Nanofiber Length Scale Differentially Impacts Meniscus and Mesenchymal Stem Cell Morphology and Nuclear Deformation

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Introduction

Aligned electrospun nanofibrous scaffolds can direct cell alignment and extracellular matrix (ECM) deposition for the engineering of fibrous tissues.[1,2] The morphological properties, proliferation, and gene expression of cells on these scaffolds are influenced by factors such as scaffold architecture and tensile deformation. Changes in fiber size at the nanoscale alter cell morphology and actin organization.[3] Similarly, mesenchymal stem cell (MSC) nuclear morphology on electrospun scaffolds is sensitive to changes in scaffold organization.[4] The degree of nuclear deformation appears to correlate with changes in gene expression.[5] While quite small, the size of fibers within standard electrospun scaffolds (500-1000 nm) is considerably larger than the length scale of the collagen fibers in the meniscus (~200 nm). Using a conductive polymer additive (PANi), we synthesized nanofibers matching this smaller length scale, and showed that MSCs on smaller fibers had a more polygonal cell shape and lower nuclear aspect ratio (NAR) compared to larger fibers.[6] The aim of this study was to consider both MSCs and meniscus cells, and to evaluate their morphology and mechanical response in an engineered microenvironment scaled to match the native tissue.

Methods

Aligned nanofiber scaffolds were fabricated by electrospinning two different polymer solutions (~350-500 μm in thickness).[2] For 'large' nanofiber scaffolds, a 14% w/v solution of PCL in 1:1 DMF:THF was used. For 'small' nanofiber scaffolds, a 3% w/v solution of polyaniline emeraldine base (PANi) doped with camphosulfonic acid in 1:1 DMF:THF was mixed in a 30:70 v/v ratio with a 14% w/v PCL solution[6] (Fig 1). Scaffolds of each fiber size were coated with fibronectin (2μg/ml) overnight and seeded with either passage 3 bovine MSCs or meniscal fibrochondrocytes (MFCs). Cell-seeded scaffolds (200 cells/mm2) were cultured for 7 days in chemically defined media containing TGF-β3.[2] Cytoskeletal and nuclear morphologies of each cell type were visualized with Actin/DAPI staining. To determine the impact of fiber scale on cell deformation, both cell types and scaffold formulations were subjected to 10% tensile strain after 1 day of culture, prior to Actin/DAPI staining.[4] Projected cell area, cell aspect ratio (CAR) (the ratio of cell length to width), and NAR (the ratio of nuclear length to width) were measured in ImageJ (n=45-50) (Fig 1). Statistical comparisons were made via ANOVA with Fisher's LSD post-hoc test ($p<0.05$).

Results

Consistent with previous observations,[6] MSCs seeded on larger nanofibers had an elongated cell and nuclear shape, as compared to the same cells seeded on smaller nanofibers. MSCs cultured on larger fibers decreased in cell area ($p<0.05$) with time in culture while MSCs on smaller nanofibers did not change with time. MFCs on larger fibers increased in cell area ($p<0.0001$) and decreased in elongation ($p<0.0001$) during culture, while on smaller fibers, cell area and NAR did not change while cell elongation increased ($p<0.0001$) (Fig 2). With stretch of the scaffold, cell area did not change in MSCs on either scaffold, while MFC cell area increased on small fibers ($p<0.0001$). Cells elongated with scaffold stretch for both cell types on both large and small fibers ($p<0.01$). Interestingly, NAR increased with stretch for both cell types on larger fibers ($p<0.05$), but did not change significantly on small fibers (Fig 3).

Discussion

All cells take instruction from their surrounding microenvironment, with features such as topography, stiffness, and organization influencing cell behavior. Different cells appear to interpret these signals to differing extents, and the predominance of these signals directing cell behavior will be important in directing new tissue formation. In this study, MFCs seeded on small fibers initially had a more rounded morphology, but elongated with time to adopt a similar morphology to MFCs seeded on larger nanofibers. Conversely, MSC morphology did not change markedly with time. For both MSCs and MFCs, nuclei were less elongated on small fibers compared to large fibers. These changes in

Poster No: 1337
2013 Annual Meeting
Orthopaedic Research Society
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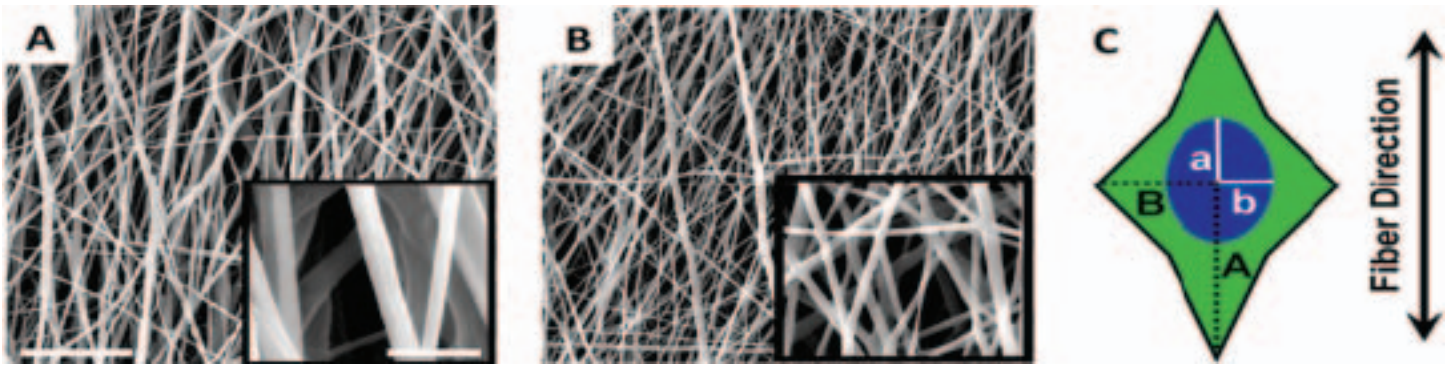

Figure 1. SEM images of large **(A)** and small scaffolds **(B)**. **(C)** Schematic of cell measurements. Green is the projected cell area, CAR is the ratio of A/B, and NAR is the ratio of a/b. Scale = 10 μm. Inset scale = 2 μm.

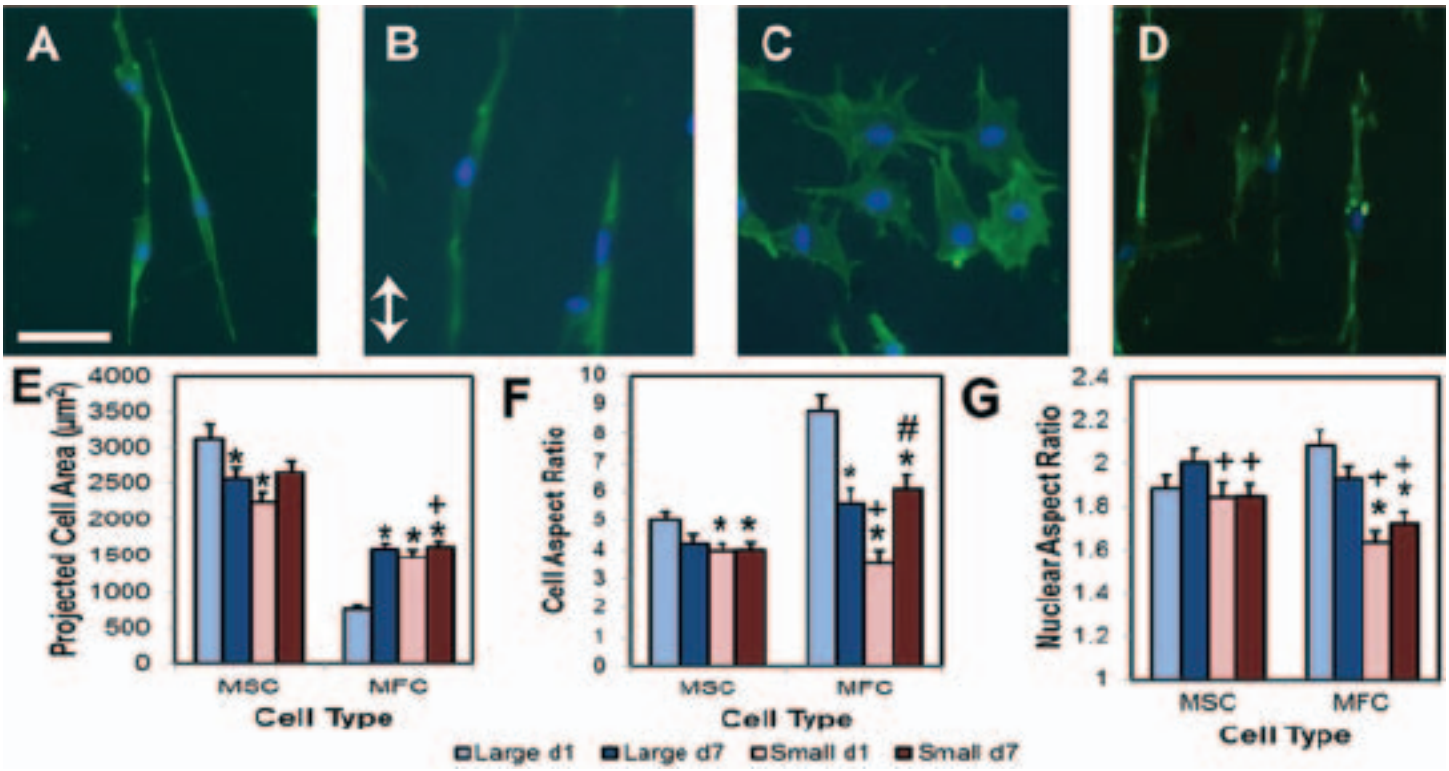

Figure 2. MFCs on large **(A,B)** or small **(C,D)** fiber scaffolds after 1 and 7 days in culture. Projected cell area **(E)**, CAR **(F)** and NAR **(G)** as a function of cell type and culture time. Data represents mean ± SEM. Scale bar = 50 μm. Arrow indicates fiber direction.

baseline morphology influenced how each cell type responded to scaffold tensile deformation. While both cell types on small and large fibers elongated in the stretching direction, only cells on larger fibers displayed changes in nuclear morphology with stretch (Fig 3). This sensitivity to nanofiber scale in translation of topographic cues may arise from an increase in the number of contact points for cells on smaller fiber scaffolds, which could alter the assembly of the internal cytoskeleton. Cells on smaller fibers tended to extend over many fibers simultaneously, while on large fibers, cells track along only a few fibers with prominent actin stress fibers. Alternatively, the smaller (and stiffer fibers) may regulate cell contractility, and thereby change load transmission mechanisms. Ongoing studies are exploring how these topographic cues translate into alterations in gene expression for each cell type and with mechanical stimulation. These results highlight the differential interaction of stem cells and fibrochondrocytes with synthetic microenvironments, and will improve tissue engineering approaches for fibrous tissue repair.

Significance

A better understanding of cell type specific response to native scale fiber morphologies may allow for the optimization of fibrous tissue engineered constructs.

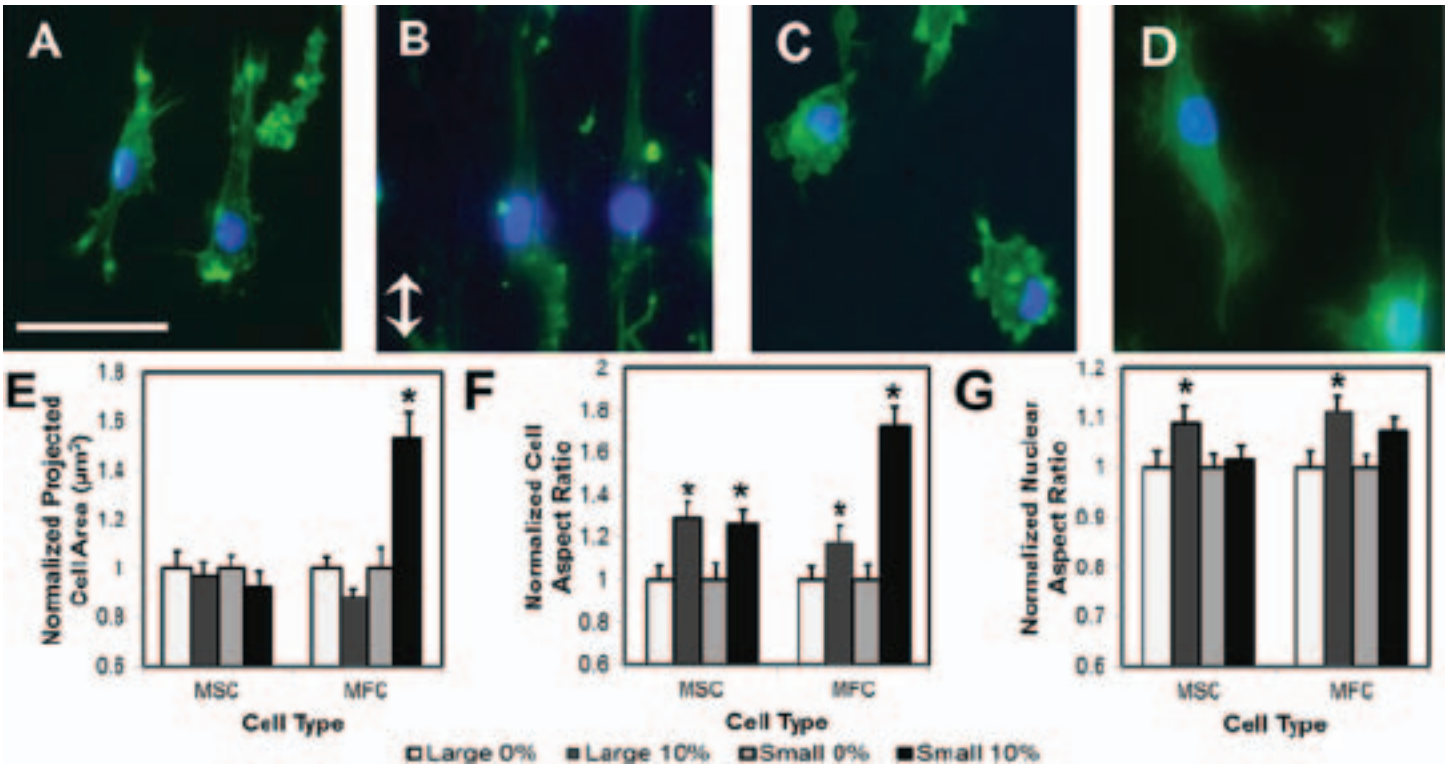

Figure 3. MFCs on large **(A,B)** or small **(C,D)** fiber scaffolds after 0% and 10% strain. Projected cell area **(E)**, CAR **(F)** and NAR **(G)** as a function of cell type and normalized to 0% strain. Data represents mean ± SEM. Scale bar = 50 μm. Arrow indicates fiber direction/direction of stretch.

Acknowledgements

This work was supported by the National Institutes of Health and the Penn Center for Musculoskeletal disorders.

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