Elaboration and Characterization of Biodegradable Scaffolds from Poly (L-lactide-co-glycolide) Synthesized with Low-Toxic Zirconium Acetylacetonate

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Abstract:

Objectives: The aim of the study was to answer the questions whether poly (L-lactide-co-glycolide) synthesized with the use of zirconium acetylacetonate: (i) is less toxic *in vitro* than that synthesized with tin compound; (ii) is it possible to produce scaffolds from such copolymer, and (iii) how these scaffolds degrade *in vitro*.

Methods: A human osteoblast line (Saos2) was used to verify the biocompatibility of the copolymer. Porous scaffolds were obtained via the solvent casting / particulate leaching technique. The scaffolds were characterized in terms of surface chemistry (FTIR-ATR, contact angle), microstructure (porosity, water uptake, SEM) and degradation in PBS (GPC, SEM, FTIR-ATR, mass loss).

Results: The copolymer synthesized with the zirconium compound performs better in contact with osteoblasts in vitro than that synthesized with tin. Porous scaffolds from a such copolymer can be easily prepared by the solvent casting/salt leaching technique. These scaffolds, having a high open porosity (88% ± 2%) and water uptake of (630% ± 50%) maintain their dimensions and porous microstructure for 8 weeks in PBS. The scaffolds degrade *in vitro*, but the rate of degradation is quite low.

Conclusion: The results of biological, textural, and physico-chemical properties of obtained porous material, regarding its behaviour in conditions simulating biological environment, show that it could be used as a scaffold for bone tissue engineering.

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Key words: Tissue Engineering; Aliphatic Polyesters; Scaffolds; *In Vitro* Cells Studies; Microstructure; Degradation

Introduction

The design and manufacture of appropriate scaffold materials for a particular application are of key importance in tissue engineering. Such properties of scaffolds as biocompatibility, adequate biodegradation time (not too fast in a first stage of contact with cells and tissues) and porous microstructure constituting interconnected system of pores are fundamental [1,2]. Elaboration of scaffolds with maximal porosity and mechanical stability for a desired period of time is of crucial importance. Several techniques to produce porous materials have been adapted for tissue engineering purposes, however the solvent casting/particulate leaching technique is still the most commonly used [2-4]. Aliphatic polyesters are widely converted into scaffolds for tissue engineering [5].

Commercial aliphatic polyesters used in medicine as resorbable sutures, pins, and screws for osteosynthesis are usually synthesized with the use of tin compounds. However it is known that the total removal of toxic tin compounds from the polymer is virtually impossible. As a result, tin in the course of degradation is released into the patient's body. Therefore, attempts to synthesize lactides, glycolide and their copolymers with the use of so called bioelements, e. g. iron, calcium, and zirconium have been undertaken [6-8]. Copolymerization of glycolide and L-lactide with the use of a zirconium compound resulted in high-molecular-weight copolymers with a segmental-chain structure and interesting mechanical properties [8]. It was already shown that the chain structure of such copolymers influences mechanical and thermal properties as well the susceptibility for degradation [8].

In this report, the copolymer of L-lactide and glycolide synthesized with the use of a zirconium compound is evaluated with respect to *in vitro* cell cultures, *in vitro* degradation and amenity to form porous scaffolds.

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Materials

Synthesis of the copolymer

Glycolide and L-lactide (Purac, Holland) were purified by re-crystallization from dry ethyl acetate and dried in a vacuum oven at room temperature. Zirconium (IV) acetylacetonate $Zr(acac)_4$ (Aldrich Corp., Germany) and stannous (II) ethylhexanoate $Sn(oct)_2$ (Sigma Chem., Germany) were used as received. Copolymerization was performed in bulk with an initiator/molar ratio of 1.25×10^{-3} at 100°C by a conventional method using a vacuum line for degassing and sealing of the ampoules according to a method described previously [8]. The obtained copolymer was ground and shaken with methyl alcohol in order to remove non-reacted monomers and then dried in vacuum at 50°C.

Preparation of films and scaffolds

Films were cast from 10% (w/v) polymer solution in methylene chloride on glass Petri dishes. The scaffolds were produced by solvent casting / particulate leaching technique. Sodium citrate (POCh, Gliwice, Poland) was mixed with 10% (w/v) polymer solution in methylene chloride in such proportion to receive a salt volume fraction of 85%. The mixture was cast on glass Petri dishes, dried overnight in air, followed by treatment at a decreased pressure for 24h. Next, the salt was leached in distilled water. This step took about 5 days, until the conductivity of the water was below 5 μS. Afterwards, that the samples were dried in the oven under decreased pressure for 24h and stored in a dessicator prior use.

Methods

Characterization of copolymer and scaffolds

The copolymer composition was determined by ^1H NMR measurements (Varian Unity Inowa spectrometer). The molecular weight and polydispersity index were determined by gel permeation chromatography (GPC) with a Physics SP 8800 chromatograph. The contact angle was measured by the sessile drop method by DSA 10 automatic system from Kruss, Germany. The result was obtained by averaging of 10 measurements.

The porosity and water uptake of scaffolds were calculated from the weight of dry samples and samples soaked with water. The samples were immersed in distilled water for 10 minutes. Next, both surfaces of the foams were wiped with wet tissue to remove water from the surfaces and the foams were weighed (m_{wet}). Finally, the samples were dried in a vacuum oven at 35°C for 24h and weighed once again (m_{dry}). Water uptake was calculated using the following formula:

$$\text{water uptake } (\%) = 100\,(m_{wet} - m_{dry})/m_{dry}.$$

The microstructure of scaffolds was studied using a scanning electron microscope (JSM 5400 from JEOL, Japan) at a magnification of 50 times. Before analysis, the samples were coated with a thin carbon layer in order to make them conductive. The chemical structure of the scaffolds was analysed using a Digital FTS 60v (BioRad) in the attenuated total reflection mode (FTIR-ATR). The samples were studied in the range of 600-4000 cm^{-1} with a 4 cm^{-1} resolution and averaging of 64 scans for each spectrum.

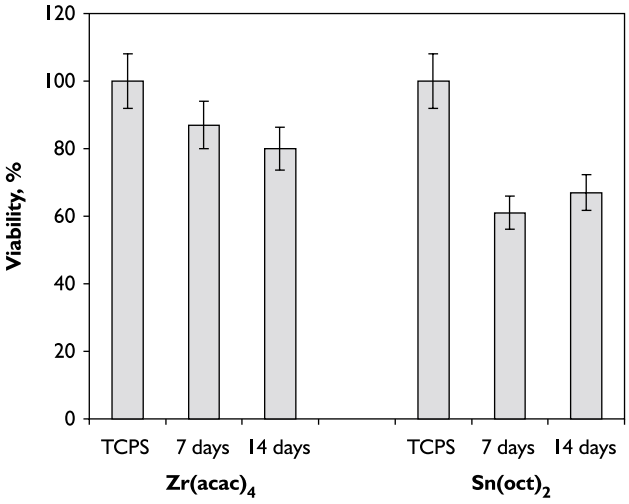

Figure 1. Viability of Saos2 osteoblasts after 7 and 14 days of culture on copolymer films synyhesized with zirconium (IV) acetylacetonate $Zr(acac)_4$ and stannous (II) ethylhexanoate $Sn(oct)_2$ in comparison with TCPS

In vitro cell culture

The films were UV sterilised, 2 min per site. The human osteoblast line Saos2 was used in the following conditions: 30 000 cells in 1 ml RPMI 160 culture medium with 15% FCS (fetal calf serum) (Gibco Laboratories, Grand Island, NY, US). The cell culture was kept in an incubator with a mixture of 5% CO_2 and air, at 37°C for 7 and 14 days. TCPS (tissue culture polystyrene) was used as a control. Viability of cells was routinely monitored by the MTT assay (Sigma) [9].

Degradation studies

Degradation of scaffolds was performed in phosphate-buffered saline (PBS) [24mM Na_2HPO_4 and 16 mM KH_2PO_4, pH=7] at 37°C. The scaffolds weighing 0.25g were incubated in 100ml of buffer in plastic vials. The buffer was changed weekly. Each week one piece of foam was taken, washed thoroughly in distilled water, dried in a vacuum oven for 24h and submitted to further investigation.

Results and discussion

Cell culture

The copolymer was intended for bone tissue engineering, therefore biocompatibility *in vitro* was performed by using the viability test on human osteoblasts. Figure 1 presents the osteoblast viability after 7 and 14 days of culture on copolymer films synthesized with $Zr(acac)_4$ and $Sn(oct)_2$ in comparison with TCPS. It is apparent that the viability of cells is much better on copolymer obtained with the use of zirconium than on tin. Taking into account that both copolymers had the same molecular structure (18:82

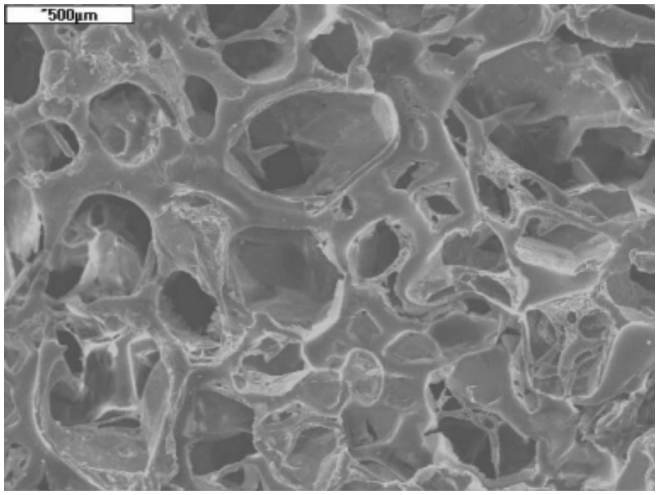

Figure 2. SEM micrograph of poly (L-lactide – *co*-glycolide) scaffold, magnification 50×

molar ratio of glycolide to L-lactide, as shown by NMR) and molecular mass (Mn=34 000D and d=2.5, as shown by GPC) it is very probable the differences in cell behaviour are due to presence of toxic residues of tin initiator.

Scaffold properties

Scaffolds were produced from copolymer with better biocompatibility, e.g. synthesized with the use of the zirconium compound. Several types of porous polymer scaffolds were obtained by solvent casting/particulate leaching. Figure 2 shows a representative microstructure of porous scaffold manufactured with sodium citrate particles. Scaffolds consist of interconnected systems of pores, hundreds micrometers across. The size of the pores is close to the size of salt particles (600 µm ± 100 µm) used as porogens. The porosity of scaffolds was 88% (± 2%) and was slightly higher than that assumed during scaffold design (V_p=85%). The scaffolds also had a very high water uptake of 630% (± 50%), despite the fact that the copolymer was quite hydrophobic (77° ± 2° as measured by sessile drop). Relatively small confidence intervals of porosity and swelling, calculated for 10 independent samples, confirm that the preparation method enables one to obtain the samples with a very reproducible microstructure. Moreover, the scaffolds possess good mechanical properties in the swollen state. High porosity and swelling ability combined with an adequate mechanical stability in a liquid environment are necessary for cell attachment and growth, easy diffusion of nutrients and waste products from the implant and for the vascularization processes [2, 10].

Degradation studies

Degradation of scaffolds in PBS for 8 weeks was analyzed by GPC, FTIR-ATR, SEM and mass loss. Figure 3a shows molecular masses (Mn and Mw) of the copolymer after synthesis, scaffold after fabrication and scaffolds versus degradation time in PBS. It is evident that the method of preparation decreases the length of copolymer chains. Nevertheless, the drop is not large (Mn about 20%, Mw about 6%), and it must be pointed out that dissolving of copolymer, preparation of salt/copolymer composite, followed by excessive washing and drying under reduced pressure already result in copolymer degradation. This fact must be taken into account in the preparation of scaffolds. Mw declines for the whole period of incubation in PBS. In contrast Mn slightly increases within three weeks of incubation, followed by a further decrease. The measured decrease of both masses is about 16% over 7 weeks of degradation.

Figure 3b presents mass loss of the scaffolds as a function of degradation. The scaffolds lose mass within three weeks up to 3%wt. This can be explained by random scission of ester bonds in a hydrolysis reaction and the leaching of low molecular weight compounds. The small increase of Mn within 3 weeks (Fig. 3a) seems to confirm this hypothesis. After that period the scaffolds do not lose mass during up to 8 weeks of incubation.

Despite the overall mass loss and the decrease in Mn and Mw FTIR-ATR did not reveal any changes in

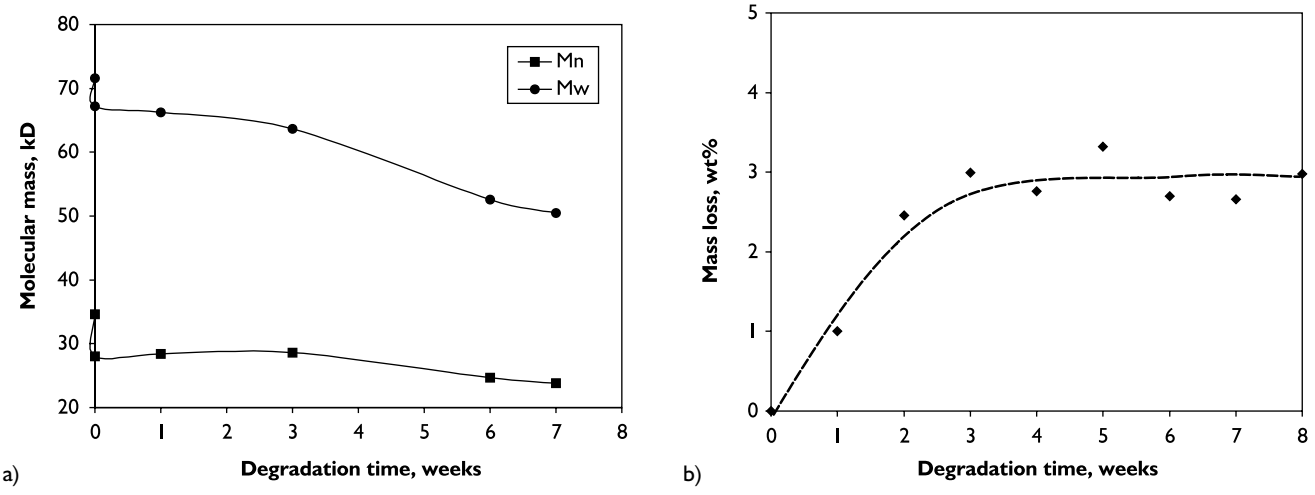

Figure 3. Number average molecular mass (Mn) and weight average molecular mass (Mw) (a) and mass loss (b) as a function of degradation time in PBS of poly (L-lactide – *co*-glycolide) scaffolds

the chemical structure of the samples before and after incubation (data not shown). Moreover, the microstructure of scaffolds as seen by SEM (data not presented) was similar to the samples before degradation (Fig. 2), pointing out that the samples did not undergo surface degradation over the studied period. It is worth noticing that within 8 weeks *in vitro* the scaffolds maintain their dimensions, porous microstructure and mechanical integrity, which are obligatory for tissue engineering applications [5, 10].

Conclusions

It was shown that copolymer synthesised with a zirconium compound as an initiator performs better in contact with osteoblasts *in vitro* than that synthesized in the commercial way, using a tin compound. Furthermore, porous scaffolds from such copolymer can be readily prepared via the solvent casting/salt leaching technique. These scaffolds, with a high open porosity and a high water uptake, sustain their dimensions and porous microstructure for 8 weeks in PBS. The scaffolds degrade *in vitro* via hydrolysis, probably by random scission of ester bonds, however the rate of degradation is quite low. Therefore the scaffolds seem to have the appropriate biological, textural and mechanical properties for tissue engineering applications.

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