fig. 4 Compressive strength in CPC materials of various porosity

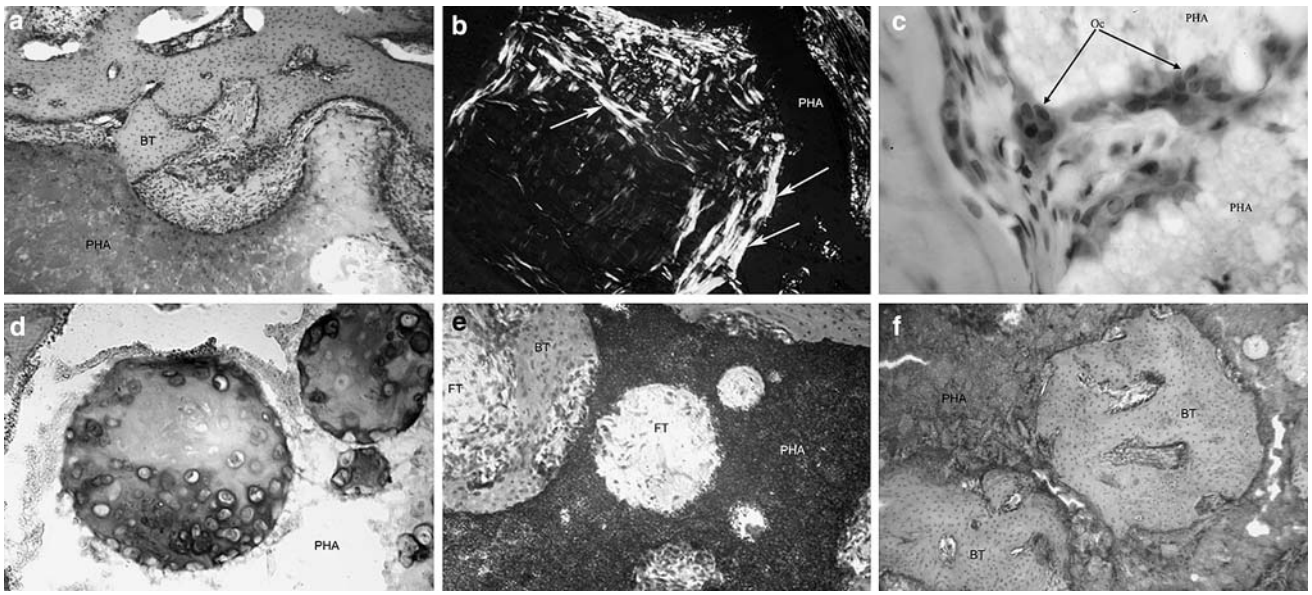

Fig. 5 PHA pores. *14th day*. Distal methaphysis of femur: (**a**) Newly formed bone tissue (BT) in outer pore. Haematoxylin and eosin. 80×; (**b**) Refraction of collagen fibers (arrow) in newly formed bone tissue in inner pore. Picrosirius red. Polarized light, 100×; (**c**) Osteoclasts (Oc) on the ceramic surface. Greater trochanter of femur. Haematoxylin and eosin. 1000×. Greater trochanter of femur: (**d**) Pores filled with chondroid. Toluidine blue, pH = 2.5. 80×. *1 months*: (**e**) Greater trochanter of femur. Newly formed woven bone (BT) and fibrous tissue (FT) in inner macropores. Haematoxylin and eosin. 80×; (**f**) *3 months*. Distal methaphysis of femur. Bone tissue (BT) in inner pores. Haematoxylin and eosin. 80×

16.5% more than that in PHA pores of *greater trochanter* (Table 1). Fibrous tissue and chondroid were still preserved along with bone. Tissue liquid was in micropores.

In 6 *months*, lamellar bone filled inner and outer macropores of the PHA implanted into *distal* and *proximal femur*. However, single inner macropores contained woven bone and fibrous tissue while micropores were filled with tissue liquid.

3.4 Implantation of the PBCP granules

7th day: In both *distal metaphysis* and *greater trochanter*, the morphologic situation was similar. The granule contacted the host bone. Its edge was surrounded by fine loop bone trabecula net with high density of osteoblasts and osteocytes. Rounded macro- and micropores of various diameters were found in the granule. They were mostly filled with tissue liquid. Connective tissue was seen in single macropores. Some micropores contained single cells among which fibroblasts, low differentiated cells and lymphocytes were observed.

In the host bone cortex and trabecules adjacent to defect areas without osteocytes were seen.

One month after implantation, the PBCP were surrounded by mature spongy bone that filled outer pores in both *distal metaphysis* and *greater trochanter*. In both cases, edges of the outer pores were not clear and cavities were seen in some places where multi-nucleus cells were located.

In *distal metaphysis*, internal pores were enlarged and possessed irregular edges. They mostly consisted of lamella trabeculas alternating with connective tissue. The bone volume increased by 24.9% comparing to the value of the previous period (Table 2). Active bone formation was marked during the first month after implantation. Macropores of various size and configuration were connected by enlarged through micropores filled with connective tissue with high density of cells. At the same time, micropores were seen which contained tissue liquid and single cells.

Total composite volume was 38.9% less than that after 7 days (Table 2).

Unlike the distal metaphysis, in *greater trochanter*, the PBCP volume decreased by 27.3% in comparison with that of 7th day and was 19.2% less than the graft volume in distal metaphysis. Edges of internal macropores remained clear. There were bone trabeculas with high density of osteoblasts in major part of pores. Lamellar bone trabeculas and connective tissue were observed in some pores. Comparing with 7th day, the bone tissue volume increased by 1520% and was 34.6% less than that in pores of composite implanted into metaphysis (Table 2).

Tissue liquid and single cells were seen in micropores of central part of the implant. Micropores were enlarged in

peripheral parts of the granule. Connective tissue with low density of cells was seen in these micropores.

Host bone distant from the defect possessed normal structural organization.

Three months after the implantation, the samples were closely surrounded by mature bone that filled external pores and penetrated inside the implant both in *distal metaphysis* and *greater trochanter*.

Decrease in ceramic material volume was characteristic for both cases though biodegradation process was more pronounced in *distal metaphysis* where the PBCP volume was 39.6% less than that in *greater trochanter*. Structure of internal pores could not be defined. A united net of bone trabeculas was formed in the implantation zone. This net was fine loop in greater trochanter and was large loop in distal metaphysis. Bone trabeculas were thin in such areas. The bone tissue volume did not change in distal metaphysis implantation zone in comparison with that in the previous stage.

Single internal macropores with uneven edges remained in the PBCP implanted into greater trochanter. Osteoigenic tissue with a high density of osteoblasts and fibroblasts were found in such pores and between some composite fragments (Fig. 6a, b). The bone formation process continued in greater trochanter. The volume of bone tissue increased by 28.4% in comparison with that in the previous period.

After *6 months*, in the PBCP implantation zone of both *distal metaphysis* and *greater trochanter*, mature bone trabeculas were found. Minor ceramic fragments and areas of bone marrow, connection tissue were situated between these trabeculas (Fig. 6c, d). The ceramic material volume in distal metaphysis was 366.1% less than that in greater trochanter. Differences in the volume of bone tissue that filled defects in both parts of femur were not discovered (Table 2). On comparison with distal metaphysis, large fragments of the PBCP were revealed in the area of implantation between bone trabeculas. Presence of osteoclasts on ceramic micro-particles attracted attention.

4 Discussion

The results of the study have proved that the processes of bone tissue formation in PHA and PBCP implantation zones were influenced by composition, porosity structure and loading conditions of the materials.

The characteristic feature of the studied materials was the presence of connecting micropores in macropore walls. It was clearly seen after 7 days implantation that there appeared narrow canals filled with tissue liquid and later with cells between large pores. Such a interconnected pore network not only increased the total porosity volume but also facilitated an invasion of cells and blood vessels into the graft creating an optimal transportation systems. This improved delivery of necessary nutrients and oxygen intensified energy supply to the bone formation processes [12].

However, as a part of internal micropores was not filled with bone or osteogenic tissue by 90 days implantation, it

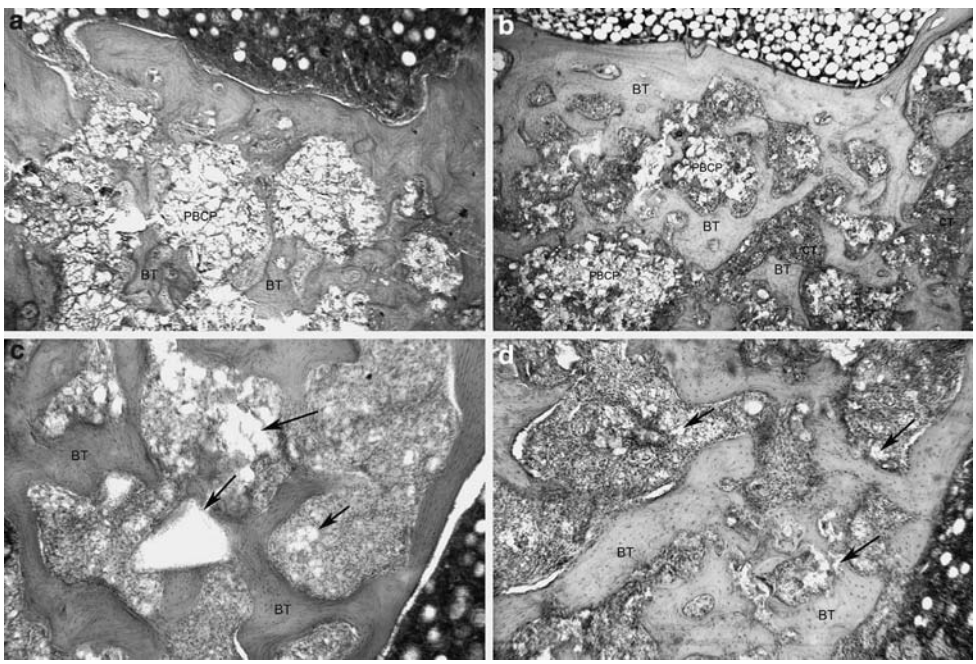

Fig. 6 PBCP pores. *3 months:* (**a**) Greater trochanter of femur. Bone tissue (BT) in inner pores. Haematoxylin and eosin. 80×; (**b**) Distal methaphysis of femur. Bone tissue (BT) and areas of connective tissue (CT) in pores. Haematoxylin and eosin. 80×. *6 months*: (**c**) Greater trochanter of femur. Trabecular bone tissue (BT). Bone marrow and connective tissue in intertrabecular spaces between large particles of granule (arrows). Haematoxylin and eosin. 80×; (**d**) Distal methaphysis of femur. Trabecular bone tissue (BT). Bone marrow and connective tissue in intertrabecular spaces between small particles of granule (arrows). Haematoxylin and eosin. 80×

could be suggested that the size of the connecting micropores was not sufficient for osteoblast penetration. Really, a multitude of micropores in the materials used were less than 10 mcm in cross-size (Fig. 1b), but pores of at least 10 mcm were necessary for osteoblasts to penetrate the graft [13, 14]. No cells was found in some internal micropores. The pores were filled with tissue liquid that could suggest that some of them were not through. After 30 days, bone tissue was seen in all large external pores and even in micropores having access to surface. While using non-resorbing grafts (PHA), the bone tissue formation and maturity also depended on loading area.

Loading conditions also influenced biodegradation of the PBCP. Ceramics bioresorbtion occurred more intensively in distal metaphysis during the whole period of observation. Influence of loading on ceramics biodegradation can be associated with the well-known fact that loading causes activation of osteoclast precursors in bone which, in their turn, are involved in processes of cell-mediated ceramics bioresorbtion [15, 16]. Osteoclasts on ceramic composite particles were observed at all stages in the study. Consequently, biodegradation of the ceramic composite (HA + β-TCP) material should be considered as a multi-stage process in which osteoclasts participate but chemical dissolution plays an important role.

Loading conditions played an appreciable role in bone tissue formation in implantation zone as well. For example, if in distal metaphysis (loaded bone zone) formation of bone tissue was seen in internal PHA macropores after 14 days, in pores of greater trochanter (slightly loaded bone zone)—no bone tissue was revealed in this period. After 1 and 3 months, the bone tissue volume was 1,66 and 1,19 times higher in internal PHA pores in distal metaphysis than that in greater trochanter, correspondingly.

The regeneration study in area of the PBCP implantation revealed that activity of osteogenesis was more in distal metaphysis. The peak of activity was observed after 1 month. In 6 months, no difference in the bone volume in the area of PBCP implantation in distal metaphysis and great trochanter was observed. This confirms the data on active bone formation in earlier periods of bioceramics implantation and its slowdown in later ones [17].

The differences in bone forming rate around the ceramic implants and in their pores in various skeleton areas should be clearly associated with the extent of physiological loading. It is known that characteristics of a material surface and mechanical loading play a critical role in proliferation and differentiation of osteoblasts and matrix synthesis by them [18]. Besides, the conditions of bone loading influence on circulation processes in lacuna canal system and thus reflect in bone tissue remodeling [19]. It is also known that bones in loaded and slightly loaded skeleton sections differ in bone tissue density and have specific organization features.

Loading regime influenced the process of bone tissue remodeling process in ceramics implantation zone. It was more active in distal metaphysis compared with greater trochanter. The peak of bone formation was observed after 1 month. The absence of significant bone tissue volume increase between 1st and 3rd months of observations in the area of PBCP implantation in distal metaphysis should be just associated with the reconstruction of bone tissue. Formation of bone tissue in greater trochanter only achieved a peak in 3 months. Soft tissue (connective tissue, bone marrow and osteoid) observed in both cases was located in intertrabecular space and alternated with fragments of PBCP implantation material.

5 Conclusions

It has been proved that bone formation processes during the implantation of PHA and PBCP containing 60 HA/40 β-TCP into loaded (distal metaphysis) and slightly loaded (greater trochanter) bone zones were more intensive in distal metaphysis.

PHA biodegradation was not observed. Bone tissue maturity in PHA pores increased with time.

The increase in bone volume was revealed in both zones of femur during the first month of PBCP implantation. In subsequent terms, the increase of bone volume in distal metaphysis was not noticed. Reorganization of bone tissue took place. In greater trochanter, the bone volume increased to 3 months. After 6 months, reconstruction of newly formed bone was seen on the account of formation of large loop bone trabecula net in distal metaphysis and greater trochanter.

An active biodegradation process was observed in the external pores of PBCP implants during the first month. Disturbance in porous structure of the composite was observed in distal metaphysis after 3 months. In greater trochanter, pores remained in the graft though their configuration was broken due to walls resorbtion and integration of the pores. After 6 months, presence of large composite fragments situated in intertrabecula spaces of greater trochanter was found to be a distinctive feature.

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