Computer Aided Biomanufacturing of Protein Scaffolds with Controlled Macroporosity

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Introduction: Macroscale porosity (0.1 mm or greater) is one of the most critical facets of an implantable device in applications that necessitate tissue integration and vascularization. It has been a great challenge to reconcile macroporosity and mechanical robustness in delicate protein based biomaterials, such as collagen. Classically, porosity in collagen biomaterials is induced by freeze drying or salt-leaching[1-3]. While these methods are useful, porosity is random, has limiting interconnectedness and degree of control on the uniformity of pore size and shape is low. We are presenting a novel biofabrication method to manufacture lattice structures with controlled pore size and shape by patterned electrocompaction of collagen molecules using electrical currents (Fig.1).

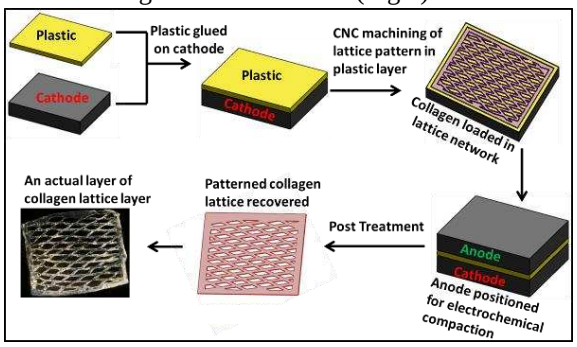

Fig.1: Patterned electrocompaction of collagen.

Methods: Fabrication of 3-D Scaffolds with Controlled Porosity: Two planar carbon electrodes were used for patterned deposition. The cathode is layered with a plastic sheet and this bilayer structure is mounted on a computer controlled micromill. The pattern designed in CAD software (Solidworks) is machined on the plastic sheet in full depth to expose the cathode surface. Type-I collagen solution was loaded in the patterned groves. The carbon anode layer was placed on top of the plastic-cathode bilayer. Upon application of electric current, collagen molecules electrophoretically mobilize[4-5] and become compacted within patterned grooves. To manufacture the final scaffold, the individual patterned layers are registered on top of each other and attached by mildly acidic collagen solution and crosslinked in genipin. Mechanical Properties: Six-layered scaffolds with 3 pore geometries were manufactured: rectangular, square and diamond shape (Fig.2, pore sizes of 1.5, 0.8 and 1.2 mm, respectively). To evaluate the effect of the number of layers on the overall mechanical properties of the scaffold, square pore scaffolds with 2 layer, 4 layer or 6 layers were manufactured. The lateral dimensions of the final scaffolds were 20 X 12 mm. Samples were tested under monotonic tension at a rate of 10 mm/min until failure. Modulus, toughness and failure stress values were measured from the stress-strain curves. Cell Seeding: MSCs (Lonza) were seeded on rectangular pore scaffold with 6 layers to assess whether cells populate the continuum of the patterned scaffold. One-way analysis of variance was performed to assess differences between groups at $p<0.05$.

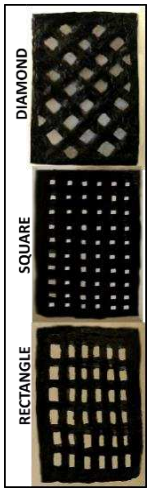

Fig. 2: Macroporosity with controlled pore size and shape

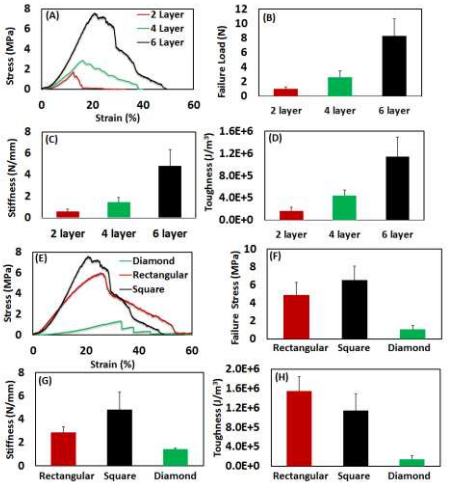

Fig. 3: Mechanical properties for different scaffold groups.

Results: Post-yield deformability improved with the number of layers (Fig.3A). A 3-fold increase in the number of layers resulted in about 8-fold increase in failure load, stiffness and toughness values (Fig.3B-D). Pore shape also affected mechanics. Failure stress, stiffness and toughness increased about 3 fold, 2.5 fold and 10 fold, respectively, from diamond shape to rectangular and square shape pore scaffold (Fig.3F-H). There was no significant difference between the mechanical properties between rectangular and square shape pore scaffolds. F-actin and DAPI stained images (Fig. 4) revealed that cells covered the entire scaffold through thickness.

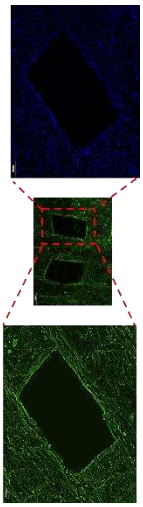

Fig 4: F-Actin (green) and DAPI (blue) staining images of ells seeded on scaffolds.

Conclusions: The current study developed a CAD/CAM based technique to manufacture pure collagen scaffolds with controlled porosity. High compaction and alignment of the collagen molecules make the construct mechanically robust. The results suggest that by changing the number of layers and shape of the structure, mechanical properties can be modulated for different tissue repair applications. The present study demonstrated that cells adhere uniformly over the scaffold. This scaffold model has the potential for repair of tendon, hernia, urinary stress incontinence and thoracic/abdominal wall.

References: 1.Haugh et al.,Tissue Eng..,2010; 2.O'Brien et al., Biomaterials, 2003; 3. Kim et al., Acta Biomater.,2008;4. Cheng et al., Biomaterials, 2008; 5.Uquillas et al. Ann Biomed Eng, 2012.