Bioprinting Methods to Create 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 the adult population surgical procedures have been used to alleviate this pain. Low back pain 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 removes the degenerative disc, but does not restore the natural kinematics of the spine by restricting movement and possibly causing subsequent IVD degeneration. 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. The use of an elastic polymeric artificial disc offers a solution to some of the problems encountered with current disc replacements.
A lamellar disc scaffold formed from elastomers would offer much better compliance and allow restoration of natural three dimensional spinal motions. 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 and growth. Currently, many different techniques have been used to create and IVD scaffold. However, none has been able to fabricate lamellar structure mimicking natural IVD histology. To this end, we used a novel rapid prototyping technique that combines ultra-fine pipettes for liquid extruding and a freezing stage for the solidification of the scaffolds. This technique permits the use of many different polymers and is suitable for fabricating scaffolds with different 3D configuration. The setup of this bioprinter is shown in Figure 1A and B.

Materials and Methods: A home-made bioprinter with a computer controlled X-Y-Z freezing stage was used for this study. Microsoft excel was used to design the scaffold (Figure 1C) and to control the three stepping motors on the bioprinter. Polymer solution was pumped to the X-Y-Z stage with a syringe pump. Ultra-fine pipette tips were created to have an inner diameter varying from 5µm 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. Polymer solution was extruded onto a plastic collecting substrate secured on the freezing X-Y-Z stage at a fine controlled rate. The bioprinter precisely controls the extrusion of the polymer allowing the creation of specific shapes or scaffolds. After the scaffold is created, the solidified scaffolds were removed from the freezing stage and freeze dried in the lypholizer for 1 hour to extract out the solvent. Mechanical properties of the scaffolds are tested using MTS equipment. Human IVD cells were seeded on the scaffolds to examine the growth of IVD cells on printed elastic scaffolds.

Results: Using our customized bioprinter, elastic polymers can be printed out and formed into lamellar structures mimicking the natural structure of IVD tissue. Fine pipettes allow fabrication of scaffolds with high resolution. Polymer stream can be controlled precisely up to a resolution of 10 µm. Concentric layers were created with spacing ranging from 100µm to 300µm for the accommodation of cells. Freezing stage allows very fast solidification of polymer solution. The temperature can be varied anywhere from -40 ^{o}C to room temperature. After extraction of the solvent, the scaffold demonstrated a very porous structure as shown in figure 1D, which helped cell attachment and growth on the scaffolds. Mechanical testing of the scaffolds demonstrated high elasticity. Human IVD cells attach and spread well inside the lamellar scaffolds.

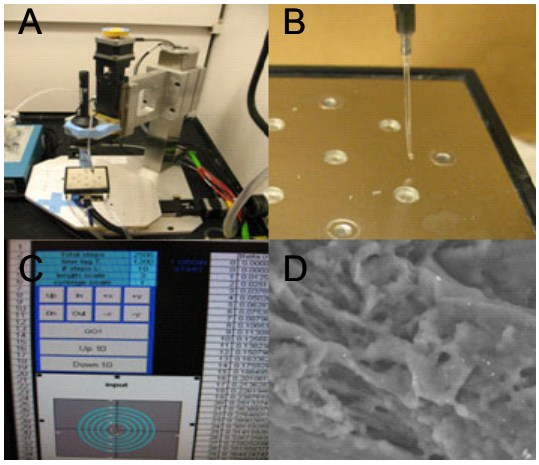

Figure 1.

Conclusions: By combining ultra-fine pipettes and freezing X-Y-Z stage, the resolution of the bioprinter can be greatly improved. By effectively lowering the polymer solution viscosity, high resolution design down to 10 um is achieved. With freezing stage, porous morphology of the scaffold can be obtained which proves to be favorable for cell adhesion and growth. The spacing between the subsequent layers of the printed scaffolds is optimal because it allows room for cell attachment while also providing space for ECM growth within the scaffold.

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

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