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MESENCHYMAL STEM CELL MORPHOLOGY IN A FIBROUS MICROENVIRONMENT WITH LENGTH SCALES MATCHING THE NATIVE MENISCUS

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INTRODUCTION:
The meniscus is a crescent shaped fibrocartilage in the knee comprised of a circumferentially aligned collagenous extracellular matrix (ECM) that functions to transmit load and enhance joint stability. The meniscus ECM varies as a function of region, where the inner meniscus is comprised of 70% collagen (60% type II vs. 40% type I), while the outer meniscus contains 80% collagen (99% type I) [1]. Similarly, cells within the inner meniscus are rounder and more 'chondrocyte-like', while those in the outer meniscus are elongated and 'fibroblast-like' [2]. Injury of the meniscus alters joint loading and hastens the onset of osteoarthritis. As meniscus healing is limited, there exists a need for functional meniscus replacements.
To that end, we have generated engineered meniscus constructs with organized and functional ECM via culture of mesenchymal stem cells (MSCs) on aligned electrospun poly(ε-caprolactone) (PCL) nanofibrous scaffolds [3]. These scaffolds are made up of ultra-fine fibers (less than 1 micron diameter) that direct cell organization and tissue formation. While promising, the length scale of collagen fibers in the native meniscus is considerably smaller. Previous work has shown that fiber size influences the activity of a number of different cell types, especially comparing fibers of several micron diameter to those < 1 micron [4,5,6] Given such findings, the goal of this study was to quantify collagen fibril diameters in different regions of the meniscus, and subsequently to evaluate how these topographical cues influence MSC morphology in an engineered, synthetic, nanofibrous context.

MATERIALS AND METHODS:
Quantification of native fibril diameters via TEM: Juvenile bovine medial menisci were isolated and sectioned transverse to the prevailing fiber direction. Outer and inner meniscus segments were fixed in 0.1 M sodium cacodylate buffer (pH 7.4), sectioned to 70 nm thickness, and stained with uranyl acetate and lead citrate. Sections were imaged via transmission electron microscopy (TEM) with a Jeol 1010 microscope. Collagen fiber diameters in the intercellular matrix (n=200) and at the cell boundary (n=108) was computed using ImageJ. Electrospinnng 'small' and 'large' nanofibers: Scaffolds were fabricated by electrospinning from PCL solutions of low (8% w/vol in 1:1 DMF:THF) and high (14% w/vol) concentrations to generate 'small' and 'large' fiber diameters, respectively [3]. Fiber diameters were imaged after sputter coating via scanning electron microscopy (SEM), and quantified using ImageJ (n=50). MSC Morphology on 'small' and 'large' nanofibers: Scaffolds of each fiber size were coated with fibronectin (2μg/mL) and seeded with passage 3 bovine MSCs. Cell-seeded constructs were cultured for up to 8 days in a chemically defined media containing TGF-β3 [3]. Cytoskeletal and nuclear morphology was evaluated via phalloidin/DAPI staining. Projected cell area, Feret's diameter (a measure of cell elongation), and nuclear aspect ratio (NAR, the ratio between the long and short axis of the nucleus) were assessed (n=10 cells per scaffold) using ImageJ. Statistical comparisons were made via Student's t-test (with $p<0.05$).

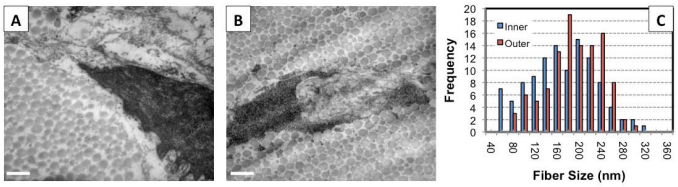

Fig 1. TEM images (A,B) and distribution (C) of collagen fibril diameters for the inner (A) and outer (B) meniscus. Scale: 500nm.

RESULTS: Quantification of collagen fiber diameters from TEM images showed a unimodal distribution for both the inner and outer meniscus (Fig. 1). The range of fiber diameters was larger for the inner meniscus (43-300nm, blue) than the outer meniscus (68-290nm, red), while the average fiber diameter was slightly greater in the outer vs. the inner meniscus (180 vs. 160 nm, $p<0.01$). Electrospun scaffolds were produced with 'large' and 'small' fiber diameters (Fig. 2). 'Large' fibers ranged from 221-1461 nm (484±216 nm), while 'small' fibers ranged from 70-485nm (201±92nm). The average diameter in 'large' fiber scaffolds was significantly greater than in 'small' fiber scaffolds ($p<0.001$). When seeded with MSCs, cells on 'large' fibers elongated in the fiber direction while those on the 'small' fibers took on a rounded/polygonal shape (Fig. 3). Quantification showed a significant increase in both Feret's diameter and NAR on 'large' compared to 'small' fibers ($p<0.001$ and $p<0.05$, respectively, Fig. 3D-E). Conversely, projected cell area decreased slightly on 'large' fiber scaffolds.

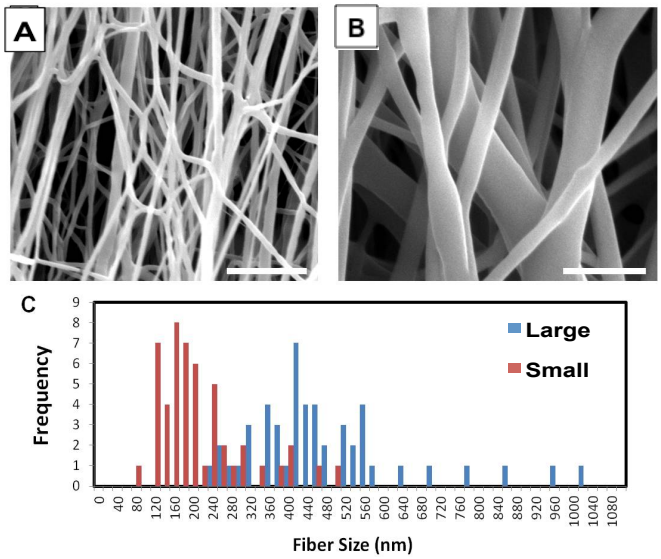

Fig 2. SEM images (A,B) and distribution (C) of 'small' (A, red) and 'large' (B, blue) electrospun PCL fibers. Scale bar: 2 µm.

DISCUSSION:

Cells within fibrous tissues, including the meniscus, are in intimate contact with surrounding collagen fibers (Fig. 1). This extracellular microenvironment conveys biochemical, mechanical, and topographic information to the cells, influencing their biologic activity. In this study, TEM imaging of the native meniscus revealed a densely packed cell-ECM interface consisting of small collagen fibers with a tight distribution. To investigate how such native fiber length scales might influence MSC behavior, we produced nanofibrous scaffolds containing aligned 'large' and 'small' fibers. The 'large' size fibers represent the size scale that we currently employ for meniscus tissue engineering studies [3], while the 'small' fibers matched the range of fiber diameters observed in the native microenvironment. The 'large'-sized scaffold fibers were on average ~2 times larger than collagen fibers in native tissue, with the largest of these synthetic fibers nearly 5× greater. When these scaffolds were seeded with MSCs, differences in cell morphology were apparent. Quantification showed a similar projected area for MSCs on both scaffolds, while both cells and nuclei were more elongated on large fibers. These results are consistent with previous reports comparing cell morphology on nano- and micron-scale fibers [4,5]. These differences in cell morphology might arise from an increased number of contact points on the 'small' compared to the 'large' fibers. Alternatively, smaller PCL fibers have a higher modulus (due to increased crystallinity) [7], and this may influence cell attachment and contractility. Having established this synthetic framework for controlling fiber diameter, current studies are now exploring how these topographic cues (that resulted in marked changes in cell morphology) translate to changes in baseline expression of markers specific to fibrocartilage, as well as how such cells respond to mechanical stimuli (i.e., tensile loading). Ultimately, this work will result in an improved understanding of cellular apperception of the microenvironment, as well as improved engineered constructs for meniscus repair applications.

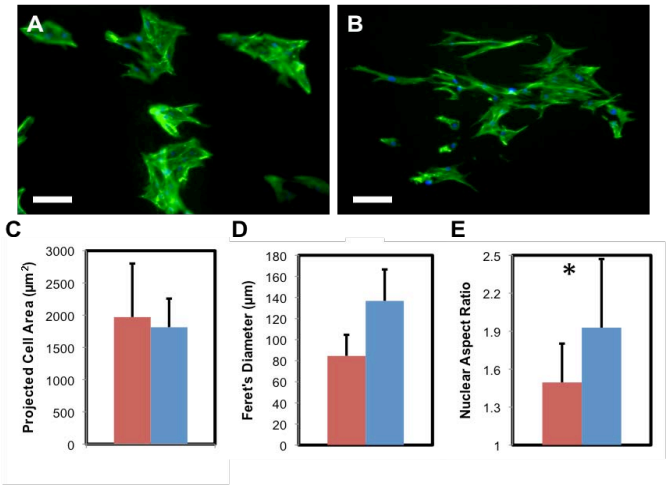

Fig 3. Actin-DAPI staining of MSCs on 'small' (A) and 'large' (B) diameter fibers on day 8 (Scale bar: 50 µm). Projected cell area (C), Feret's diameter (D), and nuclear aspect ratio (E) as a function of fiber size (red = small, blue = large; * = $p<0.05$).

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