Developing Polymer/Ceramic Scaffolds using a Factorial Design of Experiments and Response-surface Analysis

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Statement of Purpose: Tissue engineering approach has shown promise for the treatment of bone defects. Polymer/ceramic scaffolds seeded with cells and growth factors can help to speed the body's natural healing processes and decrease the rehabilitation time for patients. The effectiveness of these scaffolds is determined by examining several characteristics including porosity, interconnectivity, and mechanical properties. The goal of this study is to develop polymer/ceramic scaffolds with fully-interconnected channels through melt processing of polycaprolactone (PCL) and poly(ethylene oxide) (PEO), combined with hydroxyapatite (HA) and salt (NaCl), followed by porogen leaching using water. The effects of polymer ratio, ceramic and salt content, and pressure applied during the fabrication process have been examined in this study. A factorial design of experiments (DOE) and response-surface analysis have been used to determine the optimal scaffold fabrication parameters.

Methods: PCL (Mw: 70,000–90,000) and PEO (Mw:100,000) were purchased from Sigma-Aldrich. Compression-molding at 100°C, and different applied pressures, was used to produce PCL/PEO scaffolds with compositions of 40:60, 35:65, and 30:70 wt % (Table 1). Scaffold pore size was controlled by grinding and sieving of both polymer particles prior to melt processing and limiting particle size to between 250 µm and 425 µm. The HA supplied by Plasma Biotal LTD was composed of particles ~ 5µm in size. To produce PCL/HA scaffolds, HA particles were blended with PCL powder using a vortex mixer and compression molded. The resulting PCL/HA disks were then ground and sieved similar to the polymer powders. Scaffolds with three different HA concentrations were fabricated: 10%, 20%, and 30% w/w. Three ranges of salt particle size were examined to determine the effect of particle size on the mechanical properties and scaffold architecture: less than 100 µm, between 100 µm and 250 µm and 250 µm and 425 µm. The target salt concentration was 20% and 40% w/w. The produced constructs were placed in a bath of deionized water at 40°C for 24 hours to leach out the PEO and NaCl. The scaffolds were tested using an Instron 3345 with a 1 kN load cell. Ramp compression tests were carried out at a displacement rate of 1 mm/min to estimate the modulus at 10% strain. The scaffolds were imaged using Scanning Electron Microscopy (SEM) to examine the morphology and pore interconnectivity (Figure 1). The scaffold porosity was calculated using weight and volume measurements, and based on the combined polymer/HA matrix density (rule of mixtures).

Results: The results of mechanical testing and porosity calculations for scaffolds with various polymer ratios indicated that a higher ratio of PEO to PCL decreased the modulus and increased the porositry. Scaffolds with 30% HA showed the lowest compression modulus (Figure 2). Applying pressure during the fabrication process resulted in a higher compression modulus than similar scaffolds fabricated without pressure. Scaffold with 40% salt, with particle sizes below 250 µm, showed an increase in mechanical porperties after complete PEO and NaCl leaching, partly due to the entrapment of salt.

Table 1: Parameters for the design of experiments

		Low Point	Mid-Point	High Point
1	Polymer ratio (PCL/PEO)	40/60	35/65	30/70
2	Pressure (MPa)	0	0.25	0.5
3	HA Content (%)	10	20	30
4	2nd porogen (%)	0	20	40

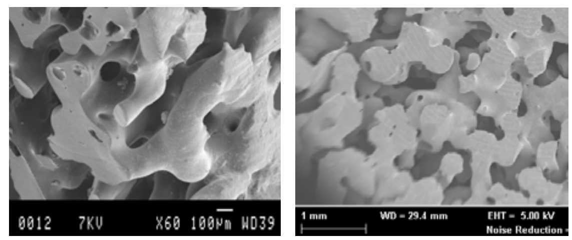

Figure 1. a) PCL scaffolds with 20% leached salt; b) PCL scaffolds with 20% HA. Leached PEO: 60%

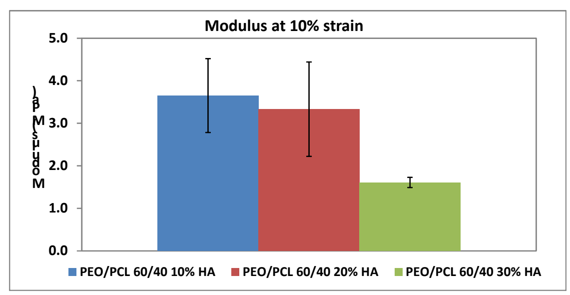

Figure 2. The effect of HA on modulus at 10% strain.

Conclusions: This study confirmed that melt processing is an effective method for creating scaffolds with desired pore size range (> 100 µm), and porosity greater than 50%. Also, the incorporation of HA into the scaffolds was successful and did not negatively impact scaffold morphology or pore formation. The factorial design of experiments (DOE) enabled us to analyze the effect of the process parameters on scaffold architecture and mechanical properties. The porosity and interconnectivity of the final scaffolds were affected by several factors including HA content, polymer ratio, NaCl concentration and particle size, as well as the pressure applied during the fabrication process. The results were used to perform a response-surface analysis so as to optimize the properties. The outcome of this study is to maintain adequate mechanical properties, while maximizing pore interconnectivity and permeability. The *in vitro* studies are currently underway to verify the suitability of these constructs for bone tissue engineering.