Tunable Electrospun Hyaluronic Acid Scaffolds to Mimic the Microenvironment of Articular Cartilage

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Statement of Purpose: Electrospinning has recently gained much interest due to its ability to mimic the nanofibrous nature of the extracellular matrix[1]. While much progress has been made in understanding how mechanics and adhesivity affect stem cell differentiation in non-fibrous hydrogels, these variables have not been extensively studied in a fibrous system, particularly towards a specific application (e.g., cartilage). Here, we electrospin methacrylated hyaluronic acid (MeHA) into swollen gel fibers and show that precise control over fiber mechanics (through extent of modification) and adhesivity (through RGD density) significantly affects mesenchymal stem cell (MSC) interactions and fate.

Methods: 35% and 100% modified MeHA were synthesized as described in[2] and then conjugated with RGD at varying concentrations (0.3, 1, or 3 mM RGD). MeHA-RGD was electrospun at a voltage of 21 kV, flow rate of 1.0 mL/hr, and a distance of 14 cm between the spinneret and collection surface (Fig 1A). The fibrous samples were then crosslinked under 10 mW/cm^2 UV light and swollen overnight in PBS. Contact-mode AFM was performed on single, suspended MeHA fibers as in[3] to measure the three-point bending modulus. For *in vitro* studies, hMSCs (Lonza) were seeded onto the scaffolds at a density of 15, 20, or 25K/cm^2 corresponding to the 3 mM, 1 mM, and 0.3 mM RGD density groups, respectively. Cell-seeded scaffolds were then cultured in chondrogenic media and stained for actin and vinculin after 1 and 14 days of culture. At 14 days, scaffolds were also homogenized in Trizol, and gene expression for chondrogenic markers was quantified using real-time PCR. Bead movements were measured using modification of the method described in[4] for all MeHA-RGD groups.

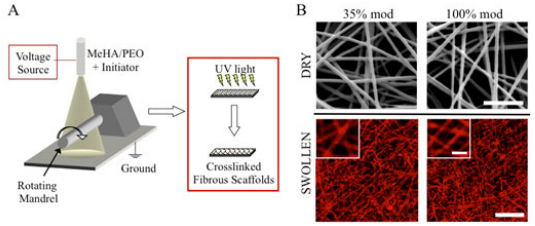

Figure 1. (A) Schematic of electrospinning setup. Macromer fibers are crosslinked with UV light after electrospinning, (B) SEM images of dry MeHA fibers (top row) and confocal images of swollen MeHA fibers (bottom row, with methacrylated rhodamine for visualization). Fiber diameter increases 3-4x after swelling. Scale bars = 10 μm for top row and 20 μm for bottom row, 5 μm for inset.

Results: Constructs were visualized using SEM of dried scaffolds and confocal microscopy of swollen constructs. Fiber diameters ranged from ~200 nm dry to ~600 nm and ~750 nm swollen for 100% modified and 35% modified MeHA, respectively (Fig 1B). The three-point bending modulus increased ~8.5 times from the 35 to 100% mod MeHA fibers. For *in vitro* studies, hMSCs were rounded on low RGD groups and spread on high RGD groups over 14 days. Vinculin staining showed clear, punctate focal adhesions with high RGD groups but diffuse or little staining with low RGD groups (Fig 2A). Morphology and focal adhesion formation trends were surprisingly similar between both soft and stiff MeHA fibers. However, gene expression showed differences in MSC response in terms of both fiber mechanics and RGD density, with lower RGD densities and lower mechanics generally resulting in higher up-regulation of chondrogenic genes (Fig 2B). Bead movements were only observed with the softer 35% mod MeHA fibers, with a general trend of increased displacements, and thus increased traction forces, with higher RGD densities.

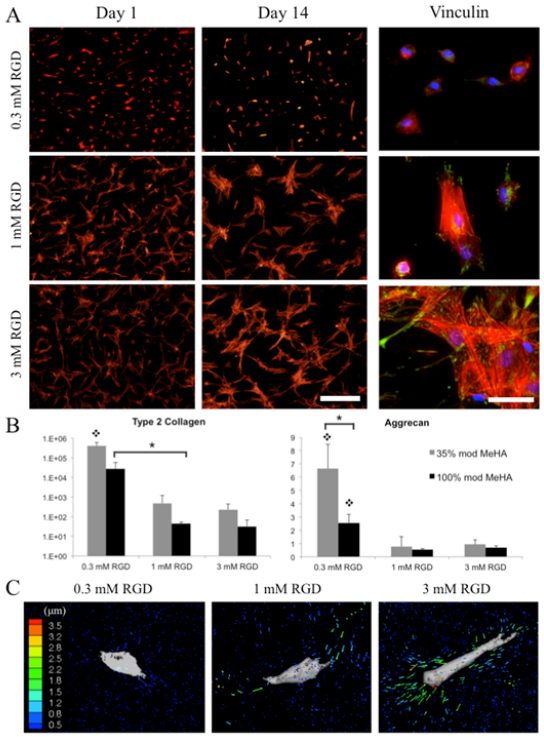

Figure 2. (A) hMSCs stained with phalloidin (red, all columns), dapi (blue, right column), and vinculin (green, right column). All images are from 35% modified MeHA groups. Scale bars = 100 μm for left and middle column, 40 μm for right column. (B) hMSC expression of typical chondrogenic genes in response to changes in % modification and RGD density; * denotes significance between indicated groups ($p<0.05$) and ❖ denotes significance compared to all RGD densities within the same % modification. (C) Representative images of bead movements of hMSCs on 35% modified MeHA fibers with varying RGD densities.

Conclusions: This work demonstrates the tunability of electrospinning MeHA, and thoroughly investigates the effects of both fiber mechanics and RGD density on MSCs towards a specific application. Both paramaters influence chondrogenesis, potentially through the ability of cells to exert traction on fibers. Ongoing work includes long-term *in vitro* studies and applying these fibrous scaffolds to an *in vivo* porcine defect model.

References: [1] Mauck RL, et al. Tissue Engineering. 2009 ;15:171-193. [2] Burdick JA, et al. Biomacromolecules. 2004;6:386-91. [3] Tan EPS and Lim CT. Applied Physics Letters. 2004;84:1603-05. [4] Legant WR, et al. Nature Methods. 2010;7:969-U113.