Synthesis of Polyurethane Foam Scaffolds for Bone Tissue Engineering

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Introduction

Biocompatible and biodegradable polyurethanes have been investigated as scaffolds for tissue engineering applications for almost twenty years[1-6]. To avoid the toxic diamine decomposition products from aromatic diisocyanates[7], aliphatic diisocyanates, such as methyl 2,6-diisocyanatohexanoate (lysine methyl ester diisocyanate, or LDI), have been used to synthesize biodegradable polyurethanes. Porous polyurethane scaffolds have been prepared from LDI and polyols by incorporating a porogen (e.g., salt[1] or gelatin[6]) or water[5], which reacts with isocyanate to form carbon dioxide, a biocompatible blowing agent. *In vitro*[8] and *in vivo* studies[1,8,9] have demonstrated that porous polyurethane scaffolds prepared from LDI and polyester (or polyether) polyols degrade to non-toxic by-products and support the migration of cells and growth of new tissue. Polyurethanes are potentially useful for injectable scaffolds because they comprise a reactive two-component system. By mixing two liquid components, a solid polymer can be formed and cured *in vivo*. To be clinically useful, the injectable scaffold must have high porosity to facilitate the ingrowth of cells and new tissue, be dimensionally stable, rise quickly, and degrade at a controlled rate to biocompatible degradation products. In this study, we synthesized porous polyurethane foams from LDI and a 70/30 poly(ε-caprolactone-*co*-glycolide) triol. Sulfated castor oil (Turkey red oil) and the polyethersiloxane TEGOSTAB 8300 were used as stabilizers to emulsify the raw materials and stabilize the rising bubbles[10-12]. Calcium stearate was added as a cell opener[10]. Triethylene diamine (TEDA, sold as TEGOAMIN33) was used as a tertiary amine catalyst.

Experimental

LDI was purchased from Kyowa Hakko USA (New York). Stannous octoate and Turkey red oil were purchased from Aldrich. ε-caprolactone was purchased from Acros Organics. Glycolide was purchased from Polysciences (Warrington, PA). TEGOSTAB 8300 and TEGOAMIN 33 were obtained from the Goldschmidt Chemical Company (Hopewell, VA). All chemical reagents were used as received. A 900-Da polyester triol (P7C3G900, see Table 1) was synthesized from a glycerol starter and a 70/30 (w/w) mixture of ε-caprolactone/glycolide monomers using previously published techniques[13]. Polyurethane foams were prepared by a two-step process using a Hauschild SpeedMixer™ DAC 150 FVZ-K vortex mixer. First the polyol-side components comprising polyol, water, catalyst, stabilizer, and cell opener were mixed in a 10-g cup at 3500 rpm for 30 s. The appropriate amount of LDI

Table 1. Polyurethane foam formulations.

	Parts per 100 parts polyol			
Component	A	B	C	D
P7C3G900	100	100	100	100
Water	3.0	2.5	2.9	3.0
TEGOAMIN 33	2.9	3.1	2.9	2.9
Turkey red oil	2.5	2.5	-	-
TEGOSTAB 8300	-	-	1.6	1.0
Calcium stearate	3.0	3.5	3.0	3.0
LDI	84.7	79.5	85.5	86.4

was then added and the two components mixed at 3500 rpm for 15 s. The mixture was then poured into an open mold where it was allowed to rise. The foam formulations are shown in **Table 1**.

MG-63 human osteosarcoma cells (American Type Culture Collection, Manassas, VA) were cultured in 100-ml spinner flasks under dynamic conditions following the method of Vunjak-Novakovic[14]. Ham's F12 nutrient mixture (F12), minimal essential medium (MEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), 0.05% trypsin-EDTA, and sterile distilled water were purchased from Invitrogen Corp. (Carlsbad, CA). Pieces of polyurethane foams (3 mm x 10 mm x 10 mm) were threaded onto K-wire and immersed in the medium. Each spinner flask was charged with 130 ml medium and seeded with 9.0×10^6 cells. The contents of each flask were maintained at 37°C and 5% CO_2. Cell viability was assessed using a LIVE/DEAD® Viability/Cytotoxicity Assay Kit (L-3224) (Molecular Probes, Eugene, OR) at days 3 and 7 following initial cell seeding. Pieces of foam were removed from their respective flasks and assayed according to the manufacturer's specifications. Visualization of adherent cells was performed with a Zeiss Axiovert 200 inverted fluorescent microscope (Oberkochen, Germany) with filters DAPI and FITC.

Table 2. Porosity of polyurethane foams.

Foam	Density kg m^{-3}	Porosity vol-%	Shrinkage %
A	43.5	96.5	5.4
B	52.1	95.8	6.4
C	49.3	96.0	2.9
D	49.9	95.9	1.0

Results

The composition of the foams was verified by IR spectroscopy, which indicated essentially complete conversion of free NCO after 24 h. The height of the rising foam was measured versus time. The rise time, defined as the time required for the foam to reach 90% of its final height, was approximately 20 minutes for Turkey red oil-stabilized foams and 35 minutes for polyethersiloxane foams (data not shown). The density, porosity, and shrinkage measured after 24 h are listed in **Table 2**. The porosity of all four foams is >95%, resulting in a highly open and porous structure which facilitates cell attachment. An SEM image of Foam B is shown in **Figure 1**. The pores range in size from 100 – 1000 μm which is within the preferred range for scaffolds. The pores are also open and inter-connected, thereby facilitating transport of cells through the scaffold. Live cells (green) attached to Foam B on day 7 (after seeding) are shown in **Figure 2** after live/dead staining. All four foams supported the attachment of live cells.

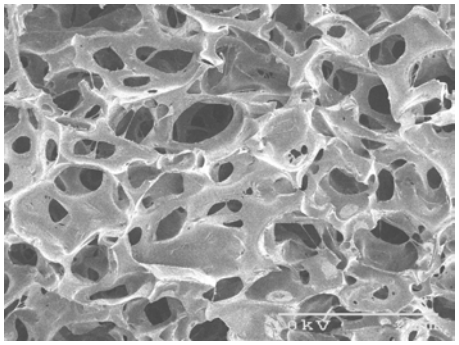

Figure 1. SEM of foam B.

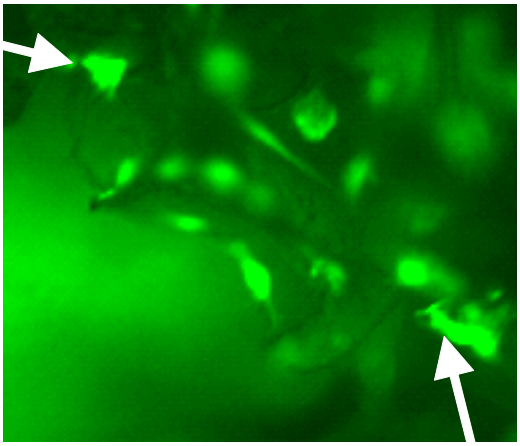

Figure 2. Cells attached to foam B.

Discussion

Porous biocompatible, biodegradable

polyurethane scaffolds can be synthesized from LDI, polyester polyol, water, Turkey red oil and polyethersiloxanes (stabilizers), DMEA (tertiary amine catalyst), and calcium stearate (cell opener). The scaffolds are a potentially injectable two-component reactive mixture which can be cured *in vivo*. The pores range in size from 100 – 1000 μm, have porosity > 95%, and support the attachment of cells *in vitro*. The rise time varies from 20 – 35 minutes and the cure time is less than 24 h. Furthermore, the maximum temperature reached during cure is 39°C, which is physiologically acceptable. Because the shrinkage is less than 7% after 24 h, the scaffolds are dimensionally stable and do not lose significant volume after curing. Toxicity concerns were addressed through the choice of raw materials used to synthesize the foams. LDI, an aliphatic diisocyanate which degrades to lysine, was used in place of MDI or TDI, aromatic diisocyanates which degrade to toxic aromatic diamines. Stannous octoate, a tin catalyst commonly used to prepare commercial polyurethane foams, was replaced with a tertiary amine catalyst.

Future work will focus on reducing the rise time, improving the mechanical properties, investigating the control of porosity by adjusting the formulation, *in vitro* and *in vivo* biocompatibility testing, and degradation studies.

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