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L-ascorbic acid modified poly(ester urethane)s as a suitable candidates for soft tissue engineering applications

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ABSTRACT

In this paper we created novel poly(ester urethane)s (PESUs) designed specifically for tissue engineering. The PESUs were derived from oligomeric α,ω-dihydroxy(ethylene-butylene adipate) (dHEBA), 1,4-butanediol (BDO) and aliphatic 1,6-hexamethylene diisocyanate (HDI) and modified with L-ascorbic acid to improve their biocompatibility. In addition, we determined their mechanical properties (such as tensile strength, elongation at break and hardness). To determine how these materials may potentially behave after implementation in tissue, we estimated the degradation behavior of obtained PESUs in various chemical environments, which were exemplified by canola oil, saline solution, distilled water and phosphate buffered saline (PBS). Finally, the PESU biocompatibility and hemocompatibility tests were carried out. Biocompatibility was determined with a MTT assay and it was performed with 3T3 cell line. The results showed satisfactory mechanical properties, good hemocompatibility and improved biocompatibility, which let us to conclude that PESUs modified with ascorbic acid might find an application in biomedical field of soft tissues engineering.

1. Introduction

Polyurethanes have found very strong position in the medical market for the last years. Some polyurethanes (PUs) with the commercial names like Pellethane, Tecoflex, Elasthane, BioSpan found the application in medicine as very good biostable materials for long term implants. They are based mostly on aromatic diisocyanates. However, new medical-grade polyurethane materials continue to be developed and obtained expand their use in medicine [1,2,3,4,5,6].

There is great interest in designing new types of polyurethanes for regenerative medicine, in particular to obtain a tissue scaffold [7,8,9]. Such scaffolds are intended to serve as a place for air and nutrients exchange as well as the template for cells that proliferate, migrate and differentiate to form new functional tissue.

In the procedure of this type of regeneration a scaffold material should be biodegradable upon the growth of new cells.

Requirements of template are suitable mechanical, physicochemical and biological properties such as biocompatibility, hemocompatibility and biodegradability. Tissue scaffold biocompatibility means that the implanted material may serve its function with an appropriate host response, without forming prominent system inflammation [10].

Properly designed polyurethanes may meet all these requirements, due to the possible changes in the chemistry of segmented structure consisting of specific hard and soft segments, which allow the adjustment of PU properties in a wide range.

PU scaffolds are mostly obtained from polyester polyols, aliphatic diisocyanates, low molecular weight chain extenders [11,12,13] and other components, called modifiers, to improve polyurethanes' biocompatibility and biodegradability [1,2].

There are several examples in the literature, describing the modification of PU with the use of natural polymers such as chitosan [14,15], starch [16], cellulose [17], gelatin [18,19] and collagen [20] leading to the desired changes in biodegradation rate of the material, as well as contributing to improving its biocompatibility [1,2].

Also known are modifications of polyurethanes with other compounds that improve biocompatibility or degradability.

Interesting examples are phospholipid copolymer modification of PU [21], elastin cross-linked peptide [22] or poly(ethylene glycol) methacrylate [23,24].

There are also literature data reporting addition of biologically active substances such as vitamin E or vitamin C [3,4,25] to increase biocompatibility of synthetic polymers.

Vitamin C (also known as ascorbic acid) is particularly interesting because of its antioxidant properties and high impact on tissue regeneration [26,27,28,29,30].

The stimulatory effect of ascorbic acid (AA) on cell proliferation was recognized by Zhang et al. and Cetina-Diaz et al. which received polyurethane materials modified with ascorbic acid for use in bone tissue engineering [3,4,31]. The PU system studied by them was derived

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from poly(ε-caprolactone)diol, 4,4′-methylene bis(cyclohexyl isocyanate) (HMDI) and various chain extenders. One of the chain extenders used was AA, another was L-glutamine.

Recently, it was proven that ascorbic acid can stimulate mesenchymal stem cells to differentiate them into adipose tissue [32,33,34] as so for soft tissue, which builds (for example) blood vessels. In addition, researchers also found that ascorbic acid used in cell culture enhances adipogenesis [35]. All of these reports have contributed to our work on the modification of the PU by the AA for soft tissue engineering.

This article describes the results of studies related to the synthesis of novel biomedical grade polyurethanes designed for soft tissue engineering. They were synthesized from oligomeric α,ω-dihydroxy(ethylene-butylene adipate) (dHEBA) polyol, 1,6-heksamethylene diisocyanate (HDI), 1,4-butandiol chain extender (BDO) and ascorbic acid (AA) to improve their biocompatibility. For comparison, unmodified poly(ester urethanes) were obtained. Both types of PESUs were tested to determine their tensile strength, elongation at break, permanent set and hardness. The samples were also subjected to the standard procedure of degradation [36,37] in three different defined media: canola oil, saline, distilled water and phosphate buffered saline (PBS). Biocompatibility and hemocompatibility were tested as well.

2. Experimental

2.1. Poly(ester urethane) synthesis

PESUs were synthesized by standard two step polymerization procedure [38,39,40]. In the first step a prepolymer was obtained with 8% of free isocyanate groups. It was derived from oligomeric α,ω-dihydroxy(ethylene-butylene adipate) (dHEBA) polyester (trade name Polios 55/20; Purinova, Poland) (77 wt.%) and aliphatic 1,6-hexamethylene diisocyanate (HDI) (Sigma Aldrich, Poland) (23 wt.%). The prepolymer reaction was carried out in the glass, 4-neck, reactor at 80 °C for 4 h. In the second step the chain extender — 1,4-butanediol (BDO) (POCH, Poland) — was added to obtain series of unmodified poly(ester urethane)s with three different molar ratio of free isocyanate groups (in the prepolymer) to hydroxyl groups of chain extender BDO (NCO/OH = 0,9:1; 1:1; 1,05:1). Dibutyltin dilaurate (DBTDL), at the amount of 0.5 wt.%, was used as a catalyst, commonly used for the synthesis of biomedical PU [41,42,43]. The reaction mixture was subjected to intensive stirring and then transferred into a mold, set at 80 °C, for 6 h. Then, the samples were left in a drier at 80 °C for 24 h to complete the reaction. After the mechanical property measurements authors selected specific composition to be modified using the AA. The optimal PU mechanical properties appeared in the case of using in the synthesis the molar ratio of NCO:OH equal 0,9:1 (Table 1).

The synthesis of modified PESU was as follows: to the obtained prepolymer 2 wt.% of solid AA (Sigma Aldrich), calculated per mass of the prepolymer (prepolymer:AA = 1:0,25), was added at room temperature. This amount of AA was established as most suitable in preliminary studies. After addition of the AA to the prepolymer, the temperature was raised to 80 °C and the reaction was continued for 1 h. Then the chain extender BDO was added to give ascorbic acid-modified poly(ester urethane) (AA-PESU). Reaction mixture, after vigorous stirring was transferred to a mold of 80 °C for 6 h. After this time, the AA-PESU left in the oven for 24 h at 80 °C to complete the reaction. Thus samples having a thickness of 2 mm were obtained. The scheme of the unmodified and AA modified PESU synthesis is shown in the Fig.1 and the reactions involved in the synthesis of polyurethanes unmodified are presented in the Fig. 2.

Table 1
Tensile strength (T_{SB}) and elongation at break (ε) of unmodified poly(ester urethane)s obtained at three different molar ratio of NCO:OH = 0,9:1, 1:1, 1,05:1.

PU symbol	$T_{SB} \pm$ SD (MPa)	$\varepsilon \pm$ SD (%)
PU-0,9/HDI/AA0	4,23 ± 0,98	27 ± 5
PU-1/HDI/AA0	6,45 ± 0,87	165 ± 39
PU-1,05/HDI/AA0	9,98 ± 0,45	351 ± 10

2.2. Characterization methods

Furier transform infrared (*FT-IR*) *spectroscopy* of the solid PESU and AA-PESU was performed at FTIR Spektrometer Tensor 27, Bruker Co. in the spectral range from 4000 to 400 cm^{-1} averaging 256 scans with a resolution of 4 cm^{-1}.

2.3. Nuclear magnetic resonance (^{1}HNMR) spectroscopy

^{1}HNMR spectra were obtained with the use of 500 MHz Varian Spectrometer Unity 500 Plus using deuterated dimethylsulfoxide (DMSO) as PU solvent and tetramethylsilane as the internal standard of $CDCl_3$ detectable at 7,26 ppm.

Tensile strength (T_{SB}) and *Elongation at break* (ε) were examined by using the universal testing machine Zwick & Roell Z020 according to PN-EN-ISO 1799:2009. The test was performed with crosshead speed of 300 mm/min ± 50 mm/min at room temperature. Medium values of these properties were evaluated on the basis of 4 samples tested.

Hardness was measured by using Shore method according to PN-EN ISO 868:2004. The hardness measurements were performed with a digital hardness meter Shore A type (Zwick/Roell, type 3101/3131). Obtained data were presented with Shore degree (°Sh A).

2.4. Degradation in canola oil, saline, distilled water and phosphate buffered saline

PESU and AA-PESU materials cut into 6 samples with an area of 1 cm^2. The prepared samples were dried and weighed in thermobalance (RADWAG MAX50/SX) set to 60 °C. The samples were then placed in glass containers filled with canola oil or distilled water or saline or phosphate buffered saline (PBS). The determination of oil sorption through a material of scaffold is a popular test procedure for a medical-grade polymer [44]. Canola oil is often used because it replaces the assay of lipids present in the living body. Furthermore, drugs delivered to the body, encapsulated in a biodegradable polymer often introduced as a lipid emulsion. Samples were incubated in a specific media at room temperature. Changes in weight of the samples were examined after 1 day of incubation for canola oil medium; after 1, 3, 7, 14 days and 1, 2, 3 months for distilled water, saline and PBS [44]. The measurement procedure was as follows: samples were taken from the container and placed between paper towels in order to reduce the medium excess. The samples were then placed on a thermobalance (set at 60 °C) and weighed to constant mass. The weight loss was calculated by the formula [7]. The results are arithmetic average of four measurements. In case of PBS study pH of the solution was controlled every two weeks with the use of Metler Toledo pH-meter.

Hemocompatibility was also studied to evaluate the biocompatibility of polyurethanes with blood. The influence of polyurethane materials on blood parameters is measured with the use of blood clotting, hemolysis and platelet deposition tests [45,46]. Well designed polyurethanes are known to be biocompatible with blood and prevent blood clotting [18]. Hemocompatibility was examined in Medical Laboratory with analyzer SYSMEX XS — 1000i. Sample of venous blood from two healthy women were used to this study. Biologic material, directly after being taken, was put into test-tube containing potassium acetate — agent which prevents blood clotting. Next step was obtaining reference parameters for blood morphology. After that to the test-tube were put samples with a size of 8 cm^2 of PESU or AA-PESU and 8 ml of blood was added. The samples before hemocompatibility test were sterilized with argon gas plasma generated over H_2O_2. The samples were