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In situ foamable, degradable polyurethane as biomaterial for soft tissue repair

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ABSTRACT

Degradable foams which can be inserted endoscopically as liquid or pasty mixtures into soft tissue defects possess a promising potential for the surgical treatment of such defects. The defects can be sealed under *in situ* foaming and simultaneous material expansion. We developed an *in situ* foamable (L-lactide-*co*-ε-caprolactone)-based, star-shaped prepolymer by ring opening polymerization of L-lactide and ε-caprolactone in the presence of meso-erythritol as starter. By conversion of the terminal hydroxyl groups of the formed oligoester with lysine diisocyanate ethyl ester (LDI) an isocyanate-endcapped, reactive prepolymer has been received. Foaming can be initiated by addition of 1,4-diazabicyclo[2,2,2]octane (DABCO), water, LDI and DMSO. By varying the composition of these additives, the foaming and curing time could be varied within a clinically acceptable range. A porosity of approximately 90%, and an average tensile strength of 0.3 MPa with elongations of 90% were determined for the foams.

In vitro cytotoxicity on cured foams was assayed on 3T3 fibroblasts and demonstrated an excellent cytocompatibility. This was also confirmed in an *in vivo* study using an established rat model, where prefabricated foams and *in situ* hardening material were inserted into subdermal skin incisions in parallel. The feature of chronic inflammation was only weakly developed in both groups and slightly more pronounced and persisted for longer time in the group of *in situ* foamed material. In both groups the foreign materials were vascularized, degraded and substituted by connective tissue. The results encourage to proceed with trials where the materials are used to fill more heavily loaded defects.

1. Introduction

The worldwide interest in biodegradable polymers for medical applications like drug-delivery devices, osteosynthesis materials (plates, pins, scews) for internal fixation of bone fractures, scaffolds for tissue engineering and repair and regeneration of various tissues and internal organs is continuously increasing. As a clinically widely introduced class of synthetic polymers polylactones or copolylactones derived from L- or DL-lactide, glycolide, ε-caprolactone or *p*-dioxanone monomers are used in most of these applications [1–4]. Many polylactones, especially the ones with high L-lactide or glycolide portion are rigid materials. For a number of implantable devices incorporated into soft tissue regions of the body like skin [5,6], adipose tissue or the cardiovascular region [7,8], elastomeric biodegradable polymers would be superior to rigid ones. There is a constant need for biodegradable materials tunable not only with regard to biodegradability but also to mechanical properties like elasticity or rubber-like behavior.

In this context, polyurethanes (PUs) represent a very important group of synthetic polymers. Classical polyurethane (PU) synthesis involves the reaction of a hydroxyl group-terminated precursor with a di-, tri- or polyisocyanate forming urethane bondings.

The relationship between structure and properties of various PU types has been extensively investigated [9–14] and due to their elastomeric behavior non-biodegradable PUs are used for long term medical implants [15–17]. In addition, also potentially biodegradable poly(ester-urethane)s have been studied in terms of their favorable mechanical properties [18,19].

Another important issue addressed to PUs is their well-known ability to form *in situ* foamable masses containing pores under specific manufacturing conditions [20,21]. Porosity is a pre-requisite for tissue

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engineering scaffolds and even a considerable advantage for other implant materials where cell ingrowth is favorable to tightly incorporate the implant into the surrounding tissue.

Numerous methods of manufacturing PU foams for implant applications are known in the literature: e.g. freeze drying of a 1,4-dioxane/water/polymer mixture [22], or leaching out porogens [23–26]. In contrast to established methods to fabricate technical PU foams, the former methods are not able to produce foams *in situ*. If a two component system with water is used [27,28] the starting mixture often becomes very low-viscous in the initial stage and hardening of the foam takes several hours.

Another problem is arising from aromatic diisocyanates often used a building components to generate PUs. It has been reported that aromatic diisocyanates produce carcinogenic and mutagenic diamines upon degradation. Although the question whether the concentrations of those harmful degradation products reach physiologically relevant levels is currently unresolved and still up for debate [29,30], the use of more acceptable cytocompatible diisocyanates is a present demand. As an alternative, lysine diisocyanate ethyl ester (LDI) derived from the natural amino acid lysine leads to biodegradable PUs forming nontoxic degradation products thus eliminating the risk of aromatic degradation by-products [31]. Both the requirements of a porous cell-compatible material structure and of high cell acceptance are addressed in the comprehensive work of Guelcher and co-workers directed to LDI and lysine triisocyanate-based, foamable, porous scaffold materials [32,33].

Considering these requirements, it was the aim of this study to develop a biodegradable and sufficiently flexible poly(ester-*co*-urethane) foam that could be applied as an injectable and viscous paste which hardens *in situ*. For this purpose a novel block copolymer was synthesized consisting of a hydroxyl-terminated biodegradable polyester prepolymer, which is end-capped with a reactive lysine-based diisocyanate moiety. To create a three-dimensional cross linked network the starter unit has to possess more than two reactive end groups. Therefore starting from a tetraol to get a four-armed prepolymer L-lactide and ε-caprolactone were used as lactone monomers. In order to avoid distribution of the monomer solution into the surroundding soft tissue it was an important task to adjust the solution viscosity. By *in situ* foaming the material is expected to fill and seal different defect geometries allowing ingrowth of body-own cells by the formed pore structure.

Isocyanates (IC) react with hydroxyl- and amino functionalities by addition to the carbon-nitrogen double bond. Urethane linkages are formed by the reaction of IC with hydroxyl groups, while urea linkages are formed by the reaction with amines. Water reacts with IC to form carbamic acid, an unstable compound, which decomposes to an amine and carbon dioxide gas, which acts as a blowing agent. Optimising this process an appropriate defined porosity of the foam was obtained.

2. Materials and methods

2.1. Materials

L-Lactide was purchased by PURAC Biomaterials (Gorinchem, Netherlands). Dibutylamine, ε-caprolactone (Sigma-Aldrich, Taufkirchen, Germany), 2,6-diisocyanato ethyl caproate (L-lysine diisocyanate ethyl ester, LDI) (Infine Chemicals, Shanghai, China) were purified by vacuum distillation. Dichloromethan, chloroform, cyclohexane, heptane, and toluene were purchased by Fisher Scientific, (Schwerte, Germany) and used without further purification, so as meso-erythritol (Acros, Geel, Belgium), DABCO (Sigma-Aldrich, Munich, Germany) and DMSO (Carl Roth, Karlsruhe, Germany). Soerensen buffer, pH 7.4, consisted of 12.13 mM KH_2PO_4, 54.53 mM Na_2HPO_4.

2.2. Polymer synthesis

LDI based poly(L-lactide-*co*-ε-caprolactone) thermoplastic PU was synthesized according to described procedures (*cf.* [34,35]). Briefly, in a first step a mixture of meso-erythritol (13.4 g, 0.11 mol), L-lactide (126.5 g, 0.88 mol), ε-caprolactone (68.5 ml, 0.66 mol) and 320 μl of stannous octoate, dissolved 20% (w/w) in toluene, was stirred under nitrogen at 150 °C. After 30 and 60 min further 160 μl of the stannous octoate solution were added. The mixture was stirred for 4 h, cooled to room temperature and dissolved in 75 ml of dichloromethane. The solution was filtrated and the crude product was precipitated twice into 1000 ml of cold heptane/acetone (9:1). Finally, the purified polymer was dried in vacuum at room temperature to constant weight. The poly(L-lactide-*co*-ε-caprolactone) prepolymer (**1**) (190.7 g, 89%) was obtained as a clear highly viscous oil.

NMR: 1.75–1.32 (m; 7.1H); 2.49–2.28 (m; 0.8H); 4.43–4.01 (m; 1.3H); 5.27–5.00 (m; 1H);

IR: 2943 (m); 1736 (s); 1453 (m); 1381 (w); 1336 (w); 1183 (m); 1128 (m); 1088 (m); 1046 (w).

In the next step, LDI (37.34 ml, 0.19 mol) was combined with **1** (0.02 mol, 40.46 g) under nitrogen and reacted at 60 °C with stirring for 4 h. After cooling to room temperature the reaction mixture was dissolved in 50 ml dichloromethane, filtrated and precipitated twice into 1000 ml cold cyclohexane. Finally, the isolated product was dried in vacuum at room temperature to constant weight. The IC-terminated-poly(L-lactide-*co*-ε-caprolactone) (**2**) (56.4 g, 98%) was obtained as a pale yellow, highly viscous oil.

NMR: 1.89–1.17 (m; 8.1H); 2.40–2.20 (m; 0.4H); 3.35–3.05 (m; 0.3H); 4.40–3.95 (m; 1.6H); 5.20–4.95 (m; 1H);

IR: 3396 (m); 2942 (m); 2247 (s); 1734 (s); 1525 (w); 1455 (w); 1372 (w); 1187 (m); 1132 (w); 1092 (m); 1046 (w); 1023 (w).

2.3. Polymer characterization

2.3.1. Size exclusion chromatography (SEC)

Molecular weights were determined as number-average molecular weight (M_n) and as weight-average molecular weight (M_w) with respect to polystyrene standards as used in the SEC method and the polydispersity index (PDI) was calculated according to PDI = M_w/M_n. The measurements were performed on a Shimadzu system (Shimadzu, Duisburg, Germany). As pre-column a PSS-SDV (100 Å, 8,0 × 50 mm) and as column a set of PSS-SDV (100 Å, 8,0 × 300 mm), PSS-SDV (1000 Å, 8,0 × 300 mm) and PSS-SDV (100,000 Å, 8,0 × 300 mm) were used. A refraction detector RID 10A (Shimadzu) was utilized.

All samples were analysed at room temperature. Chloroform (stabilised with 1% amylene) was used as eluent, delivered at a flow rate of 1.0 ml min^{-1}. The samples were dissolved in chloroform at a concentration of 5 mg ml^{-1}. The injection volume was 100 μl.

2.3.2. Nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy

^1H and ^{13}C NMR spectroscopy was used to characterize the chemical structures and compositions of the synthesized copolymer. The spectra were recorded on a Bruker DRX 400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) at room temperature, using tetramethylsilane as an internal reference and $CDCl_3$ as solvent.

IR spectra were recorded in the range from 4000 to 548 cm^{-1} by a Bio-Rad FTS 175 FT-IR Spectrometer with Golden Gate Reflection ATR (LOT Oriel, Darmstadt, Germany).

2.3.3. Isocyanate content (ICC)

The ICC was determined by titration according to the German standard DIN EN ISO 11909 [36] with slight modifications. Briefly, about 1 g (weight exactly determined) of **2** was dissolved in toluene and filled up to 100 ml. Twenty ml of this solution (containing the mass m being 1/5 of the initially weighed mass) were mixed with 10 ml of 0.2 M dibutylamine in toluene, stirred for about 15 min, then 75 ml of dry