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FABRICATION OF COMPOSITE POLYURETHANE/HYDROXYAPATITE SCAFFOLDS USING SOLVENT-CASTING SALT LEACHING TECHNIQUE

ABSTRACT

Scaffolds are porous three-dimensional structures which are used to fill bone losses and make them possible to cells to grow. Many different structural and biological properties are required from them: porosity, mechanical strength and biocompability. The present research is aimed at development of composite polyurethane/hydroxyapatite scaffolds by using the solvent-casting salt leaching method. The SEM examinations were applied to assess the structure of obtained scaffolds.

Keywords: *scaffolds, polyurethane - hydroxyapatite scaffolds, solvent-casting, salt leaching*

INTRODUCTION

The modern scaffolds have to mimic biological functions in extracellular matrix, to hold the structure and functions of growing tissues, to assist cells adhesion, growing and differentiation [1]. Nowadays the porous metal alloys are used in musculoskeletal surgeries even if not always a permanent implant is necessary. Then a conception of temporary implant has become more popular in enhancing the tissue regeneration. The major property of temporary implant material is biodegradation. The requirements for such materials are complex, but they emphasize three main requirements: to provide controlled degradation, to possess suitable mechanical properties and to be completely substituted with host tissue [2].
Scaffolds in tissue engineering are porous three-dimensional constructions fabricated from synthetic or natural materials, in which host tissues can grow in. Scaffolds can be bioinert and don't react with patient's tissues or they can be releasing chemical substances [3].
Scaffolds are made from appropriate biocompatible materials, which degrade slowly and undergo resorption in organism. Most of them assure the three-dimensional space for cells to grow and differentiation [1].
Actually standard in bone loses treatment is based on transplantation of patients tissues. The possibilities of finding a proper donor's bone are limited, and the transplantation of bone fragments is linked with many surgeries and patients` traumas.

Scaffolds are structures that can be substitutes for bone tissues [4]. Scaffolds must fulfill many requirements: high porosity, proper pore size, high apparent surface area, substantial biodegradation degree and its rate, suitable mechanical properties, in particular compression strength, biocompability, positive interaction with cells [5] (Fig.1).

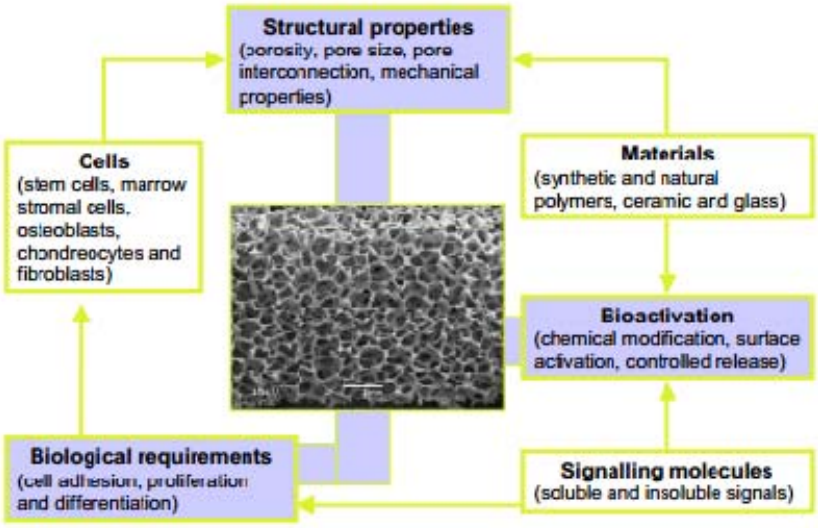

Fig. 1. Schematic picture of scaffolds required properties [6]

Scaffolds fabrication methods

There are many techniques which make it possible to transform solid materials into scaffold. They may be divided into two groups: conventional and modern fabrication techniques. Conventional technics include: gas foaming [7], phase separation [8], solvent-casting and particulate leaching [7, 9] and melt molding [7]. Modern technics are: 3D printing, electrospinning, Fused Deposition Modeling (FDM), Selective Laser Sintering (SLS), stereolitography (SLA) [6, 7] and phase inversion [10, 11].

Solvent-casting and particulate-leaching. To obtain small polymer scaffold to tissue engineering purposes often used method is solvent-casting and particulate-leaching [9]. In this method polymer solution is mixed with salt particles of a specific diameter. After that the solvent evaporates leaving a polymer matrix with salt particles in it. Then the composite is immersed in water where salt particles leach to fabricate a porous structure. Using this technique it is possible to obtain highly porous scaffold (up to 93%) with pores diameter up to 500 μm. The biggest disadvantage is that it can be used only to fabricate thin membranes (up to 3 mm thick) [7]. This method is used when biomaterials are under tests and only small amounts of polymers are available [9].

This research is aimed at developing the polymer-hydroxyapatite scaffold by one of possible methods. Based on the above short state-of-work, the solvent-casting salt leaching technique was chosen as relatively inexpensive, the novelty of this work was an use of biodegradable polyurethan [7].

Polyurethane

Many different polymers were used in biomedical applications. Among them polyurethanes are the interesting family of the materials. Polyurethanes are segmented multiphase elastomers. They are unique because of possibility to fabricate different polyurethanes with a great variety of chemical and physical properties.

Polyurethanes are considered to be excellent biomaterials because of their proper mechanical properties and good biocompatibility. The degradation of polyurethanes is determined by their preparation and composition. Almost always aliphatic diisocyanate are used as substrates when degradation of polymer is necessary, because their degradation products are nontoxic. Polyester polyols are biocompatible and biodegradable polymers used in synthesis of the biodegradable polyurethane [12-14].
Polyurethane have ability to stimulate cell adhesion and proliferation and to support differentiation. That makes it as good candidates for bone substitutes [15].

MATERIALS AND METHODS

Materials

Polyurethane (PUR) was synthetized by two-step pre-polymer method. As substrates at first step, polyethylene glycol PEG (2000 Da molecular mass) and hexamethylene diisocyanate HDI were used. The reaction runs for 4 h at temperature 75 °C. The used excess of HDI was 8%. At the second step the chain extender 1,4 – butanodiol BDO and the catalyst, dibutylin dilaurate, were added to the pre-polymer.
Next PUR was poured into the non-adhesive form heated to 90 °C. After taking out, the PUR was curing for 24 h at 80 °C.
The hydroxyapatite powder was purchased from Sigma Aldrich (p.a. ≥ 90%). Dimethyl sulfoxide (DMSO) and n,n – dimethylformamide (DMF) were purchased from POCH (Polskie Odczynniki Chemiczne SA).

Fabrication of composite polyurethane/hydroxyapatite scaffolds

To obtain scaffolds the solvent-casting salt leaching technique was used, because it is an easy method which does not require specialistic equipment. The solution was prepared by dissolving the proper amount of PUR in 9 g of DMSO or DMF at 108.4 °C. The solutions were cooled in ice-cube forms and the proper amount of salt NaCl and the saturating amount of hydroxyapatite powder were added to the solutions. Table 1 shows the used amounts of components. The forms were then placed in the freezer for 24 h, after that time were taken out and put into warm air, and after one day - into cold water. Water was changed at first for three days every hour and after that every 8 h. This procedure lasted for 14 days. Then samples were dried in ambient air for another 14 days. The specimens of 10% PUR were degrading into the water and they were breaking down.

Table 1. *Amounts of used components*

No.	PUR mass [g]	Used solvent	Mass of solvent [g]	Mass pct. [%]	Mass of HAp [g]
1	1	DMSO	9	10	0
2	1	DMSO	9	10	0.5
3	1.5	DMSO	9	15	0
4	1.5	DMSO	9	15	0.5
5	2	DMSO	9	20	0
6	2	DMSO	9	20	0.5
7	2	DMF	9	20	0
8	2	DMF	9	20	0.5
9	1.5	DMF	9	15	0
10	1.5	DMF	9	15	0.5

Characterization of scaffolds

The cross-section morphology of the composite membranes were observed with a scanning electron microscope (Philips-FEI XL 30 ESEM).

The porosity was calculated using a Multiscan software. Firstly the microphotos of porous structures were made, then the software was used to express in a binary form the photos. It was based on distribution of the photos into two parts: white and black. The percent of white objects was used to define the porosity. Three measurements of each photo were made and the average was calculated.

RESULTS

Obtained results for all samples are diverse. For specimen No. 3 no porous structure was obtained. For specimen No. 4 had highly porous structure, with pores of irregular shape and size, ranged in 75-150 μm or 400-500 μm. The specimen No. 6 showed similar microstructure: highly porous, with pores ranged in 50-175 μm and 400-575 μm diameter. On the other hand, the specimen No. 5 showed very small number of pores (Fig. 2). Summarizing the porous structure was obtained only in presence of HAp.

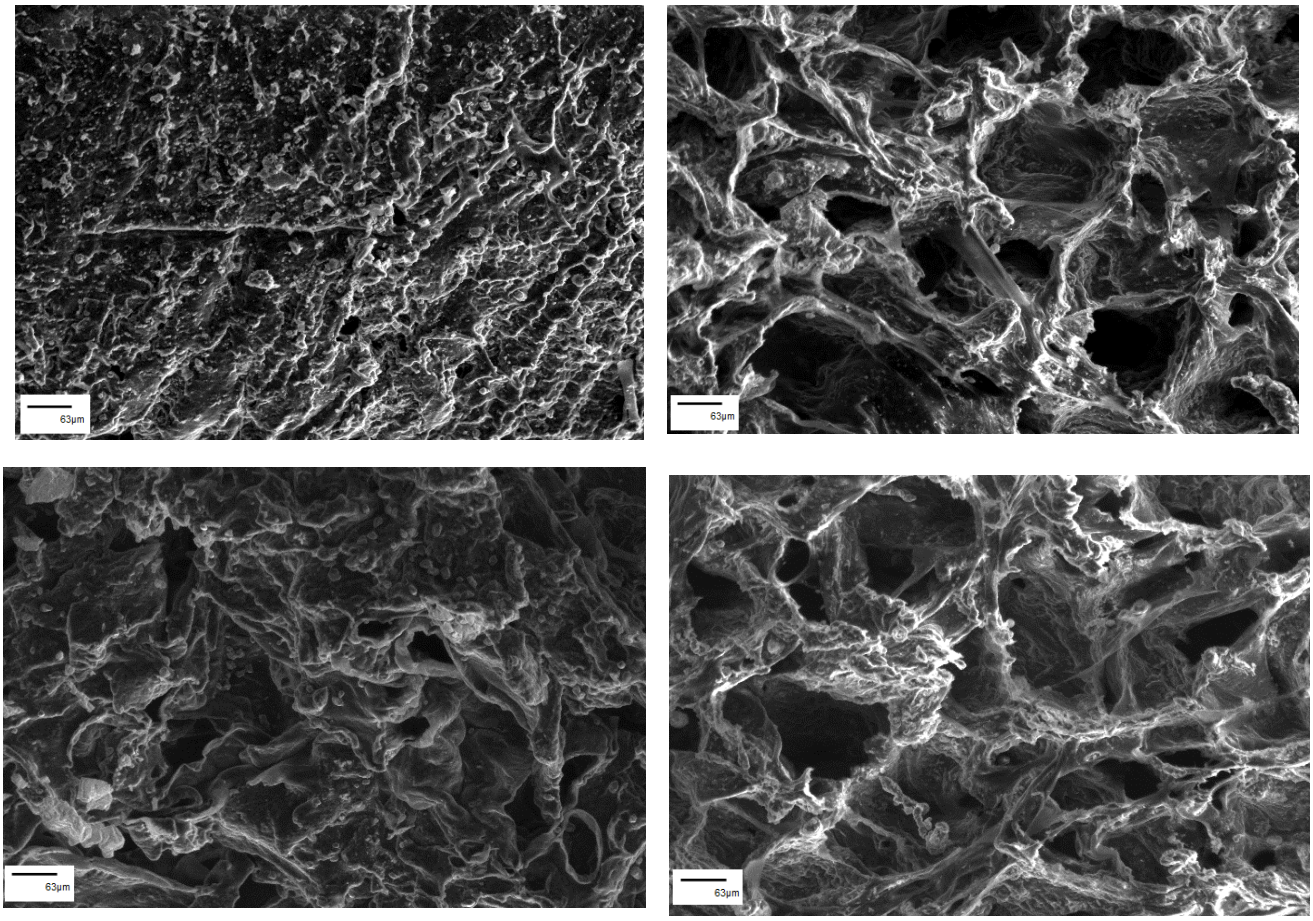

Fig. 2. Microstructure of specimens No. 3, No. 4, No. 5 and No. 6 (from left top to the right bottom)

Specimens No. 7, No. 8 and No. 10 demonstrated always porous structure (Fig. 3), but the use of DMF as a solvent changed the type of porosity: the surface looked smoother and the pores were not numerous. The pore diameters ranged between 400-575 μm.

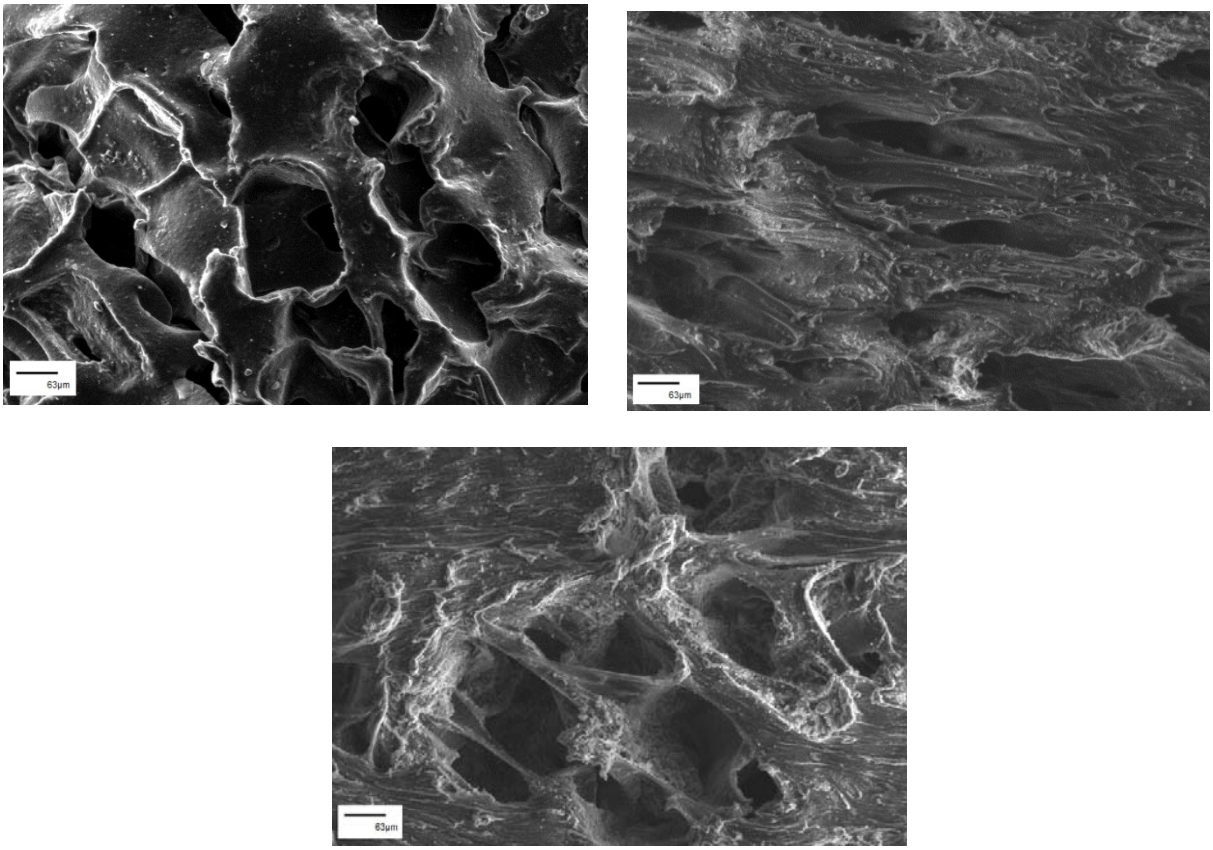

Fig. 3. Microstructure of specimens No. 7, No. 8 and No. 10 (from left top to the bottom)

Porosity measurements

Table 2. shows measured porosity of the specimens.

Table 2. *Measured porosity of specimens*

Specimen number	Porosity [%]
No. 4	71.66 ± 2.39
No. 6	51.14 ± 3.63
No. 7	57.33 ± 6.53
No. 8	59.26 ± 7.5
No. 10	38.69 ± 4.23

Specimens No. 3, 5, 9 had no pores. Specimens No. 4 and 6 were prepared using DMSO as a solvent. The difference between them was the percentage of PUR. When it increase the porosity had declined (for almost 20%).

Specimen No. 7 had no HAp in structure whereas No. 8 had HAp. Both were prepared using DMF as a solvent. The porosity was nearly the same.

In the specimen No. 10 DMF was the solvent and it had HAp in the structure. The content of PUR was 15%. The porosity had highly declined compared to the specimen No. 8.
Specimens No. 3 and 5 were prepared using DMSO and No. 9 DMF. No. 3 and 9 had 15% PUR content when No. 5 had 20%. All of them had no HAp presence.

DISCUSSION

The obtained structures were hardly dependent on used solvent, amount of PUR, presence of HAp.
Scaffolds fabricated using DMF as a solvent had smaller number of pores, than ones on the DMSO. Fabrication of scaffolds without any HAp addition were almost impossible. Samples with HAp powder had pores in their structure, but results were hardly repeatable. In different samples series, in spite of using the same solvent, PUR and amount of HAp, they had different porosity and pores sizes.
PUR amount also have influence on received scaffolds morphology. Too low content of PUR effect on complete destroying of samples in water during the leaching process. Contrarily, when the amount of PUR increased the porosity of obtained samples had declined.
Two colonies of pores (with bigger and smaller diameters) were presented, because of two different processes. The bigger ones were presented because of leaching salt particles from the samples. The smaller, occurred because of the melting temperature of used solvent. For DMSO it is 18,5 °C [16] so when samples were put into the freezer it starts to crystallize. These pores appeared because of leaching the solvent's crystals.
In porosity measurements the high dispersion of the values was observed. The measurement uncertainty was between over 3% (for sample No. 4) to almost 13% (for sample No. 8). Pores were distributed uniformly.
In that method impossible was to predict or design the final structure of samples. The structure depend on many factors e.g. used solvent, PUR or HAp amount. Furthermore, necessary time to prepare scaffolds was really long (almost the month).

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