Fragmented Electrospun Fibers Provide Environment to Promote Mesenchymal Stem Cell Chondrogenesis

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Introduction: Osteoarthritis (OA) is a degenerative joint disease that results in the loss of articular cartilage and normal joint function. After damage, articular cartilage has a limited capacity to heal. Current therapies only alleviate symptomatic pain, and surgical interventions do not restore normal hyaline cartilage. Therapies such as tissue engineering and biomaterial scaffolds can be utilized to facilitate the growth of cells and produce a normal hyaline cartilage phenotype. Electrospinning is routinely used to create fibers on the nano- to micron-scale, and can potentially be used to resemble the fibrous cartilage ECM. However, electrospinning can be limited in creating large, 3D scaffolds. Alternatively, 3D printing can produce large constructs however, depending upon the biomaterial composition, may not be able to achieve features down to the nanoscale. Combining electrospinning and 3D printing technologies may hold promise. In this study, electrospun fibers were fragmented in order to create an injectable substrate suitable for use in 3D printing. To our knowledge, the examination of scaffolds consisting of free floating fibers not embedded in a gel or other biomaterial has yet to be explored. Fragmented, electrospun gelatin fibers that varied in fiber densities were investigated for their effect on mesenchymal stem cell (MSC) chondrogenesis. Cells attached to fibers and were maintained in culture for up to 28 days. Cells produced collagen type II, with low levels of collagen type I, where fiber density played a role on the amount of collagen type II produced indicating fiber density may impact chondrogenesis

Methods: Gelatin mats were first created by electrospinning following previously reported protocols (1). Briefly, gelatin was solubilized in an acetic acid/de-ionized water solution and the solution was electrospun to form fibrous mats. Mats were crosslinked using EDC-NHS. The electrospun mats were fragmented using a processing blender in excess ethanol, and subsequently dried to produce fibers. Fiber morphology and dimension was determined using scanning electron microscopy (SEM). The fibers were dispersed in PBS, ejected into a 96-well plate at 1.5 (Low), 2.5 (Medium), and 3.5 (High) mg/mL densities.. The fibers seeded with human mesenchymal stem cells (MSCs). Cultures were maintained in chondrogenic induction media (CCM+), consisting of high glucose DMEM, sodium pyruvate, proline, glutamine, antibiotic-antimycotic, ITS-Premix, and transforming growth factor beta 3 (TGF-β3) at 10 ng/mL, and evaluated for up to day 28. Cell number was evaluated via the Quant-iT dsDNA Pico Green assay kit (Invitrogen) and total GAG content using the DMMB assay (N=4 per group per time point). The production of collagen types I and II was quantified using enzyme linked immunosorbent assays (N=4 per group) (ELISA, Chondrex, USA). Immunostaining for collagen types I and II was performed and viewed under a fluorescence confocal microscope (Nikon). Statistical analysis was performed using one-way or two-way analysis of variance (ANOVA) to determine significant differences between groups and time ($p<0.05$). Multiple comparisons within groups were made using Tukey's posthoc test ($p<0.05$). Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

Results: Fragmented, electrospun mats resulted in fibers having uniform fiber morphology (Figure 1). Cell number was maintained throughout 28 days on all fiber densities and cells produced GAG over time, where cells on Medium density fibers had the highest GAG production ($p<0.05$). In the presence of LOW density fibers, cells appeared to contract around the fibers, showing visible condensation of fiber bundles over 28 days. Immunostaining confirmed that collagen type II was present (Figure 2). By 28 days in culture, all groups produced significantly more collagen type II than type I (Figure 3) with the High and LOW densities showing significantly more production of collagen type II than the Medium density group.

Discussion: The fragmented fibers supported chondrogenesis as shown by collagen type II and GAG production. The high expression of collagen type II with low expression of collagen type I suggests the fibers may support a more homogeneous hyaline cartilage matrix formation. Future considerations will include further analysis of differentiation by gene expression including hypertrophic markers and utilizing the fragmented fibers as a printing medium for the formation of a 3D printed construct. These fragmented fibers could also be developed for other avenues of scaffold delivery, such as an injectible formulations.

Significance: Tissue regeneration approaches that can rebuild lost hyaline cartilage are needed. The results of this study demonstrate the potential of using fibers as an injectable substrate and for use in the formation of larger, 3D printed constructs for cartilage regeneration.

References:

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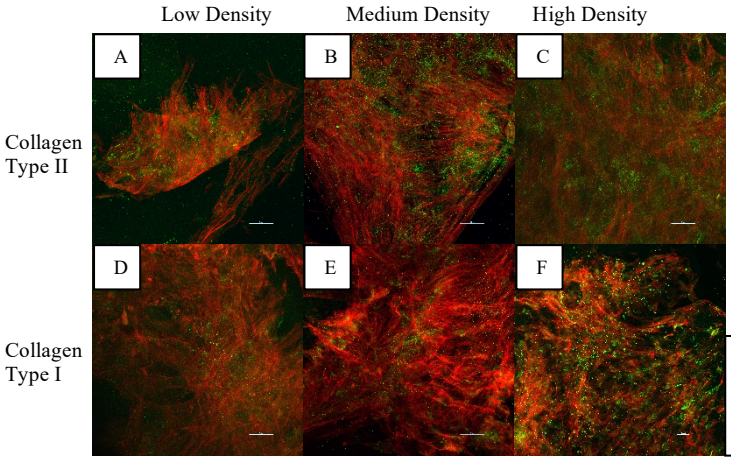

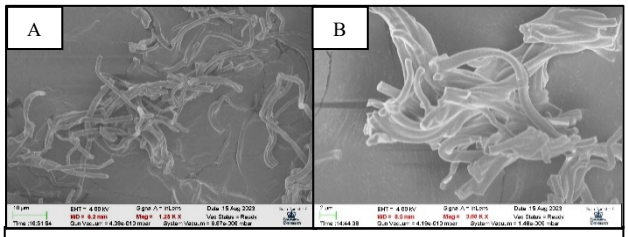

Figure 1. SEM images of fragmented, electrospun gelatin fibers. (A) 1250x and (B) 3000x magnification..

Figure 2 (left). Confocal images of MSCs cultured on low (A,D), medium (B,E), and high (C,F)-density gelatin fibers. Top row: Red = Actin, Green = Collagen Type II. Bottom row: Red = actin, Green = Collagen Type I.

Figure 3 (right). (A) collagen type II and (B) collagen type I production and (C) Collagen Type II to Type I ratio for cells cultured on low, medium, and high fiber densities supplemented with CCM+ for 28 days. [a.] Significantly different from other groups ($p< 0.05$).