MECHANICAL BEHAVIOR OF AND CELLULAR PROLIFERATION IN ELECTROSPUN SCAFFOLDS OF COLLAGEN TYPE II

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Introduction: Articular cartilage appears to have a limited ability for intrinsic repair and thus development of a suitable technique for repairing damaged tissue is essential to prevent further deterioration of the articular surface. A microprocessing technique in tissue engineering called electrostatic spinning or electrospinning is capable of developing scaffolds that biomimic the extracellular matrix (ECM) of biological tissues (patents pending). (1, 2) Electrospinning of natural biomaterials can produce scaffolds with a preferred fiber orientation and with fiber diameters in the nanometer range. The purpose of this study was to create tissue engineered scaffolds of collagen type II, the major collagen within articular cartilage ECM, using electrospinning techniques and to quantify the fiber structure, cell seeding, and mechanical behavior of the scaffolds.

Methods: Scaffold Preparation: Collagen Type II from chicken sternae (SIGMA; St. Louis MO) was dissolved in hexafluoro propionol (HFP) (SIGMA). A 5 ml syringe was filled with the collagen II / HFP solution and placed onto a syringe pump (Model 100, KD Scientific Inc., New Hope, PA). The syringe tip was connected to a 30 kV voltage supply (Spellman CZE1000R; Spellman High Voltage Electronics Corp., Hauppauge, NY) in order to charge the solution. A square (38 mm L x 5 mm W x 38 mm H) rotating, aluminum mandrel was placed perpendicular to the syringe tip. The solution was discharged from the syringe at a constant rate and fibers collected on the mandrel. After removal from the mandrel, the created scaffold was sectioned into dogbone scaffold specimens (long axis parallel to preferred fiber direction) and either mechanically tested or crosslinked for cell seeding and cell culturing. Crosslinking was achieved with a 24 hour, 3% vapor glutaraldehyde fixation technique. The seeding scaffold specimens were sterilized with a 5 minute ethyl alcohol wash followed by a 5 minute phosphate buffered saline wash. These sterilized scaffolds were then placed in 10 ml of culture media for at least 4 hours prior to seeding.

Cell Source: Immortalized adult human articular chondrocytes were cultured in DMEM/F-12 (GIBCO) with 10% fetal bovine serum (FBS) and 1% penicillin plus streptomycin. (3) The cells were passaged every four to five days and used for cell seeding.

Scaffold Cell Seeding: The sterile scaffolds were placed into a rotating seeding chamber along with approximately 5x10⁶-1x10⁶ cells and 50 µg/ml of ascorbic acid. The scaffolds were rotated for two hours in a 37°C and 5% CO₂ incubator to complete the seeding.

Scaffold Culturing: Seeded scaffold specimens were then placed in 10 ml of culture media for at least 4 hours prior to seeding. Scaffold specimens were periodically changed every 2-3 days.

SEM Analysis: Uncrosslinked and crosslinked seeded scaffold specimens were processed for SEM analysis. The scaffold specimens were immersed and fixed at 4°C in 2% glutaraldehyde prepared in Sorenson’s buffer. The scaffolds were rinsed in cacodylate buffer and 1% penicillin plus streptomycin. (3) The scaffolds were static cultured for 7 days and used for cell seeding.

Mechanical Testing: Uncrosslinked scaffold specimens were mechanically tested with an MTS tensile testing machine (MTS Systems Corp.; Eden Prairie, MN). Testing was performed at an elongation rate of 1 mm/min. The stress-strain analysis was completed using MTS TestWorks software TestWork4 version 4.06A. Statistical Analysis: Comparisons between groups were performed with a two sample t-test (n=4) and a Wilcoxon Rank-Sum test using NCSS 2000 statistical software.

Results: SEM analysis of the scaffolds revealed some longitudinal orientation of the collagen fibers and the adherence and proliferation of the chondrocytes on the scaffold. (Figure 1A, 1B) Tensile testing of the uncrosslinked scaffolds revealed a tangent modulus of 172.5 MPa and an ultimate tensile strength of 3.3 MPa. (Figure 2A) The average scaffold thickness for uncrosslinked specimens was 0.20 mm and the average scaffold thickness for crosslinked specimens was 0.52 mm. A minimum fiber diameter of 70 nm was measured in the uncrosslinked scaffold, while the average fiber diameter was 455 nm. The average fiber diameter of the crosslinked scaffold was 1.58 µm. (Figure 2B) The average pore size for uncrosslinked specimens was 6.94 µm². Significant difference was noted between the uncrosslinked scaffold and the crosslinked scaffold thickness and fiber diameter (p < 0.001).

Figure 1 (A) SEM photograph of collagen type II dry scaffold uncrosslinked. Arrows represent the preferred fiber orientation. B) SEM photograph of crosslinked type II scaffold crosslinked and seeded with chondrocytes.

Figure 2 (A) Tangent modulus and ultimate tensile strength of uncrosslinked collagen type II scaffold (n=4) (B) Scaffold thickness (n=4) and fiber diameter of collagen type II uncrosslinked scaffolds and crosslinked scaffolds after 7 days of cell culture.

Discussion: We have shown the fabrication technique of electrostatic spinning of collagen type II offers a viable option to the creation of a physical structure similar in fiber dimensions to that of naturally occurring ECM. The average pore size is conducive for chondrocytes to readily adhere, proliferate on the scaffold surface, and infiltrate the interior. Future work includes optimizing the electrostatic spinning parameters to increase the percentage of aligned fibers and attain a consistent fiber diameter. In vivo research studies will require a substantially thick scaffold with increased mechanical integrity for manipulation during the seeding, culturing, and implantation process. Additional analysis through biochemical and immunohistochemical assays will determine the extent of chondrocyte proliferation and their ability to synthesize native ECM thus maintaining their phenotype. In summary, electrospun scaffolds of collagen type II, seeded and cultured with chondrocytes, may prove to be a viable option for repairing damaged articular surfaces.


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