Sphere-templated Scaffolds of Acetone-Treated Fibrinogen for Tissue Engineering

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

Statement of Purpose: In tissue engineering, the scaffold plays a pivotal role in the success of a construct. Enhancement of cellular attachment, proliferation, and organized development of native structures are just a few of the key features of a beneficial scaffold. A good scaffold will also comply with the mechanical properties of the implant location and degrade as cells are able to create new extracellular matrix (ECM) materials to take over load bearing. The transfer of structural support from the scaffolding material to newly created ECM is an important balance. If the burden of support is transferred to the ECM too soon, the material may fail, and if the scaffold remains around too long, the body often walls it off in a dense and avascular fibrous capsule, reducing nutrient transport to the area. We present here a method to tailor the mechanical properties of fibrinogen, a naturally angiogenic protein, using combinations of thrombin, genipin, and acetone treatments combined with sphere-templating.

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, interconnected bead structure. Next, a solution of fibrinogen was pulled into the scaffold and precipitated with acetone. To achieve this, a 200mg/mL solution of fibrinogen (Sigma, USA) was drawn into the templates using a vacuum chamber. The fibrinogen was then precipitated with acetone to form a robust scaffold for various time periods of between 9 and 120hrs. This step also removes the PMMA template leaving behind the fibrinogen based scaffold. Other formulations for the scaffold include polymerizing to fibrin via diffusion of 13U/mL thrombin (Sigma, USA) and calcium through the scaffold or crosslinking with 0.625% Genipin in PBS.

Scaffold Characterization: Dogbone shaped fibrin and fibrinogen scaffolds were mechanically tested on a 5543 Instron mechanical tester with a 10N load cell and pneumatic grips. Samples were stretched at a rate of 20mm/min during 3 pre-conditioning cycles and then until failure. Pore size and interconnectedness of pores were measured using digital volumetric imaging (DVI) and surface characteristics were examined using scanning electron microscopy (SEM). DVI was also used to calculate overall porosity of the scaffolds. Cellular attachment was also 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:* Acetone-treated fibrin scaffolds are very different morphologically from acetone-treated fibrinogen scaffolds. SEM shows distinct fibers on the surface of the pores of the fibrin samples not present in other samples. All processing techniques were able to retain the microporous structures formed using the pMMA template. These studies suggest that while acetone-treated fibrin scaffolds retain the nanofibrillar structure of native fibrin, the acetone-treated fibrinogen scaffolds are more likely to represent denatured intact fibrinogen molecules that undergo self-association to form a solid matrix.

Mechanical Properties: We were able to tailor the Young's Modulus of the fibrinogen scaffolds to between 6.95+/-1.38kPa and 17.68+/-2.66kPa through different acetone treatment times. When the scaffolds were strengthened with thrombin polymerization this jumped to between 45.82+/-4.31kPa and 82.27+/-4.54kPa. Treatment with genepin, a natural crosslinker, brought the modulus to between 22.28+/-3.98kPa and 98.86+/-10.4kPa. The mechanical properties of the porous fibrinogen and fibrin scaffolding materials were very similar to what has been previously reported for cardiac tissue (67kPa) as well as solid fibrin hydrogels of similar concentrations.[1-3]

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

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 the protein interactions are strengthened when placed in acetone and that the scaffold's mechanical properties can be varied to match the tissue they are intended to replace throughout a large range of strengths. The innate advantages of fibrinogen combined with the acetone strength treatment and micro-porous templating provide a promising way to support cell growth.

References:

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This Research was supported by the Singapore University of Washington Alliance (02-1-95-45-025) and the NIH Bioengineering Cardiovascular Training Grant (T32 EB001650)