Reference Scaffolds for Tissue Engineering

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Statement of Purpose: A reference scaffold is being developed for use as a standard during characterization of new scaffold constructs. In this work, the structure and permeability of the reference scaffolds is presented. Freeform fabrication (FFF) was chosen to make the reference scaffolds since this technique affords the most precise control of scaffold structure. Scaffold structure was assessed using X-ray microcomputed tomography (μCT) and permeability was measured using an automated liquid permeameter. The scaffold structural parameters of porosity and pore size as well as scaffold permeability were found to be very reproducible. The reference scaffolds will be available from National Institute of Standards and Technology in the future to serve as calibration standards during characterization of tissue engineering scaffolding materials.

Methods: Reference scaffolds were fabricated from poly(ε-caprolactone) (weight averaged molecular weight 65,000) (PCL) using a precision melt extrusion deposition system [1]. Temperature in the feed chamber was 95 °C while the nozzle was 90 °C. Nozzle diameter was 0.178 mm, nozzle speed was 10 mm/s and flow rate was 0.017 mL/min. Strut diameter was approximately 200 μm. Scaffolds were fabricated into cylinders with 5 mm height and 20 mm diameter (Fig. 1). Scaffolds with three different strut spacings were fabricated (strut-edge to strut-edge distance; gap width): 200 μm, 300 μm and 450 μm. Scaffold morphology was characterized by μCT (μCT 40, Scanco Medical). The microfocus X-ray source was set at 45 kVp and 177 μA to give a spot size of 5 μm. The samples were scanned at a 15 μm voxel resolution with an integration time of 0.2 s. 3D reconstructions were made using Sigma 1.2, Support 2 and Threshold 30. The porosity, pore size and wall thickness were determined by direct distance transformation methods [2]. Scaffold permeability was measured with an automated liquid permeameter (LP-101-A, Porous Materials, Inc.) using oil as the liquid (ASTM Oil Viscosity Reference Standard S6). The rate of fluid flow through the scaffolds was measured over a range of pressures (500 Pa to 2000 Pa) and used to calculate permeability using Darcy's Law.

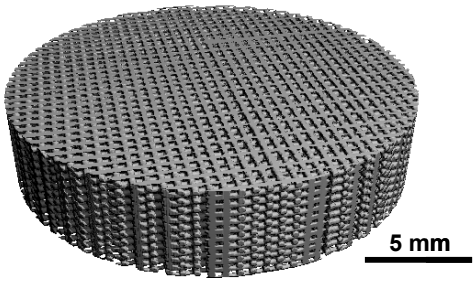

Fig. 1. μCT image of reference scaffold (300 μm strut spacing).

Results: Scaffold porosity and pore size increased with increasing strut spacing (Table 1) and differences between the scaffolds with different strut spacings were significant [$p < 0.05$; ANOVA (analysis of variance) with Tukey's comparison test]. These results fit the expectation that increasing strut spacing will lead to increased porosity and pore size. In addition, the standard deviations for the μCT-calculated porosity and pore size were ≤ 5 %, indicating that scaffold fabrication was precise.

Table 1. Scaffold Structural Parameters Determined by μCT*

Strut Spacing (μm)	200	300	450
Porosity (%)	44.1 (2.4)	58.5 (3.3)	67.2 (2.0)
Pore Size (μm)	125 (5)	234 (28)	362 (16)

*Averages (n = 3) with standard deviations are given (same as the combined standard uncertainty for the purposes of this work).

The permeability of the scaffolds increased with increasing strut spacing (Fig. 2) and all of the permeabilities for the different strut spacings were significantly different from one another ($P < 0.005$; ANOVA with Tukey's comparison test). These results fit the expectation that increased strut spacing will lead to increased permeability.

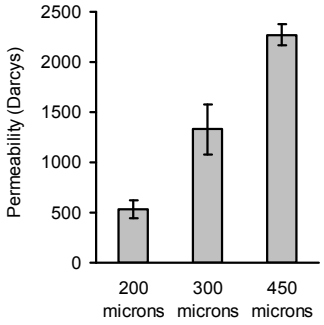

Fig. 2. Permeability of reference scaffolds (n = 3; error bars are standard deviation which is the same as the combined standard uncertainty for the purposes of this work).

Conclusions: Freeform fabrication yielded scaffolds with reproducible structural parameters (porosity, pore size) and permeability indicating that the scaffolds could be applied as reference materials for use during characterization of new scaffold materials.

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