In vitro biocompatibility and biodegradation of poly (esterurethane urea) scaffolds

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Introduction: Porous poly(esterurethane urea) scaffolds have been reported to degrade to non-toxic by-products and support the migration of cells and ingrowth of new tissue *in vivo*.[1, 2] These two-component materials can be synthesized by reactive liquid molding, thereby making them potentially suitable for injectable applications. The objective of this study is to investigate the effects of water concentration and polyester polyol composition on *in vitro* biocompatibility and biodegradation. The clinical goal is to develop injectable poly (esterurethane urea) scaffolds that deliver biologically active components to enhance healing of bone fractures.

Materials and Methods: Poly (esterurethane urea) foams were prepared by reactive liquid molding of two components: (a) lysine methyl ester diisocyanate (LDI), and (b) a hardener comprising polyester polyol, water, catalyst, stabilizer, and pore opener. Three 900-Da poly(ε-caprolactone-*co*-glycolide-*co*-lactide) triol compositions with half-lives ranging from 30 – 230 days were synthesized from a glycerol starter using published techniques[3]. The water content in the hardener was varied from 0.5 – 2.75 parts per hundred parts polyol (pphp). Water reacts with LDI to form gaseous carbon dioxide, which functions as a blowing agent. The reactions of LDI with water and polyol were catalyzed by 3 pphp triethylenediamine.

The height of the rising foam was measured versus time and shrinkage was calculated as the reduction in foam volume after 24 h. Pore size was determined by SEM. The density and the compressive stress required to generate a 50% deflection were measured according to ASTM D3574. To evaluate *in vitro* biocompatibility, MG-63 cells were seeded on the scaffolds under dynamic conditions[4]. Cell viability was determined by live/dead staining and cell counts were measured by CyQuant assay. Dynamically seeded MC3T3 osteoblast cells was fixed in 2.5% glutaraldehyde and processed for SEM. Degradation experiments were carried out to understand the polymer degradation. Foams were incubated in PBS at 37 °C in 5 % CO_2 for 4 to 8 weeks and the media collected and tested for cytotoxicity (LDH assay) and live/dead staining. The polymer degradation products were analyzed using micro FT-IR (Nicollet Coninuμm™).

Results and Discussion: Polyurethane foam scaffolds cast as reactive liquid mixtures rose and gelled in approximately 20 minutes. By varying the concentration of water and surface-active components in the hardener, porous scaffolds with porosities ranging from 85 – 95% and pore sizes ranging from 200 – 1000 μm were prepared. The foams were soft with compressive stresses at 50% deflection ranging from 1 – 3 kPa, which limits their utility to non load-bearing applications. The maximum temperature reached during cure was less than 40°C. Counts of MG-63 cells seeded on polyurethane scaffolds are shown in **Figure 1**. The statistically significant difference between cell counts on Days 1 and 4 for Foams A – D indicates that the cells proliferated on foams stabilized by sulfated castor oil. Cells proliferated to a lesser extent on Foams E – F, which were stabilized by polyethersiloxanes. Although the cell proliferation assays show less cell proliferation with Foam E, SEM revealed good cell adhesion, and cell ingrowth **(Figure 2.)** FT-IR of the degradation products did not reveal any free isocyanate or toxic components. LDH assay and live/dead staining of the cells after incubation with the polymer

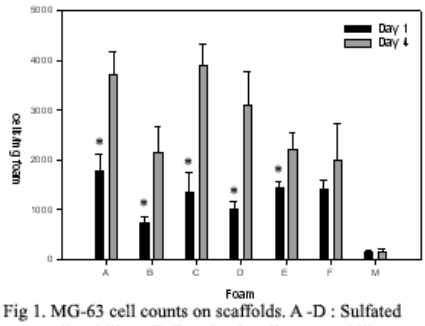

Fig 1. MG-63 cell counts on scaffolds. A -D : Sulfated castor oil stabilizer. E -F: polyethersiloxane stabilizer. M: polyethylene control. * Significant difference ($p < 0.025$)

degradation products confirmed 70-80% viable cells suggesting the polymer degradation products are less toxic, further confirming our FT-IR observation.

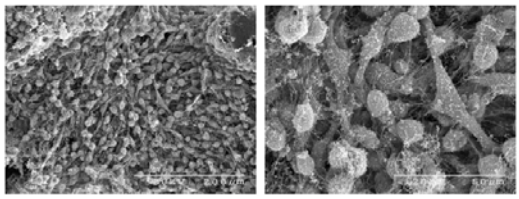

Figure 2. MC3T3 osteoblast cells seeded dynamically on Foam - E after 21 days in osteogenic medium

Conclusion: Polyurethane foam scaffolds have been prepared by casting reactive liquid mixtures in open molds. Because the foams rise and gel in about 20 minutes with minimal temperature increase, they are potentially injectable. Porosity and pore size can be tuned to targeted values by varying the composition of the hardener. Foams prepared from sulfated castor oil stabilizers support the attachment and proliferation of MG-63 cells *in vitro*. These materials have potential application as injectable delivery systems of transcriptional osteoblast regulators to enhance bone fracture healing.

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