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Tailoring the degradation kinetics of poly(ester carbonate urethane)urea thermoplastic elastomers for tissue engineering scaffolds

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ABSTRACT

Biodegradable elastomeric scaffolds are of increasing interest for applications in soft tissue repair and regeneration, particularly in mechanically active settings. The rate at which such a scaffold should degrade for optimal outcomes, however, is not generally known and the ability to select from similar scaffolds that vary in degradation behavior to allow such optimization is limited. Our objective was to synthesize a family of biodegradable polyurethane elastomers where partial substitution of polyester segments with polycarbonate segments in the polymer backbone would lead to slower degradation behavior. Specifically, we synthesized poly(ester carbonate)urethane ureas (PECUUs) using a blended soft segment of poly(caprolactone) (PCL) and poly(1,6-hexamethylene carbonate) (PHC), a 1,4-diisocyanatobutane hard segment and chain extension with putrescine. Soft segment PCL/PHC molar ratios of 100/0, 75/25, 50/50, 25/75, and 0/100 were investigated. Polymer tensile strengths varied from 14 to 34 MPa with breaking strains of 660–875%, initial moduli of 8–24 MPa and 100% recovery after 10% strain. Increased PHC content was associated with softer, more distensible films. Scaffolds produced by salt leaching supported smooth muscle cell adhesion and growth in vitro. PECUU in aqueous buffer in vitro and subcutaneous implants in rats of PECUU scaffolds showed degradation slower than comparable poly(ester urethane)urea and faster than poly(carbonate urethane)urea. These slower degrading thermoplastic polyurethanes provide opportunities to investigate the role of relative degradation rates for mechanically supportive scaffolds in a variety of soft tissue repair and reconstructive procedures.

1. Introduction

Scaffolds that are mechanically compatible with the tissue to which they are applied can avoid potential problems with stress concentrations at tissue–scaffold interfaces (if too stiff) or scaffold mechanical failure (if too weak). For dynamic tissues, such mechanical compatibility becomes of greater importance, as evidenced by the interest in appropriately compliant vascular scaffolds [1,2] and the general growth in interest in biodegradable elastomers for soft tissue repair in the biomaterials community [3,4]. Commonly investigated biodegradable elatomers include poly(glycerol sebacate) [5,6], poly(hydroxyalkanoate)s [7], poly(ether ester)s [3,8] and poly(trimethylene carbonate) based polymers [9,10] and polyurethanes [4,11]. The latter group is regularly synthesized from soft segments of aliphatic polyesters or polycarbonates, hard segments of aliphatic diisocyanates and potentially chain extension with diols or diamines that may possess hydrolytic or enzymatic lability [4,12–14]. Thermoplastic biodegradable polyurethanes also generally exhibit good processability and have been used to create porous or fibrous scaffolds in a variety of shapes using methods such as salt leaching [15,16], phase separation [17,18] and electrospinning [19,20]. Further, these polymers are amenable to tuning of properties by adjusting the composition of the soft segment, hard segment or chain extender and also by adjusting the molar ratio of individual components.

Although not often directly addressed, in most instances where degradable scaffolds are being applied in vivo, investigators lack specific knowledge as to the optimal period over which the scaffold should be present. The trade offs between too short of a duration (e.g. mechanical failure) and too long (e.g. inappropriate tissue

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development, fibrosis) are recognized, but require the availability of broader biomaterial options to begin to explore these effects for various tissue applications. In work aimed at creating elastic scaffolds for soft tissue application, we have previously reported on a series of biodegradable poly(ester urethane) ureas (PEUUs) based on a poly(caprolactone) soft segment [21], a family of faster degrading poly(ether ester urethane)ureas (PEEUUs) where the soft segment was a poly(ether ester) triblock copolymer [22], and a third family where specific enzymatic liability was introduced by an elastase-specific peptide chain extender [23]. These polyurethanes provide a range of degradation behaviors, but are generally limited in that the slowest degradation behavior is exhibited by PEUU. Having access to similar degradable polyurethane elastomers where the degradation rate could be tuned to be slower than PEUU would allow investigation of elastomeric scaffold solutions to soft tissue repair where a longer presence is required of the material support.

Our objective in this report was to synthesize a group of degradable polyurethane elastomers where the partial substitution of polyester segments with polycarbonate segments in the polymer backbone would lead to slower degradation behavior. Specifically, we synthesized poly(ester carbonate)urethane ureas (PECUUs) using a blended soft segment of polycaprolactone (PCL) and poly(1,6-hexamethylene carbonate) (PHC) and a hard segment of 1,4-diisocyanatobutane (BDI) with chain extension by putrescine. Different molar ratios of PCL and PHC were investigated as soft segments. The chemical structure, mechanical properties, in vitro degradation and cytocompatibility of PECUU films were studied and porous PECUU scaffolds were generated using the salt leaching method. These scaffolds were characterized mechanically and for cytocompatibility and were then implanted into a rat subcutaneous model to examine in vivo degradation relative to polyurethane scaffolds lacking either polycarbonate or polyester in their backbone.

2. Materials and methods

2.1. Materials

Polycaprolactone diol (PCL, $M_n = 2000$, Sigma) and poly(1,6-hexamethylene carbonate) diol (PHC, $M_n = 2000$, Sigma) were dried under vacuum at 50 °C to remove the residual water before synthesis. 1,4-diisocyanatobutane (BDI, Sigma) and putrescine (Sigma) were distilled before usage. Dimethyl sulfoxide (DMSO, Sigma), *N,N,*-dimethylformamide (DMF, Sigma), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Oakwood Products) and phosphate buffered saline (PBS, Lonza) were used as received. Stannous octoate ($Sn(Oct)_2$, Sigma) was dried by adding molecular sieves.

2.2. Synthesis of poly(ester carbonate urethane)ureas

Poly(ester carbonate urethane)urea (PECUU) was synthesized from PCL, PHC and BDI using putrescine as a chain extender by a two-step solvent synthesis method (Fig. 1). The (PCL + PHC):BDI:putrescine molar ratio was defined as 1:2:1. Briefly, variable molar ratios of PCL and PHC (PCL/PHC ratios of 100/0, 75/25, 50/50, 25/75 and 0/100) were completely dissolved in DMSO in a 3-neck flask with argon protection and then BDI was added to the solution, following 4 drops of $Sn(Oct)_2$. The flask was placed in an oil bath at 70 °C. After 3 h, the prepolymer solution was cooled at room temperature and then a putrescine/DMSO solution was added dropwise into the agitated solution. The final polymer solution concentration was controlled to be approximately 4% (w/v). Then the flask was placed in the oil bath and kept at 70 °C overnight. The polymer was precipitated in an excess volume of cool deionized water and then dried in a vacuum at 60 °C for 3 d. The polyurethane ureas synthesized from the different PCL/PHC molar ratios defined above are referred to as PEUU, PECUU 75/25, PECUU 50/50, PECUU 25/75 and PCUU, respectively. The yields of all final products were approximately 95%.

2.3. Film casting and porous scaffold fabrication

The synthesized polymers were completely dissolved in HFIP after which a ~3% polymer solution was poured into a polytetrafluoroethylene (PTFE) dish. After near complete HFIP evaporation, the film was dried in a vacuum oven at 60 °C for 3 d to remove residual solvent. Initial solution volumes were selected to achieve film thicknesses of 80 μm.

For scaffold fabrication, polymer samples were completely dissolved in HFIP to obtain a 20% (w/v) solution. This solution (1 mL) was blended uniformly with 4.5 g salt particles (NaCl, Sigma), which had particle sizes ranging from 100 to 150 μm obtained by from serial treatment of obtained material with American standard sieves. The polymer/salt mixture was poured into a cylindrical glass mould. After complete solvent evaporation, the mixture was immersed in an excess of 30% ethanol solution to remove the salt particles from the scaffold. The ethanol solution was changed frequently over 2 d of immersion. The scaffold was then placed in pure deionized water to exchange the ethanol solution for 3 h, and then was frozen at −80 °C, followed by lyophilization for 2 d to obtain a porous scaffold for subsequent characterization, cell seeding or implantation.

2.4. Polymer and scaffold characterization

Fourier transform infrared (FTIR) spectra were obtained at room temperature with a Nicolet FTIR spectrometer. A polymer solution in DMF was cast directly onto the NaCl window with subsequent evaporation of DMF under IR irradiation. The glass transition temperature (T_g) and melting temperature (T_m) were determined by differential scanning calorimetry (DSC-60; Shimadzu) with a scanning rate of 20 °C/min over a range of −100 to 200 °C with nitrogen flow. The water contact angle ($n = 8$ per polymer) in air was detected on the film surface using a sessile drop method on a UCA contact angle instrument (UCA optima, AST Products Inc.).

The polymer inherent viscosity was measured using an Ubbelohde viscometer at 22 °C [24]. Each sample was dissolved in 15 mL HFIP at a concentration of 0.1 g/dL and then filtered using a 0.45 μm polytetrafluoroethylene filter. Each sample was tested 5 times and the inherent viscosity was calculated as $\ln(t_p/t_s)/C_p$, where t_p represents the polymer solution flow time, t_s represents the HFIP flow time and C_p is the polymer concentration.

Scaffold morphology was observed with scanning electronic microscopy (SEM, JEM-1011, JEOL). Porosity was measured using an ethanol displacement method [25], where a scaffold sample was immersed in a graduated cylinder containing a known volume of ethanol (V_1). After 5 min, the total volume of ethanol and the ethanol-impregnated scaffold was recorded as V_2. The ethanol-impregnated scaffold was removed from the cylinder and the residual ethanol volume was recorded as V_3. The porosity of the scaffold was calculated as $(V_1 - V_3)/(V_2 - V_3) \times 100\%$.

2.5. Mechanical testing

Strips (2 × 20 mm) were cut from the polymer films and mechanical properties were measured on an MTS Tytron 250 MicroForce Testing Workstation at room temperature. The crosshead speed was set at 10 mm/min according to ASTM D638-98. Four samples were tested for each polymer. The instant strain recovery was measured under the same conditions with samples marked at their distal ends, stretched to 10% strain and held for 1 min, and then released. This stretch cycle was repeated 3 times and then the length change was recorded after the release of tension. The original length (L_0) and the length immediately after releasing the tension (L_1) were measured by a caliper. Instant strain recovery was calculated as $(1 - (L_1 - L_0)/L_0) \times 100\%$. The mechanical properties of porous scaffolds were also measured using the above protocol. Four samples were tested for each type of polymer scaffold.

2.6. In vitro degradation

Polymer samples having an initial weight of ~30 mg (W_0) were cut from cast films and then immersed in 20 mL glass vials loaded with 10 mL PBS at 37 °C. At predetermined time points, samples were removed, dried in a vacuum oven at 60 °C for 3 d, then weighed (W_1). The percent mass remaining was calculated as $W_1/W_0 \times 100\%$. Further, the inherent viscosity (IV) of each polymer sample after 4 and 8 wk degradation time was measured using an Ubbelohde viscometer method as described above. The residual IV was calculated as $IV_1/IV_0 \times 100\%$, where IV_0 represents the original inherent viscosity before degradation and IV_1 represents the inherent viscosity after degradation.

2.7. In vitro cell culture

Polymer films were cut into 6 mm diameter disks using a standard punch and sterilized using 70% ethanol solution and UV irradiation in a laminar flow cell culture hood (Class II A/B3 Biological Safety Cabinet). Vascular smooth muscle cells previously isolated from Lewis rat aorta (RSMCs) were seeded on the surface of each polymer film in a 96-well cell culture plate for evaluating cellular attachment and cellular growth, using 2×10^4 and 5×10^3 cells respectively. The culture medium (DMEM supplemented with 10% fetal bovine serum and 5% penicillin/streptomycin solution; Lonza) was changed every 2 days. A mitochondrial activity assay (MTT assay, Sigma) was used as an indirect method to quantify cellular attachment at 1 d and cellular numbers at 2, 3 and 4 d, with the presumption of similar metabolic activity across the surfaces and over time. Tissue cultured polystyrene (TCPS) was utilized as a positive control. To qualitatively verify the results based on the MTT