Biofabrication of a Lamellar Elastic Scaffold for Intervertebral Disk Regeneration

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Statement of Purpose: Low back pain has affected over 80% of the adult population, which costs society nearly $90 billion each year.[1] The primary cause of low back pain results from a degenerated intervertebral disk (IVD). Conventional methods to alleviate this pain include spinal fusion and artificial disk replacement. These procedures do not restore natural kinematics of the spine and may cause stress shielding due to their large compliance mismatch. An elastic polymeric replacement composed of chitosan/gelatin (CS/GEL) offers a solution to these problems by offering a better compliance. This is the first study attempting to fabricate a lamellar structure mimicking natural IVD microstructure. CS/GEL materials consisting of a lamellar structure will mimic natural morphology found in the annulus fibrosus of IVDs, which has proved one of the most difficult challenges for IVD tissue regeneration, along with imitating the mechanical properties. Lamellar scaffolds guide cell adhesion, in 3D, along a defined microstructure enabling functional matrix to be created similar to that of native tissue. We used a novel biofabrication method combining micropipettes for liquid extrusion and a freezing stage for the solidification of the scaffolding material (Fig. 1A).

Methods: A computer controlled 3D bioprinter with a temperature controlled stage was created for this study. CAD was used to precisely recreate the lamellar structure of the IVD scaffold and control the 3D printing device. Elastic degradable CS/GEL materials were extruded through micropipettes onto the temperature controlled freezing stage where they solidified rapidly through an increase in polymer solution viscosity. SEM was used to evaluate the ability of this biofabrication technique to precisely control polymer deposition to create biomaterial scaffolds with defined microstrucutre similar to native IVD tissue. IVD cells were seeded on the CS/GEL scaffolds to examine the growth and alignment on the printed constructs and to compare the designed scaffold to native tissue. CS/GEL scaffolds compressive dynamic mechanical properties were also measured to prove elastic compliance. Confined compression was also used to compare biomaterial behavior with native human IVD tissue at a range of physiological frequencies (0.25-5 Hz).

Results: Using our biofabrication method, CS/GEL materials can be printed into elastic/lamellar structures mimicking the morphology of IVD tissue (Fig. 1B). The polymer stream can be precisely controlled down to a resolution of 20 µm using the micropipettes. Concentric layers were created with spacing of ~30 µm to mimic the structure within the native IVD (Fig. 1C). IVD cells attached, infiltrated, and aligned along the lamellar 3D scaffolds in a concentric manner similar to native IVD tissue (Fig. 1D). 3D rendering of the scaffold showed cell alignment within scaffold lamellae. The biocompatibility of the CS/GEL materials was verified. Mechanical testing of the scaffolds demonstrated high elasticity, with a J-shaped stress-strain curve while confirming no deformation after cyclic loading (Fig. 2). Dynamic loading also proved that scaffolds exhibited excellent mechanical properties, highly similar to native tissue (Fig. 2&3). Frequency sweeps across a range of physiological frequencies proved there was no significant difference between the scaffolds and the native IVD tissue (Fig. 3).

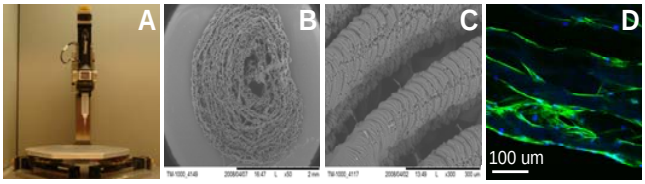

Fig. 1: Custom bioprinter (A), Scaffold mimicking IVD microstructure (B), 3D lamellar layers (C), Cells aligning on 3D lamellar scaffold showing temporal spacing similar to the navtive IVD (D).

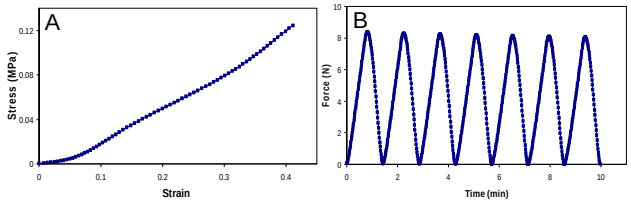

Fig. 2: J-shaped Stress-Strain curve (A), dynamic loading showing no material deformation with ability to support constant loads (B).

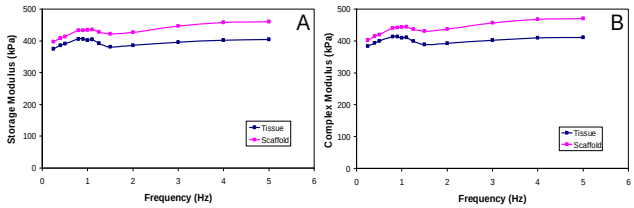

Fig. 3: Graph showing similar storage modulus (A) and complex modulus (B) of human IVD tissue and CS/GEL scaffolds across physiologic frequencies.

Conclusions: The micropipettes and freezing stage improve the resolution of this device down to 20 µm by effectively lowering the CS/GEL viscosity. CS/GEL scaffold morphology and spacing can be controlled, proving optimal as it mimics the complex IVD microenvironment. Mechanical testing proved that the CS/GEL material has similar elastic properties to native IVD tissue. The scaffolds were resilient to deformation after cyclic loading across physiologic frequencies. This is the first technique shown to recreate IVD microstructure with a high resolution scaffold.

References: 1. Luo, X. et al. Spine 2004;29:79–86