3D Printing of an Elastic Lamellar Scaffold for Intervertebral Disc Regeneration

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Statement of Purpose: Low back pain has affected over 80% of the adult population. In about 5% of this population, surgical procedures have been used to alleviate this pain, which costs society nearly $90 billion each year [1]. One primary cause of low back pain results from a degenerative intervertebral disc (IVD). Conventional methods to alleviate this pain include spinal fusion and artificial disc replacement. Spinal fusion does not restore the natural kinematics of the spine and restricts movement. Current artificial discs help replace the degenerated disc and restore some movement. However, most disc replacements generate wear particles, cause stress shielding on the vertebrae, and loosen *in vivo,* causing implant failure. A regenerative medicine strategy may offer solutions to above problems. A lamellar disc scaffold formed from degradable elastomers would offer much better compliance and allow regeneration of natural IVD structure. A lamellar structure mimics the natural histological structure found in the annulus fibrosus of natural IVDs. Lamellar structures also allow a greater surface area for cell adhesion, alignment, and growth. This is the first technique attempting to fabricate a lamellar structure mimicking natural IVD morphology. We used a rapid prototyping technique that combines ultra-fine pipettes for liquid extrusion and a freezing stage for the solidification of the scaffolds mimicking natural IVD structure (Fig. 1A&B). This technique permits the use of many different polymers and is suitable for fabricating scaffolds with different 3D configurations.

Methods: A custom 3D bioprinter with a computer controlled freezing stage was used for this study. Microsoft Excel Visual Basic Editor was used to design the scaffold and to control the bioprinter. Polymer solution was pumped to the 3D freezing stage with a syringe pump, where the temperature is finely controlled. Ultra-fine pipette tips were created to have an inner diameter varying from 20µm to 50µm. The pipette tips were secured on the printing head. Elastic degradable polyurethane and degradable chitosan-gelatin were used as model polymers for this study, as their use within the body has been thoroughly characterized. Polymer solution was extruded at a fine controlled rate onto glass slides secured on the freezing stage. The freezing stage increases the polymer solution viscosity, solidifying the polymers rapidly as they are extruded out of the pipette tip. This new technique allows the 3D printer to precisely control the extrusion of the polymer, allowing for the fabrication of scaffolds with specific characteristics. Human IVD cells were seeded on the scaffolds to examine the growth and alignment on the printed scaffolds. The scaffolds compressive dynamic mechanical properties were measured to prove elastic compliance.

Results: Using our customized 3D printer, polymers can be printed into elastic/ lamellar structures mimicking the natural structure of IVD tissue. The polymer stream can be precisely controlled down to a resolution of 20 µm using the fine pipettes. Concentric layers were created with spacing ranging from 30µm to 150µm to mimic the spacing within the native IVD (Fig. 1C). The scaffolds demonstrated a very porous microstructure, aiding cell attachment and growth on the scaffolds. Human IVD cells spread out and aligned along the lamellar scaffolds in a concentric manner similar to native IVD tissue (Fig. 1D&E). 3D rendering of the scaffold showed cell attachment within scaffold lamellae (Figure 1F). Cytocompatibility assays verified the biocompatibility of the scaffolds. Dynamic mechanical testing demonstrated highly elastic nature of the lamellar scaffolds (Fig. 2).

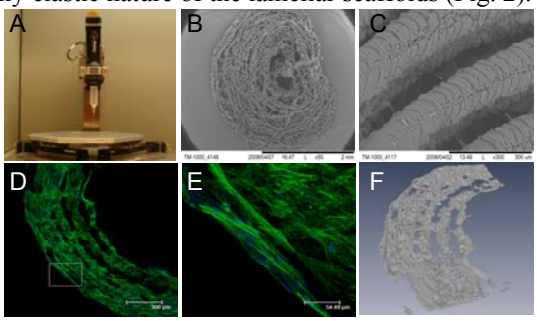

Fig. 1: A) Customized bioprinter, B) Scaffold mimicking IVD structure, C) 3D lamellar layers, D) Cells grow on 3D scaffold, E) High magnification of cell alignment along scaffold, F) 3D rendering of cells growing on scaffolds.

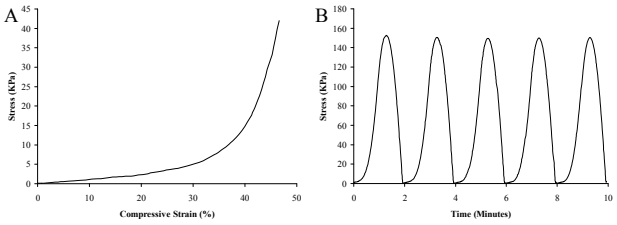

Fig. 2: Stress-Strain curve (A), Dynamic loading showing highly elastic characteristics of the lamellar scaffold (B).

Conclusions: The resolution of the 3D printer can be greatly improved by combining ultra-fine pipettes and a freezing stage. By effectively lowering the polymer solution viscosity, high resolution fabrication down to 20 um is achieved. Porous morphology of the scaffold is obtained to promote cell adhesion, alignment, and growth. Mechanical testing proved that the scaffolds have similar elastic properties to native IVD tissue, and are resilient to deformation after cyclic loading. This technique creates a high resolution scaffold with IVD cell alignment in an elastic and lamellar fashion providing a step towards tissue regeneration for the IVD.

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