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CHANGES OF HAp/PLLA BIOCOMPOSITES AND TISSUE REACTION AFTER SUBCUTANEOUS IMPLANTATION

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Summary. *Experimental and clinical studies have shown that HAp granules and powder may be successfully applied in reconstruction of bone defects. Composite HAp/PLLA biomaterial embodies good characteristics of each of these biomaterials. Differences in porosity, microstructure, compressive consistency as well as in bioresorbility of HAp/PLLA are achieved by using PLLA of different mole masses. The aim of this study was to analyse light microscopy of the tissue reaction and changes of implants made of combination of syngeneic bone fragments and HAp/PLLA biocomposites with PLLA molecule mass of 430 000 (HP2) and 50 000 (HP3). The mice were divided into 4 experimental studies, 8 animals each. Two groups underwent implantation, one group of mice was falsely operated, and one group was used for general control. Pathohistological analysis of implants was performed three months after subcutaneous implantation. Phagocytosis of HAp/ PLLA and bone was noticed on both implants' preparations. Blood vessels were also noticed, confirming the process of angiogenesis around the implants' particles. The holes around particles of biocomposites and bone fragments represented the signs of their resorption. The presence of multinuclear cells between implants' particles was observed and it was more prominent in HP3 implants. Osteogenesis is more intense in HP3 and ectopic hematopoesis is present. Our results show that designed HAp/PLLA biocomposites have good biocompatibility, their combination with bone fragments of a vital bone enables osteogenesis and hematopoesis and these processes are more prominent if PLLA biocomposite with lower molecular mass was used.*

Key words: *Subcutaneous implantation, hidroxyapatite, polylactide, biocomposite*

Introduction

Implantation of biomaterial is nowadays one of important trends in solving the problem of bone tissue loss. Calcium hydroxiapatite (HAp), as the most representative bone element, has become a serious candidate for such implantations. Experimental and clinical studies have shown that HAp granules and powder may be successfully applied to reconstruction of bone defects (1,2). It has been shown that HAp has good compatibility and bioinactivity and appropriate consistency. On the other hand, HAp is not so used in bone discontinuity due to its fragility, because natural bone shows elasticity and great resistance to stretching owing to the presence of fibrillary protein component and collagen. Synthetic polymer poly-L-lactide (PLLA) is often used as polymeric material and has some features, which make it useful for combining with HAp (3,4). Fibers of PLLA may strengthen HAp and its good bioresorbility provides space for tissue extension (5). Composite biomaterial HAp/PLLA embodies good features of each of these biomaterials (6). Differences in porosity, microstructure, compressive consistency as well as bioresorbility of HAp/PLLA may be achieved by using PLLA with different mole masses (7,8,9). The most appropriate way of recognizing the usability of biocomposite is its implantation into the organism. Subcutaneous implantation is relatively often used in such studies, because it is simple and microenvironment of implant enables the occurrence of ectopic osteogenesis (10).

The aim of the study

The aim of the study was to analyse by means of light microscopy, the tissue reaction and the changes of implants made of combination of bone fragments and HAp/PLLA biocomposites of different molecule mass (50 000 and 430 000) three months after subcutaneous implantation.

Material and methods

Experimental animals: Syngeneic Balb/c mice were used (male, 3 months old, and weight -24-26 g.). The animals were fed by *ad libidum* and kept on constant temperature of 23^{o}C.

Implanted material: Biocomposites made of 80% of HAp and 20% of PLLA and syngeneic bone fragments were used. Biocomposite with molecule mass (MM)

PLLA of 430 000 was labeled as HP2, and biocomposite with MM PLLA of 50 000 was labeled as HP3. Before implantation composites were crushed up in a very fine powder in mortar, disinfected in 70% alcohol and rinsed out in sterile physiological solution. The bone for implantation was taken from another mouse and crashed up in a sterile physiological solution. The same amounts of biocomposite powder and bone fragments were mixed with fibrin glue, which enabled formation of a lump, 1mm in diameter, which was then implanted.

Subcutaneous implantation: The lump made of combination of bone fragments and HAp/PLLA biocomposites was subcutaneously implanted through the top of the wide needle 4/ 18 into intracapsular region of the back. Before implantation, the skin of the mouse was disinfected with alcohol and after implantation mice were intraperitoneally given antibiotic.

Experimental design: One mouse underwent the implantation of only one material, so that 2 experimental groups of 8 animals were made. The groups were labeled as HP2, with PLLA 430 000, and HP3, with PLLA 50 000. Three months after implantation, the animals were sacrificed by cervical dislocation and after that the extraction of the implant was performed.

Pathohistological analysis: The samples of implant were fixed in Brasil-Bouine fixative for 3 hours and post-fixed in 4% buffered formaline during the night. Fixed implants were decalcinised electrolitically in water solution 8 vol% HCl and 10 vol% of formic acid. The process of decalcination was conducted for two hours at 100 V and 50 mA. The tissue was then dehydrated in alcohol and implanted into paraplast. Sections of 3-6 micrometers were stained by haematoxillin and eosin (HE). Dehydrated portions were fixed on glass and then analyzed on light microscope. In particular, the connection between implant and surrounding tissue was observed.

Results

HP2. Phagocytosis of HAp and bones is noticed on preparations. Blood vessels are also visible, confirming the process of angiogenesis in surrounding particles of implants. There are groups of cells in form of granulomas in some spots. A tiny connective capsule was formed around the implant. Figure 1 illustrates the holes as the consequence of resorption of biocomposites and the presence of multinuclear cells between particles in connective tissue. Large particles of material have not been resorbed yet, but phagocytosis and signs of resorption are present on peryphery. Figure 2 shows the presence of several multinuclear cells, the signs of resorption, and bone formation.

HP3. Phagocytosis of HAp and bone fragments can be noticed on preparations. Mature fibrous tissue of capsule with few cell elements is formed around the implant. Ectopic hematopoesis with megacariocytes is very prominent in some spots. Figure 3 shows wide spaces around grains of HAp, which is probably the consequence of intensive resorption. Cell density is relatively low in spite of good vascularization. Figure 4 shows ectopic hematopoesis and osteogenesis. In one part of bone fragment the biocomposite is present surrounded by an empty field, which is probably the consequence of resorption. The signs of resorption are present on bone fragment as well. A white and red line is present in hematopoetic tissue.

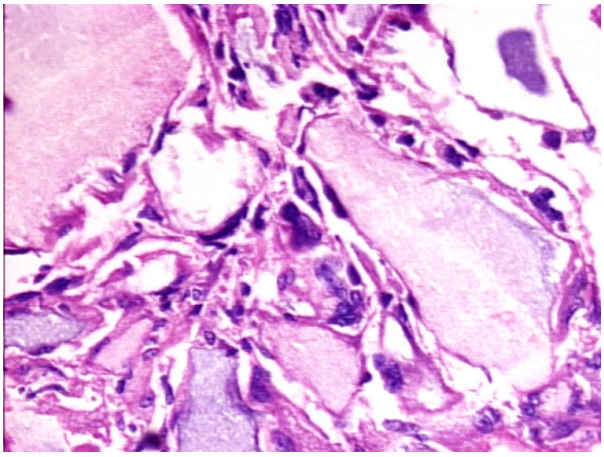

Fig. 1. Resorption of implanted biomaterial HP2 (40×, HE)

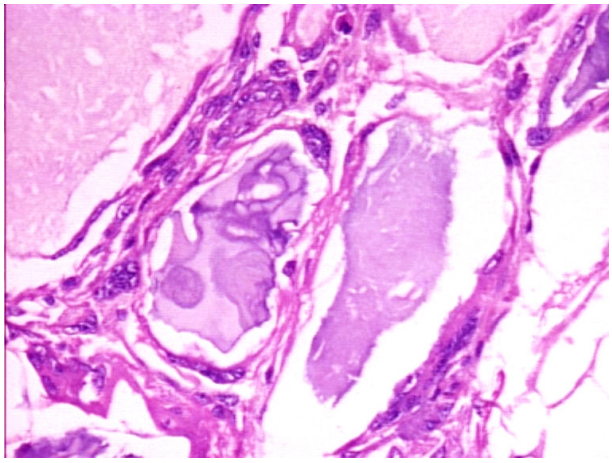

Fig. 2. Resorption of implanted bone fragments close to HP2 particules (40×, HE)

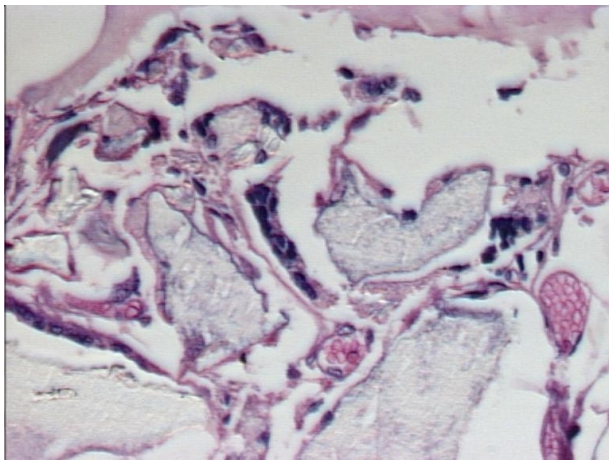

Fig. 3. Good tissue vascularization around implanted biomaterial HP3 (40×, HE)

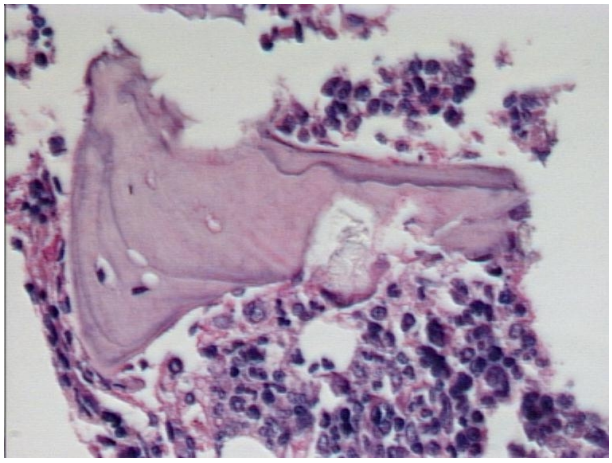

Fig. 4. Ectopic haematopoiesis and osteogenesis in implant close to biocomposite HP3 (40×, HE)

Discussion

Subcutaneous implantations represent a common model for the examination of the characteristics of biomaterial. These experiments enable the perception of biocompatibility, bioresorbility, interaction with the tissues in the organism, and some other biofunctional characteristics. The possibility of this model to induce osteogenesis is very valuable. In such a way it is possible to recognize very important factors and conditions necessary for reparation of bone tissue (10,11). Our model, therefore, represents a simulation of interskeletal implantation, because the fragments of vital bone are mixed with the examined ceramics, stick with fibrin glue, and implanted bone fragments imitate autologous ones due to the donor who is syngeneic animal.

Both examined materials had good bioresorbility. Osteogenic and angiogenic activity has been found in the presence of implanted fragments of vital bone. More prominent resorption of multinuclear cells by phagocytosis was noticed on HP2 material, while greater amount of material was resorbed in case of HP3, which had been already shown in earlier experiments (12). On the other hand, ectopic osteogenesis and hematopoesis, as well as vascularization were more prominent in implants with HP3 material. Since these materials differ only in PLLA molecule mass, the processes that depend on molecule mass of polylactides influence the stated differences. As it was shown in our previous study (12), resorption was faster if molecule mass was lower. This might be the reason that three months after implantation such implants had less engaged phagocytic system. Obviously, cell conditions with such cytokine balance preserve intensive hematopoesis and certain osteogenesis.

In the study of cylindric subcutaneous implants of poly-L-lactide, poly-L/D-lactide and poly- L/DL-lactide Mainil et al. reported that the fall of molecule mass of implants was the most intense one month after implantation, but none of the polymers was completely resorbed within a year. Inflammatory reaction around implanted biomaterials was dependent on the form of the implant and features of its surface, porosity and chemical stability (13). In our experiment it was confirmed that tissue reaction on polylactides used in this study was manifested by formation of the capsule around the implant and the presence of fibroblast, macrophages, polymorphonuclear cells and cells characteristic for reaction to the foreign body. Thereby, cell reaction to the implant was more intense around polymers that undergo a faster decomposition, which was registered. Generally, cell reaction to implant was more intense on disconnected endings of bone fragments and rough surfaces of implants than around flat surface.

Conclusion

The examined composite materials made of hydroxiapatite and polylactide show good biocompatibility. Resorbtion was more prominent on biocomposite with lower molecule mass of polylactide. Osteogenesis, hematopoesis and angiogenesis are performed subcutaneously in the presence of both examined biocomposites. Three months after implantation the signs of these processes are more visible in the presence of polylactide particles of lower molecule mass.

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PROMENE BIOKOMPOZITA HAp/PLLA I REAKCIJA TKIVA POSLE SUBKUTANE IMPLANTACIJE

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Kratak sadržaj: *Eksperimentalna i klinička istraživanja su pokazala da se granule i prah HAp mogu uspešno primeniti za rekonstrukciju koštanih defekata. Kompozitni biomaterijal HAp/PLLA objedinjuje dobre osobine koje poseduje svaki od ovih biomaterijala. Razlike u poroznosti, mikrostrukturi, kompresionoj čvrstoći kao i bioresorbilnost HAp/PLLA postižu se korišćenjem PLLA različitih molskih masa. Cilj rada je bio da se svetlosnom mikroskopijom analiziraju odgovor tkiva i promene implantata napravljenih od kombinacije singenih koštanih fragmenata i biokompozita HAp/PLLA sa PLLA molekulske mase od 430 000 (HP2) i 50 000 (HP3). Miševi su bili podeljeni u 4 eksperimentalne grupe od po 8 životinja. U dve grupe je urađena implantacija, jedna grupa miševa su bili lažno operisani, a jedna grupa je služila kao opšta kontrola. Posle 3 meseca od subkutane implantacije rađena je patohistološka analiza implantata. Na preparatima oba tipa implantata se vidi fagocitoza HAp/PLLA i kosti. Takođe su vidljivi krvni sudovi koji svedoče o procesu angiogeneze u okolini partikula implantata. Prazna polja oko partikula biokompozita i koštanih fragmenata predstavljaju znake njihove resorpcije. Vidljivo je prisustvo multinuklearnih ćelija između partikula implantata, a to je izraženije u HP3 implantatima. U HP3 je intenzivnija osteogeneza i prisutna je ektopična hematopoeza. Naši rezultati pokazuju da dizajnirani biokompoziti HAp/PLLA imaju dobru biokompatibilnost, da je u njihovoj kombinaciji sa koštanim fragmentima žive kosti moguća osteogeneza i hematopoeza i da su ovi procesi izraženiji ako je u biokompozitu PLLA manje molekulske mase.*

Ključne reči: *Subkutana implantacija, hidroksiapatit, polilaktid, biokompozit*