TOMOGRAPHIC AND HISTOLOGICAL ASSESSMENT OF BONE REGENERATION IN THE EXPERIMENTAL DEFECTS IN RABBIT FEMORAL TROCHLEA TREATED WITH RESORBABLE SCAFFOLDS

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Introduction

Nowadays a great attention is drown to resorbable materials, for example copolymers of lactide and glycolide (PLG), as potential materials enhancing bone tissue regeneration [1]. It is related to the fact that one may control their biodegradation by changing lactide/glycolide ratio, chain microstructure, molecular mass, and crystallinity. PLG materials can be processed into scaffolds which mimic the structure of spongy bone, thus provide temporary matrix enhancing osteogenic cells adhesion, proliferation and differentiation. Moreover, the PLG scaffolds can be modified with hydroxyapatite (HAp), the most abundant mineral component in natural bone, in order to ensure better conditions to adhering cells and to promote the mineralisation of newly-formed tissue. Our previous experiments showed that the scaffolds produced from PLG materials support growth and differentiation of osteogenic cells in vitro [2,3].

This study aims at analyzing bone tissue regeneration in critical-size experimentally created bone defects in rabbits treated with different PLG scaffolds using computed tomography and histological technique.

Materials and methods

PLG 85:15 (M_n=100 kDa, d=2.1) was synthesized according to the method described previously [4]. Two types of cylindrical porous scaffolds (diameter 4 mm, height 5 mm) with porosity of 85% and size of the pores of 250-320 μm and 400-600 μm respectively, were obtained by a newly modified solvent casting/salt particulate leaching method [3]. The scaffolds were also modified with HAp by biomimetic SBF method [5]. The research protocol for the in vivo study was approved by the local ethic committee. The scaffolds after being sterilized with oxygen peroxide plasma (Sterrad 120, ASP, J&J) were implanted into experimentally created critical-size osteochondral defects in New Zealand rabbit femoral trochlea. Four animals were used and each animal received three implants. The fourth defect was left empty. After 26 weeks from the surgery the animals were sacrificed, the femora containing implants were excised, placed in formalin and submitted to computed tomography (CT) examinations. The evaluations were done on GXCB-500/i-CAT (Gendex Dental System, Italy) tomograph at a voxel size of 200 μm. The bone mineral density (expressed in Hounsfield units) was also evaluated from the image data for 15 μm2 areas. Afterwards, tissue blocks with implanted scaffolds and empty defect were excised, decalcified in buffered EDTA and embedded in Paraplast. Histological slices of 9 μm in thickness were prepared and stained by Masson-Goldner method.

Results and discussion

FIG.1 shows representative CT images of tissue defects treated with three different types of scaffolds and that of empty defect. The images show that bone mineralization was more advanced in the defects filled with the scaffolds having the poresize of 250-320 μm (FIG.1A) and those enriched with HAp (FIG.1B). On the other hand, defects filled with the scaffolds with bigger pores, e.g. 400-600 μm in diameter (FIG.1C), and that of empty defect (FIG.1D) exhibited lower mineralization. The quantitative data of bone mineral density (BMD) for each defect and that measured for healthy spongy bone in rabbit femoral trochlea are presented in FIG.2. The results show that BMD tended to be the highest for the scaffolds having the pores in the range of 250-320 μm enriched with HAp, while the lowest for the empty defect. Moreover, a tendency that the scaffolds with smaller pores elicited better bone mineralization than those with bigger pores of 400-600 μm was found, although these results were not statistically different.

Histological pictures after Masson-Goldner staining of

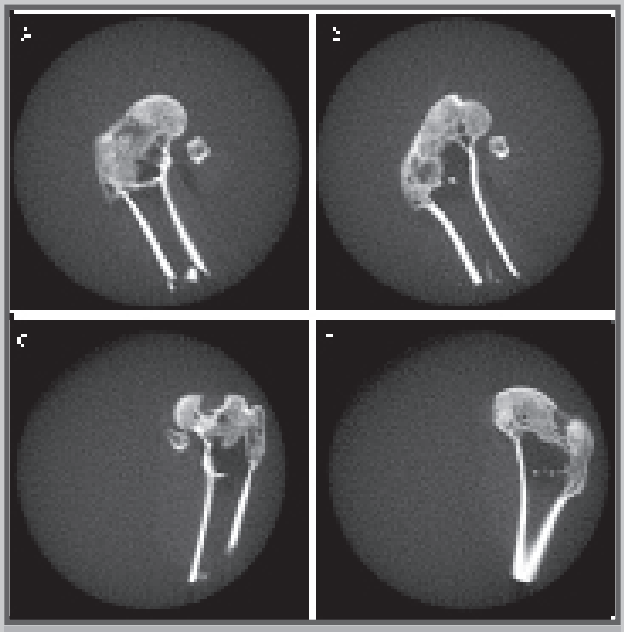

FIG.1. Computed tomography pictures of tissue defects in rabbit femoral trochlea after 26 weeks from surgery treated with scaffolds: A – poresize 250-320 μm, B – poresize 250-320 μm and HAp, C – poresize 400-600 μm, and D – empty defect.

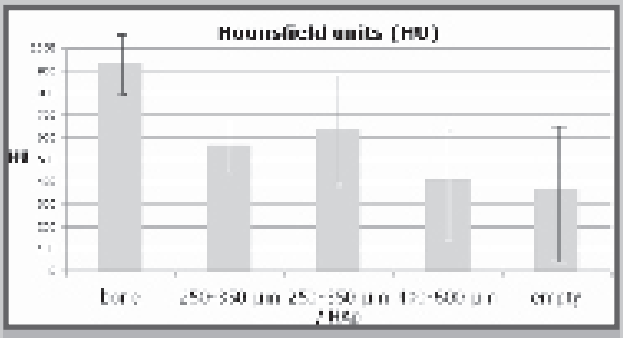

FIG.2. Bone mineral density in tissue defects in rabbit femoral trochlea after 26 weeks from surgery treated with scaffolds: A – poresize 250-320 μm, B – poresize 250-320 μm and HAp, C – poresize 400-600 μm, and D – empty defect; averages ± standard deviation.

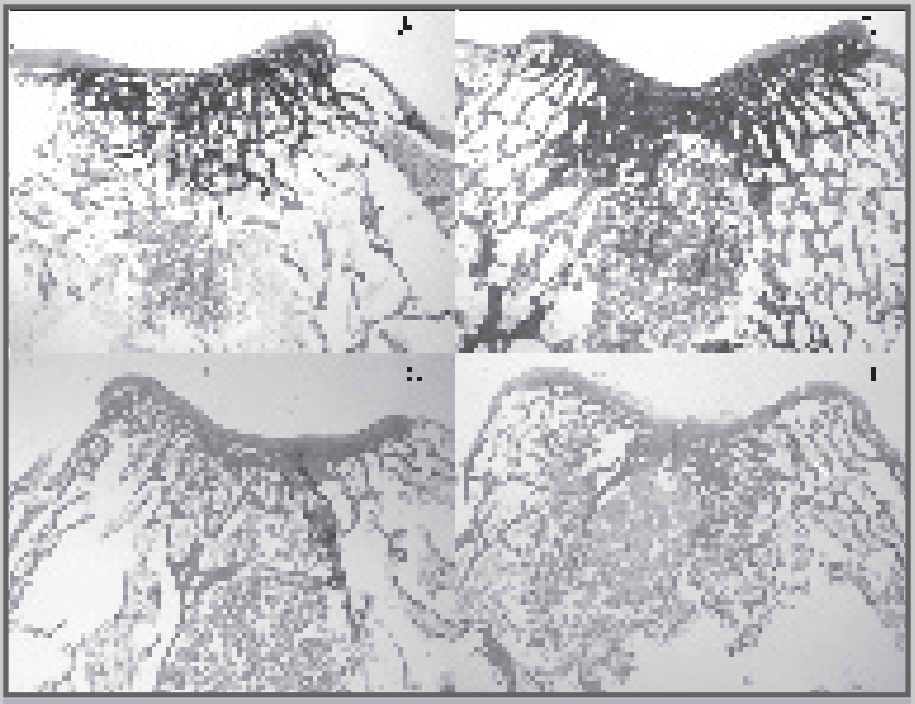

FIG.3. Histological pictures of tissue defects in rabbit femoral trochlea after 26 weeks from surgery treated with scaffolds: A – poresize 250-320 μm, B – poresize 250-320 μm and HAp, C – poresize 400-600 μm, and D – empty defect; Masson-Goldner staining, optical microscope Zeiss StereoDiscovery, original magnification 20x.

all examined materials and that of empty defect are presented in FIG.3. The results show that on the base of the implanted scaffolds newly formed bone trabeculae were observed. The remnants of the scaffold material were also visible in the implantation site. Bone regeneration was more advanced in the case of the scaffolds having the poresize of 250-320 μm (FIG.3A) and of 250-320 μm enriched with HAp (FIG.3B), than that of bigger pores , e.g. 400-600 μm in diameter (FIG.3C). It was also found that the defect reconstruction started from the peripheral bone ends and incorporated into the center of the scaffolds. In the case of empty defect the central part was filled with fibrous connective tissue (FIG.1D).

Our findings based on tomography and histological studies imply that in rabbit model the scaffolds with smaller pores better support bone regeneration and mineralization that those with bigger pores. It is contradictory to our previous findings received from in vitro studies of osteoblast-like cells in both static and dynamic conditions [3,6]. In those experiments the scaffolds with bigger pores (400-600 μm) were the most appropriate for cell adhesion, proliferation and osteogenic differentiation. We attributed it to higher pore interconnectivity and scaffold permeability, thus assuring better diffusion of nutrients, wastes and/or material degradation products in the whole volume of the scaffold. In tissue defects it seems that these parameters characterizing the microstructure of the scaffolds are not the most crucial to support tissue regeneration. Probably relative surface area of the scaffolds, which is higher for the scaffolds with smaller pores than for those with bigger pores, better enhances osteogenic cells migration from the periphery into the center of the bone defect. As a result bone regeneration proceeds faster. Additional beneficial effect in bone mineralization was found if the scaffolds were modified with hydroxyapatite. This outcome is not surprising and it was already found in many previous studies [7].

To sum up, the results show that the scaffolds with pores in the range of 250-320 μm enriched with HAp have the highest potential for regeneration of critical size bone defects.

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