POLYMERIC SCAFFOLDS WITH CONTROLLED MICROSTRUCTURE AND SURFACE CHEMISTRY: MECHANICAL AND PHYSICO-CHEMICAL CHARACTERISATION AND IN VIVO EVALUATION

E. Pamuła[1]*, E. Menaszek[1,2], M. Orzelski[3], P. Silmanowicz[3], M. Krok[1], Ł. Rumian[1], P. Dobrzyński[4]

[1] AGH University of Science and Technology,
Faculty of Materials Science and Ceramics,
Department of Biomaterials,
Al. Mickiewicza 30, 30-059 Krakow, Poland
[2] Jagiellonian University, Collegium Medicum,
Department of Cytobiology,
ul. Medyczna 9, 30-688 Krakow, Poland
[3] University of Life Sciences, Faculty of Veterinary Medicine,
Department and Clinic of Animal Surgery,
ul. Głęboka 30, 20-612 Lublin, Poland
[4] Polish Academy of Sciences,
Center of Polymer and Carbon Materials,
ul. M. Curie-Skłodowskiej 34, 41-819 Zabrze, Poland
* e-mail: epamula@agh.edu.pl

[Engineering of Biomaterials, 105, (2011), 2-4]

Introduction

Scaffolds made of resorbable polymers, for example copolymers of lactide and glycolide (PLGA), are very promising biomaterials for bone and cartilage tissue engineering and regeneration [1]. It was found that they provide temporary matrix enhancing cells adhesion, proliferation and their osteogenic/chondrogenic differentiation [2,3]. Our previous experiments showed that the scaffolds produced from PLGA support growth and differentiation of osteogenic cells in vitro [4,5]. Interestingly, the scaffolds with bigger pores (400-600 μm) were found to be the most appropriate for cell adhesion, proliferation and differentiation in vitro [5]. 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 [5]. Moreover, we have shown that the PLGA scaffolds can be modified with hydroxyapatite (HAp), the most abundant mineral phase in natural bone, which assured better conditions to adhering cells and promoted their osteogenic differentiation [6].

The aim of this study was to analyze tissue healing in critical-size osteochondral defects treated with different PLGA scaffolds in a rabbit model. Particularly we wanted to explore the influence of pore size and presence of HAp in the PLGA scaffolds on bone and cartilage tissue healing in a short-term study.

Materials and Methods

Scaffolds manufacturing

PLGA 85:15 (M_n=100 kDa, d=2.1) was synthesized according to the method described previously [7]. 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 [5]. The scaffolds were also modified with HAp by biomimetic SBF method [6].

Scaffolds characterization

The microstructure of PLGA and PLGA/HAP scaffolds was studied with the use of a scanning electron microscope (JEOL 5400) under magnifications of 50x and 2000x. Before the analysis, the samples were sputter-coated with a thin carbon layer to make them conductive. Elemental composition of the scaffolds was studied by energy dispersive X-ray (EDX) spectroscopy (Link AN 10000, UK).

The water contact angle was evaluated by drop shape analysis system (DSA 10 Mk2, Kruss) equipped with a digital camera. UHQ-water droplets of 2 μl were put on the surface of the scaffolds and immediately the picture of the droplet was taken with a digital camera. The results are presented as the average ± SD (Standard Deviation) for six individual droplets placed on each type of the scaffolds.

The mechanical properties of the scaffolds were evaluated using a compression test on the universal testing machine ZWICK 1435. The cross-head speed was set at 1 mm/min. Stress–strain curves were collected from load and displacement measurements. The compressive modulus was defined as the initial linear modulus and the compressive strength at 60% strain was recorded. The results are presented as the average ± SD for six individual scaffolds originating from each experimental group.

In vivo experiment

The scaffolds after sterilization with oxygen peroxide plasma (Sterrad 120, ASP, J&J) were implanted into experimentally created critical-size osteochondral defects in the New Zealand rabbit femoral trochlea. The research protocol for the in vivo study was approved by the local ethic committee. Four animals were used and each animal received three implants. The fourth defect was left empty. After 4 weeks from the surgery the animals were euthanatized, the femora containing implants were excised, placed in formalin, 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

Properties of the scaffolds

FIG. 1 shows the microstructure of the scaffolds registered by scanning electron microscope. The scaffolds have interconnected pores of a size close to the size of porogen particles used in the process of their preparation. Under higher magnifications, i.e. 2000x, round cauliflower-shape deposits on the pore walls are visible on the scaffolds submitted to incubation in SBF (FIG. 1C, inserts). The deposits were observed both on the surface of the scaffolds as well in the cross sections. EDX spectroscopy analysis confirmed that on PLGA scaffolds after contact with SBF calcium and phosphorus were detected (FIG. 1F, TABLE 1). The Ca/P ratio was 1.6, which is similar to that of hydroxyapatite. The SEM and EDX results obtained in this study were similar to those presented on our previous paper [5].

The results of wettability show that the water contact angle on raw PLGA scaffolds is relatively high, and does not depend on the pore size (TABLE 1). On the contrary, on the scaffolds with hydroxyapatite deposits, the water droplets immediately penetrated the scaffolds, thus making impossible registration of the shape of the droplets. It means that presence of HAp on the pore walls decreased hydrophobicity of the material, thus improving water penetration within the volume of the scaffolds.

TABLE 1. Chemical composition by EDX, water contact angle, strength and modulus of PLGA scaffolds.

	Chemical composition [at%]				Water contact angle	Strength σ	Modulus E
	C	O	Ca	P	[degree]	[MPa]	[MPa]
250-320 μm	61	39	nd	nd	126.7 ± 9.3	0.58 ± 0.10	0.63 ± 0.12
250-320 μm /HAP	17	66	10.6	6.4	nd	1.14 ± 0.31	1.72 ± 0.37
400-600 μm	58	42	nd	nd	120.4 ± 6.8	0.60 ± 0.09	0.68 ± 0.15
nd – not detectable							

TABLE 1 presents the results of compression test of the scaffolds. The results of modulus tended to be higher than those obtained in our previous experiment [5], but the differences were not statistically significant. The compressive strength and modulus of the scaffolds measured at 60% strain were significantly higher for the scaffolds with HAp. Size of pores did not influence both compressive strength and modulus.

In vivo evaluation

FIG. 2 shows macroscopic appearance of tissue defects treated with three different types of scaffolds and that of empty defect. The images show that tissue healing was more advanced in the defects filled with the scaffolds having the pore size of 250-320 μm and those enriched with HAp (FIG. 2A). On the other hand, healing process was inhibited in the defects filled with the scaffolds with bigger pores, e.g. 400-600 μm in diameter, and in the case of empty defect (FIG. 2B).

Histological pictures after Masson-Goldner staining of all examined materials and that of empty defect are presented in FIG. 3. The results show that on the base of the implanted scaffolds with the pores of 250-320 μm (FIG. 3A) and of 250-320 μm enriched with HAp (FIG. 3B) newly formed bone trabeculae were observed. Bone regeneration was more advanced in the case of the scaffolds enriched with HAp (FIG. 3B). Newly-formed hyaline cartilage was also visible in those samples (FIG. 3A, B).

On the contrary, in the case of the scaffolds with bigger pores, i.e. 400-600 μm in diameter (FIG. 3C), only some remnants of the scaffold material and connective tissue were present. It was presumably due to the fact that histological procedure (consisting of several staining, washing and drying steps) was hazardous for fine and delicate newly-formed tissue. This is an indirect proof that bone/cartilage tissue healing for this defect was inhibited in comparison with the defects treated with scaffolds with small pores. In the case of empty defect bone/cartilage tissue healing was least advanced (FIG. 3D).

The results of this study show that in osteochondral rabbit model in a short-term period the scaffolds with smaller pores better supported tissue healing than 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, in which bigger pores (400-600 μm) were the most appropriate for cell adhesion, proliferation and differentiation of osteogenic and mesenchymal stem cells [3,8].

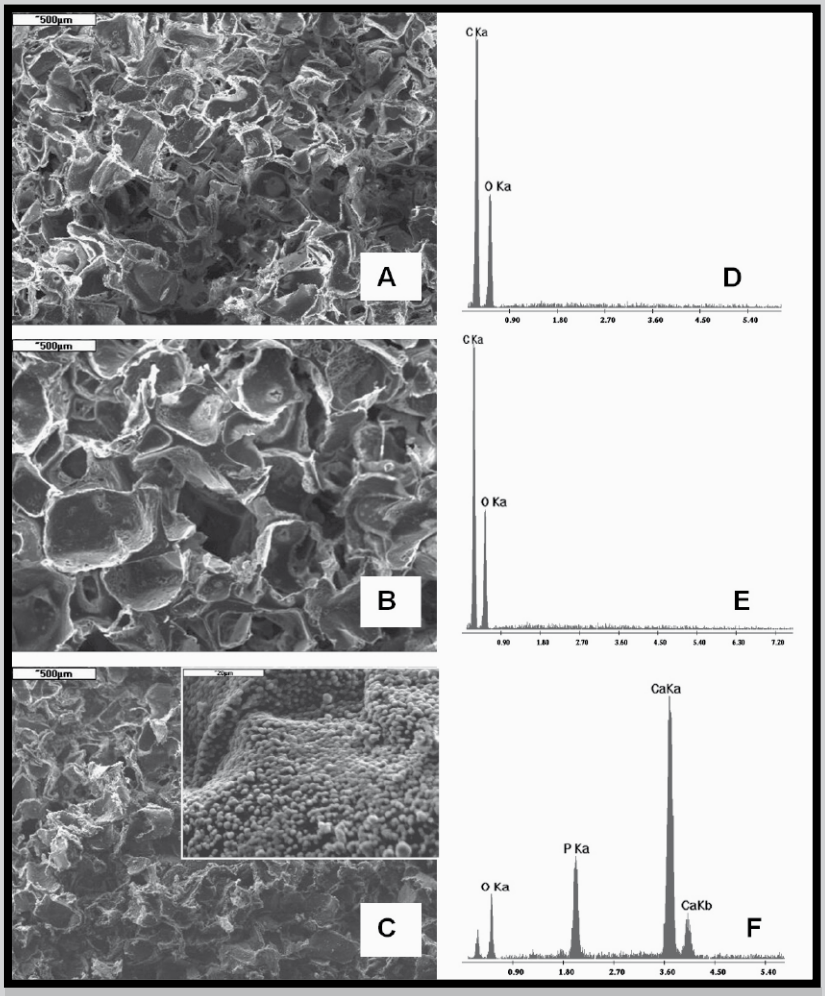

FIG. 1. SEM microphotographs (A, B, C) and EDX spectra (D, E, F) of PLGA scaffolds with pore size of 250-320 μm (A, D), 400-600 μm (B, E), and 250-320 μm with HAP deposits (C, F).

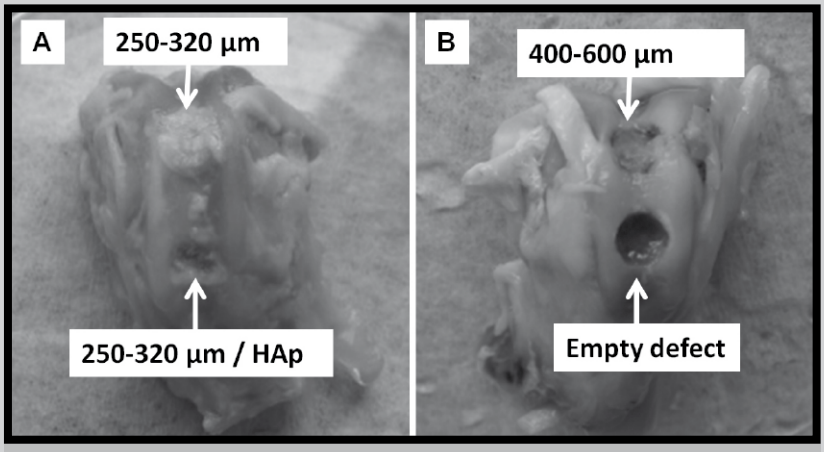

FIG. 2. Macroscopic appearance of tissue defects in rabbit femoral trochlea (A – left leg, B – right leg) after 4 weeks from surgery treated with scaffolds with size of pores: 250-320 μm, 250-320 μm and HAp, 400-600 μm, and empty defect.

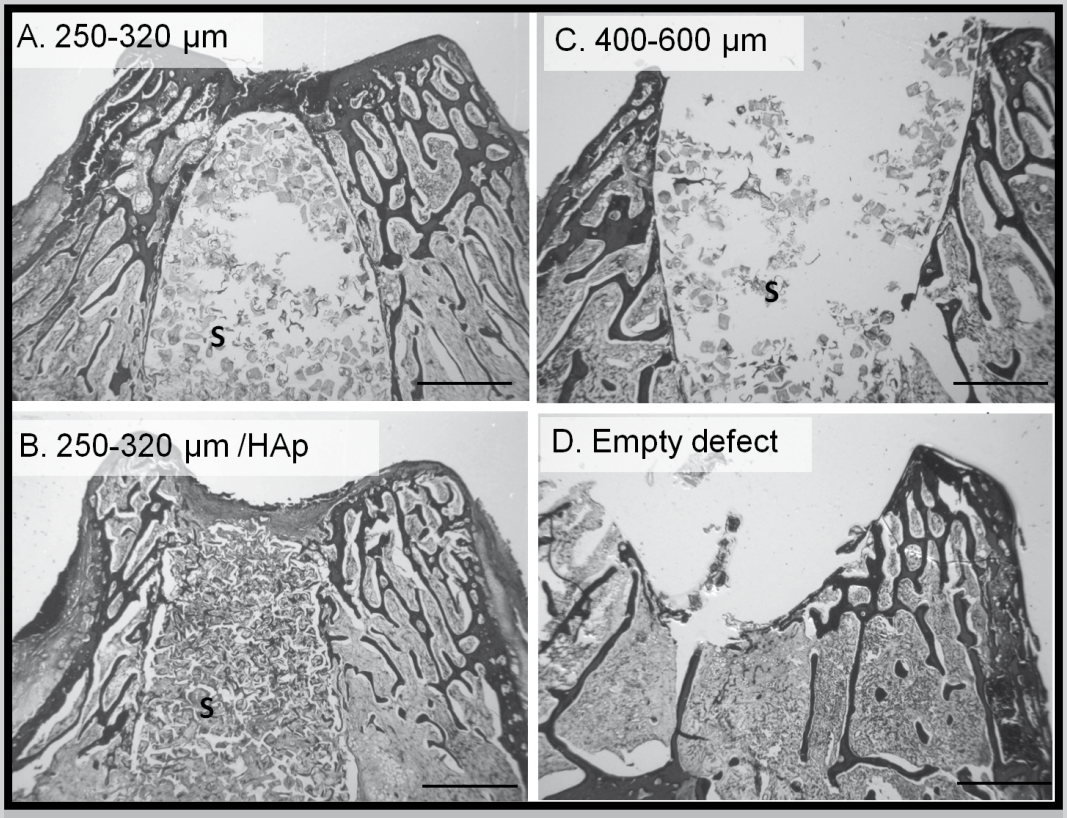

FIG. 3. Histological pictures of tissue defects in rabbit femoral trochlea after 4 weeks from surgery treated with scaffolds: A – poresize 250-320 µm, B – poresize 250-320 µm and HAp, C – poresize 400-600 µm, and D – empty defect; Masson-Goldner staining, s – scaffold, optical microscope Zeiss StereoDiscovery, original magnification 20x, bar = 2 mm.

Probably relative surface area of the scaffolds, which was higher for the scaffolds with smaller pores than for those with bigger pores, better enhanced osteogenic cells migration from the periphery into the centre of the bone defect. As a result tissue healing and regeneration proceeded faster. Interestingly, the results of this study correlate well with to the results of the experiment conducted in the soft tissue model in rats, in which three types of scaffolds of defined pore-size (about 200, 400 and 600 µm) were applied [9]. It was found that cell penetration, extent of inflammation, and tissue regeneration were inversely proportional to the size of pores in the scaffolds, being the most advanced in the scaffolds with smaller pores [9].

Conclusions

In summary, the results show that it is feasible to produce PLGA scaffolds with defined microstructure, surface characteristics and mechanical properties. The PLGA scaffolds with pores in the range of 250-320 µm containing HAp deposits have the highest potential for critical-size osteochondral defects healing in a short-term study.

Acknowledgements

This study was financed from the Polish Budget Founds for Scientific Research within the years 2009-2012, as a research project N° N507280736.

References

[1] Seyednejad H., Ghassemi A. H., van Nostrum C. F., Vermonden T., Hennink W. E.: Functional aliphatic polyesters for biomedical and pharmaceutical applications. J. Control Release. 2011 May 30; 152(1):168-76.
[2] Kruger E. A., Im D.D., Bischoff D.S., Pereira C.T., Huang W., Rudkin G.H., Yamaguchi D.T., Miller T.A.: In vitro mineralization of human mesenchymal stem cells on three-dimensional type I collagen versus PLGA scaffolds: a comparative analysis. Plast. Reconstr. Surg. 2011 Jun;127(6):2301-11.
[3] Park K., Cho K. J., Kim J. J., Kim I. H., Han D. K.: Functional PLGA scaffolds for chondrogenesis of bone-marrow-derived mesenchymal stem cells. Macromol. Biosci. 2009 Mar 10;9(3):221-9.
[4] Pamula E., Bacakova L., Filova E., Buczynska J., Dobrzynski P., Noskova L., Grausova L.: The influence of pore size on colonization of poly(L-lactide-glycolide) scaffolds with human osteoblast-like MG 63 cells in vitro. J. Mater. Sci. Mater. Med. 2008 Jan;19(1):425-35.
[5] Pamula E., Filová E., Bacáková L., Lisá V., Adamczyk D.: Resorbable polymeric scaffolds for bone tissue engineering: the influence of their microstructure on the growth of human osteoblast-like MG 63 cells. J. Biomed. Mater. Res. A. 2009 May;89(2):432-43.
[6] Douglas T., Pamula E., Hauk D., Wiltfang J., Sivananthan S., Sherry E., Warnke P.H.: Porous polymer/hydroxyapatite scaffolds: characterization and biocompatibility investigations. J. Mater. Sci. Mater. Med. 2009 Sep;20(9):1909-15.
[7] Dobrzyński P., Kasperczyk J., Janeczek H., Bero M.: Synthesis of biodegradable copolymers with the use of low toxic zirconium compounds. 1. Copolymerization of glycolide with L-lactide initiated by Zr(acac). Macromolecules 2001; 34: 5090-5098.
[8] Wojak I., Pamuła E., Dobrzyński P., Zimmermann H., Worch H., Scharnweber D., Hintze V.: Coating of poly(l-lactide-co-glycolide) scaffolds with collagen/glycosaminoglycan matrices and their effects on osteoblast behavior. Eng. Biomat. 2009; 86: 9-13.
[9] Menaszek E., Pamuła E.: The soft tissue response to different forms of PLGA implants. 7th Advanced Summer Course in Cell-Materials Interactions, June 19-23, 2006, Porto, Portugal.