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

Iris L. Kim, Harini G. Sundararaghavan, Jason A. Burdick
Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA

Statement of Purpose: Electrospinning has recently gained much interest due to its ability to mimic the nanofibrous nature of the extracellular matrix and allow for better control over matrix organization and mechanical properties[1]. While much progress has been made in understanding how mechanics, adhesivity, and topography affect stem cell differentiation, these variables have not been extensively studied in a 3D fibrous system, particularly towards a specific application (e.g., cartilage). Here, we electrospin and then crosslink methacrylated hyaluronic acid (MeHA), which offers precise control over mechanics (through extent of modification) and fiber alignment (through collection apparatus), the latter of which is important for articular cartilage applications due to the depth-dependent alignment of collagen fibers, cell morphology, and biochemical composition in native cartilage.

Methods: MeHA of varying modifications was synthesized as described in[2] and then electrospun at a voltage of 22.5 kV with a distance of 15 cm between the spinneret and grounded rotating mandrel. *Fiber alignment* distribution was precisely controlled by varying the speed of the rotating mandrel from 1.2 to 10 m/s as shown in Figure 1(A). To mimic the structure of articular cartilage, electrospun mats composed of both an aligned and unaligned portion in a continuous manner were created by first spinning for four hours at a speed of 10 m/s and then spinning for 12 hours at a speed of 1.2 m/s. *Mechanics* of electrospun mats of varying % modifications (~40%, 60%, and 80%) of MeHA were measured using the weighted bead method as in[3]. For *in vitro* studies, hMSCs (Lonza) were seeded by pipetting a concentrated cell solution ($20x10^6$ cells/mL) onto freeze-dried electrospun MeHA constructs of varying alignment or % modifications. After 7 and 14 days in chondrogenic media, scaffolds were homogenized in Trizol, RNA was extracted, and gene expression for chondrogenic markers was quantified using real-time PCR.

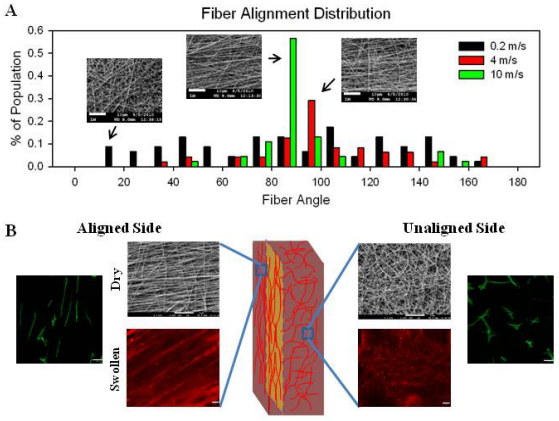

Figure 1. (A) Fiber alignment distribution based on rotating mandrel speed, (B) SEM and confocal images of depth-dependent alignment scaffolds (scalebar = 10 μm), as well as cell morphology based on fiber alignment (scalebar = 100 μm).

Results: Constructs with depth-dependent alignment were visualized using SEM of dried scaffolds and confocal microscopy on swollen constructs with methacrylated rhodamine. Figure 1(B) shows a clear difference in alignment between the two regions in both dry and swollen states. Importantly, fiber alignment was also shown to direct the morphology of hMSCs. Mechanics of fibrous scaffolds were found to be linearly-dependent on % modification of MeHA, ranging from about 10-60 kPa as % modification ranged from 20-90%. For *in vitro* studies, cells were relatively well-distributed throughout the scaffolds after two weeks of *in vitro* culture, Figure 2(B). hMSC up-regulation of chondrogenic markers increased with higher mechanics, as shown by statistically significant up-regulation between the 40% and 80% modification groups for Sox-9, type 2 collagen, and significant down-regulation of type 1 collagen ($p<0.05$). Since local mechanics in native cartilage ECM is around 20-25 kPa, a modification of about 40-50% would be the expected ideal. However, the mechanics measured may not recapitulate those observed by a seeded cell.

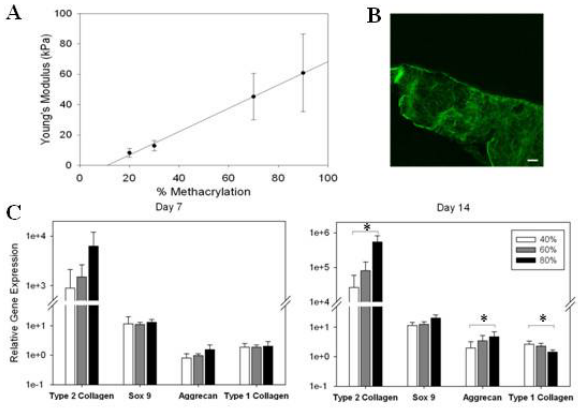

Figure 2. (A) Cross-section of cell-seeded scaffold, showing even distribution of cells (stained with FITC-phalloidin) after 2 weeks of *in vitro* culture (scalebar = 100 μm), (B) Dependence of mechanics on % modification of MeHA, (C) hMSC expression of typical chondrogenic genes in response to changes in scaffold mechanics; asterisks denote significance ($p<0.05$).

Conclusions: This work demonstrates the tunability of electrospinning MeHA as a promising scaffold system for articular cartilage and other applications. As discussed, fibrous constructs with depth-dependent alignment were synthesized and hMSCs were shown to respond significantly to changes in scaffold mechanics. Although not detailed here, adhesivity can also be controlled through variations in the amount of RGD reacted to the MeHA backbone. Ongoing work includes demonstrating the effects of these variable properties on hMSC chondrogenesis and long-term studies of deposited matrix alignment and mechanics within depth-dependently aligned constructs.

References: [1] Mauck RL, et al. Tissue Engineering. 2009:15:171-193. [2] Burdick JA, et al. Biomacromolecules. 2004:6:386-91. [3] Reinhart-King CA, et al. Langmuir, 2003:19:1573-79.