Fabrication and Characterization of Sphere-templated Fibrin Scaffolding

Linnes, Michael P. Ratner, Buddy D. Giachelli, Cecilia M.
University of Washington

Statement of Purpose: A significant amount of research has recently been done investigating the use of fibrin in tissue engineering. Fibrin is a material that can be autologously sourced, is highly angiogenic, and is what the body uses as a temporary scaffold during wound healing. Fibrin is most commonly used as a continuous hydrogel that can both entrap cells and be molded into numerous shapes. We present here a new use for fibrin as a promising tissue engineering application. Sphere-templated fibrin is a biocompatible and biodegradable scaffold with a high surface area for cellular attachment, a high void volume, and interconnected pores for nutrient transfer and cellular infiltration.

Methods: *Scaffold Fabrication:* Sphere templated fibrin scaffolding was created in three steps. First, templates were made using polymethyl-methacrylate (PMMA) beads that were sieved to a uniform distribution, sonicated to pack the beads into the mold, and sintered at 145°C for 24hours in order to form a precise interconnectedbead structure. Next, a fibrin gel was formed around the template. To achieve this, a 200mg/mL solution of fibrinogen (Sigma, USA) was drawn into the templates using a vacuum chamber. The fibrinogen was then polymerized via diffusion of 13U/mL thrombin (Sigma, USA) and calcium through the scaffold. Finally, the PMMA beads were solvent extracted leaving behind only the fibrin scaffold.

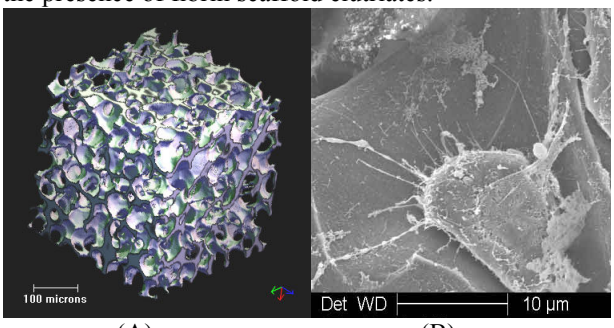
Scaffold Characterization: Rectangular strips of the fibrin scaffold were mechanically tested on a 5500 series Instron mechanical tester. Samples (1cm x 2mm x 2mm)were stretched at a rate of 10mm/min between grips designed for small sample applications. Pore size and interconnectedness of pores were measured using digital volumetric imaging (DVI) and scanning electron microscopy (SEM). DVI was also used to calculate overall porosity of the scaffolds. Cellular attachment was viewed using SEM.

Cytotoxicity: A standard cytotoxicity test (ISO 10993-5) was performed using the elution method. Images of the NIH-3T3 fibroblasts were taken at 0, 24, and 48 hours to measure proliferation and survival in the presence of the scaffolds elutriates.

Results / Discussion: *Scaffold Morphology:* Figure 1A is a 3-D DVI image showing the morphology of a sphere templated fibrin scaffold created using PMMA beads of 30-38µm in diameter. The DVI software allows for precise calculations of pore size and interconnected necking size in a 3-D scaffold rendition. The average pore size was found to be 34 +/-2.95µm and the average interconnected neck size was 17 +/-2.39µm. The porosity of the scaffold was found to be 74% using the DVI software (RESView™ v3.2). SEM images also showed the ability of the scaffold to support cellular attachment (Figure 1B).

Mechanical Properties: Tensile testing of the sphere templated scaffolds yielded a Young's Modulus of 61.8 +/-9kPa and a max strain percent of 173% +/-15 (n = 9). The mechanical properties of the porous fibrin scaffolding material are very similar to what has been previously reported for solid fibrin hydrogels of similar fibrinogen concentrations[1, 2]. There is no significant loss of overall distensibility or strength in the 74% porous templated material compared with the solid hydrogels. This may be due to the alignment of fibers caused by the spherical constraints during polymerization.

Cytotoxicity: The Cytotoxicity assay showed no decrease of proliferation of the NIH-3T3 fibroblasts when placed in the presence of fibrin scaffold elutriates.



(A) (B)

Figure 1: (A) DVI image of fibrin scaffold with 30-38µm pores. (B) SEM image of cellular attachment to porous scaffolding material.

Conclusions: The aim of this study was to characterize the morphological and mechanical properties of a novel scaffolding material. We were able to show that PMMA sphere templates allow for very reproducible pore sizes and interconnectedness of the fibrin hydrogel. Also, the templating procedure did not cause any loss in strength of the bulk properties of the material. We were also able to show that the processing of the scaffold did not leave behind any cytotoxic byproducts that would be detrimental to future tissue engineering applications. This fibrin templating method can be tailored for a number of applications in the future including the creation of larger sized pores (100-500µm) for bone tissue engineering[3] or small pores (20-50µm) for increased angiogenesis.

References:

1. Velada, J. L., Hollingsbee, D. A., Menzies, A. R., Cornwell, R. and Dodd, R. A. Biomaterials. 2002; 23: 2249-2254.
2. Sierra, D. H., Eberhardt, A. W. and Lemons, J. E. J Biomed Mater Res. 2002; 59: 1-11.
3. Karageorgiou, V. and Kaplan, D. Biomaterials. 2005; 26: 5474-5491.

This Research was supported by the Singapore University of Washington Alliance