Application of Polymer Processing Methodologies in the Preparation of Scaffolds for Tissue Engineering

Newell R. Washburn, Carl G. Simon, Kimberlee Potter*, Joy Dunkers, Alamgir Karim, and Eric J. Amis
National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
*National Institutes of Health, Bethesda, MD, USA

Introduction

Polymer processing methodologies have been applied toward the preparation of scaffolds for tissue engineering. The use of polymer blending and co-extrusion to create polymeric materials with appropriate morphology, mechanical properties, and hydrophilicity is presented.

Materials and Methods

Poly(ε-caprolactone) (PCL) and single-phase blends of PCL with poly(D,L-lactide) (PLA) were mixed with poly(ethylene oxide) (PEO) in a twin-screw extruder to form a two-phase blend with micron-sized domains. After subsequent annealing the PEO was leached out with water, resulting in a porous material.[1] Scaffold morphology was assessed using scanning electron microscopy (SEM) and optical coherence tomography (OCT). Compressive moduli were measured as a function of void volume fraction. Scaffolds were seeded with MC3T3-E1 cells in medium supplemented with serum. Biocompatibility was assessed by monitoring the long-term behavior of cells on the scaffold surface using a live/dead cell assay as well as standard cytotoxicity tests. Cell proliferation was monitored visually using OCT and magnetic resonance imaging (MRI).

Results and Discussion

In figure 1 is shown an SEM image of a 30% PCL/70% PEO sample that was annealed for 20 minutes at 80 °C followed by dissolution of the PEO with water. The image spans 2000 μm indicating the pores

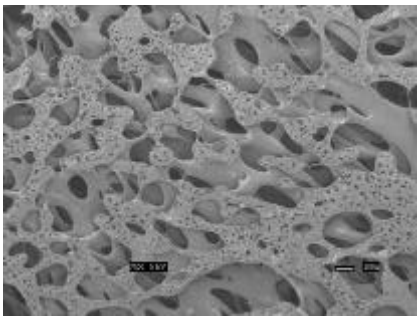

Figure 1. SEM image of annealed PCL scaffold.

are in excess of 100 μm, the length scale of interest for many tissue engineering applications. It also appears that the PCL and the void space both form continuous networks, which is important for tissue growth.

Scaffold microstructure was also investigated using optical coherence tomography (OCT). Using contrast-matching techniques it is possible to obtain data 700 μm into the material. These data are consistent with the assertion that the pore network is continuous.

The compressive modulus of the scaffold displays a lower modulus (1 MPa) at strains less than 5-10% and a higher modulus (10-30 MPa) at higher strains, depending on the void volume fraction. The materials with high void content tend to collapse under lower loads.

Results of osteoblast seeding on the scaffolds suggest that it is possible to use twin-screw extrusion to blend polymers without the introduction of toxic contaminants. While the crystalline PCL scaffolds largely retain their morphology during long exposures to aqueous environments, the surface appears to be too hydrophobic to promote extensive cell adhesion. Initial seeding studies suggest that cell adsorption will not occur without thorough conditioning of the polymer surface by serum proteins. In figure 2 is shown a scaffold similar to that in figure 1 that has been seeded with osteoblasts and cultured for 9 days. A cluster of cells is visible but it appears that they have not completely covered the scaffold surface. Since the cells on the base of the well plate appeared to reach confluence, we conclude that the scaffold surface is too hydrophobic to promote cell adhesion and proliferation in this medium but the material itself is not toxic.

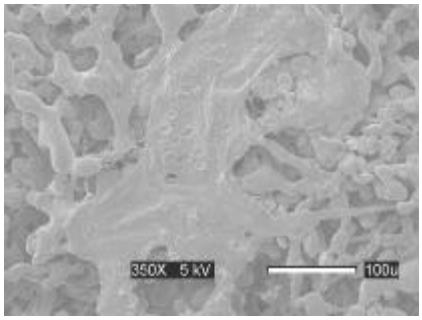

Figure 2. SEM image of osteoblasts on PCL scaffold.

The improvement of the scaffold properties is pursued by blending the PCL with PLA, which forms a single-phase blend.[2] This blend has a lower water-polymer interfacial tension and appears to promote cell adhesion over the pure PCL scaffold. In addition, the mechanical properties of the scaffold are enhanced by blending with the glassy PLA. The results of osteoblast seeding onto the PLA/PCL blend are also presented.

Magnetic resonance imaging and OCT are used to characterize the long-term proliferation of osteoblasts on the scaffolds. These complementary, non-destructive techniques provide information about changes in the scaffold structure and tissue formation over time.

References

(1) Washburn, N. R. *et al.*, in press.
(2) Meredith, J. C., Amis, E. J. *Macromol. Chem. Physic.* **201**, 733-739 (2000).