Engineered Nanofiber Crimp Alters Scaffold Mechanics and Mesenchymal Stem Cell Mechanotransduction

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Introduction

Tendons and ligaments are composed of highly aligned collagen fibers that, at the micron-scale, have an intrinsically crimped micro-architecture.[1] This crimping results in a non-linear mechanical response, which provides low-force deformation at small strains (in the so-called 'toe' region) and resists excessive deformation via the higher 'linear region' modulus that follows.[2] While existing biomaterials can reproduce features of this non-linear response,[3] these materials fail to provide the micro-scale topography that cells within these tissues encounter, which may be important for proper mechanobiological signaling. Recently, we developed electrospun nanofibrous scaffolds that exhibit non-linear mechanics and mimic the native crimped fibrous tissue environment.[4] In this study, we used a fiber-reinforced structural constitutive model to characterize the mechanics of these scaffolds as a function of fiber crimp and investigated the effect of crimping on mesenchymal stem cell (MSC) mechanotransduction.

Methods

Composite aligned nanofibrous scaffolds were generated by co-electrospinning poly-L-lactide (PLLA, 8.5% w/v in HFP) and poly(ethylene oxide) (PEO, 10% w/v in 90% EtOH) onto a common rotating mandrel. Composite PLLA/PEO scaffolds were washed (to remove the PEO fiber fraction) or washed and heated to 65° between two glass plates (to induce fiber crimp). Scaffolds were separated into three groups: washed (DW), heated and then washed (DHW), or washed and then heated (DWH). Scanning electron microscopy (SEM) was used to calculate the ratio between the fiber contour and end-to-end lengths, which defines the strain required to uncrimp the fibers. Scaffolds (40x10 mm2) were tested in uniaxial tension using a Bose 5500 (n = 3-4/grp) either parallel or perpendicular to the fiber direction. The linear modulus was calculated using a bilinear fit and the mechanical data prior to sample yield was fit with a hyperelastic constitutive model incorporating crimped fibers embedded in a neo-Hookean matrix.[5,6] Additional scaffolds (70x5mm2) were coated with fibronectin (20ug/ml), seeded with passage 1 bovine MSCs (100k cells), and cultured for 2 days in chemically defined media. Scaffolds were stained with Hoechst (nuclei) in DMEM (20 min; 37° C) and then stretched (n = 4/grp) in 1% increments to 8% strain using a microscope-mounted tensile device. Microscale Lagrangian strains were calculated from nuclear triads and a Poisson's ratio was calculated for each triad. In parallel unstained samples, stretch was applied as above and protein was collected for Western blotting with p44/p42 MAPK (ERK) or phospho-p44/p42 MAPK (pERK) antibodies to determine MAPK/ERK activation. Differences were evaluated via one-way ANOVA and Bonferroni post-hoc tests with $p < 0.05$.

Results

Scaffolds that were heat-processed to induce crimp (DHW and DWH) exhibited markedly different mechanical properties compared to the non-heated DW group, with the greatest change observed for the DWH samples (Figure 1A). The model successfully fit all scaffold groups; however, a non-zero matrix term was required to fit only the DWH samples, given their extensive toe-region (Figure 1B). Conversely, the DW and DHW groups could also be fit by the crimped fibers alone (data not shown). While SEM measurements showed that fiber crimping increased for the DHW and DWH scaffolds, the fiber uncrimping indicated by the model fits increased only for the DWH group (Figure 1C). The fiber modulus predicted by the model agreed with the measured linear modulus, which significantly decreased for both heat-processed groups (Figure 1D). The matrix modulus determined from testing perpendicular to the fiber direction was similar across all groups; however, the matrix modulus determined from testing parallel to the fibers in the DWH group was significantly higher (Figure 1E). Finally, the Poisson's ratio was generally comparable between scaffold types (Figure 1 F). Cells stretched on these scaffolds showed increased ERK phosphorylation with tensile stretch on both the DHW and DWH samples, but not on the DW scaffolds (Figure 2).

Discussion

This study aimed to determine whether the micron-scale crimping produced in nanofibrous scaffolds via heat-processing produced commensurate changes in scaffold mechanics

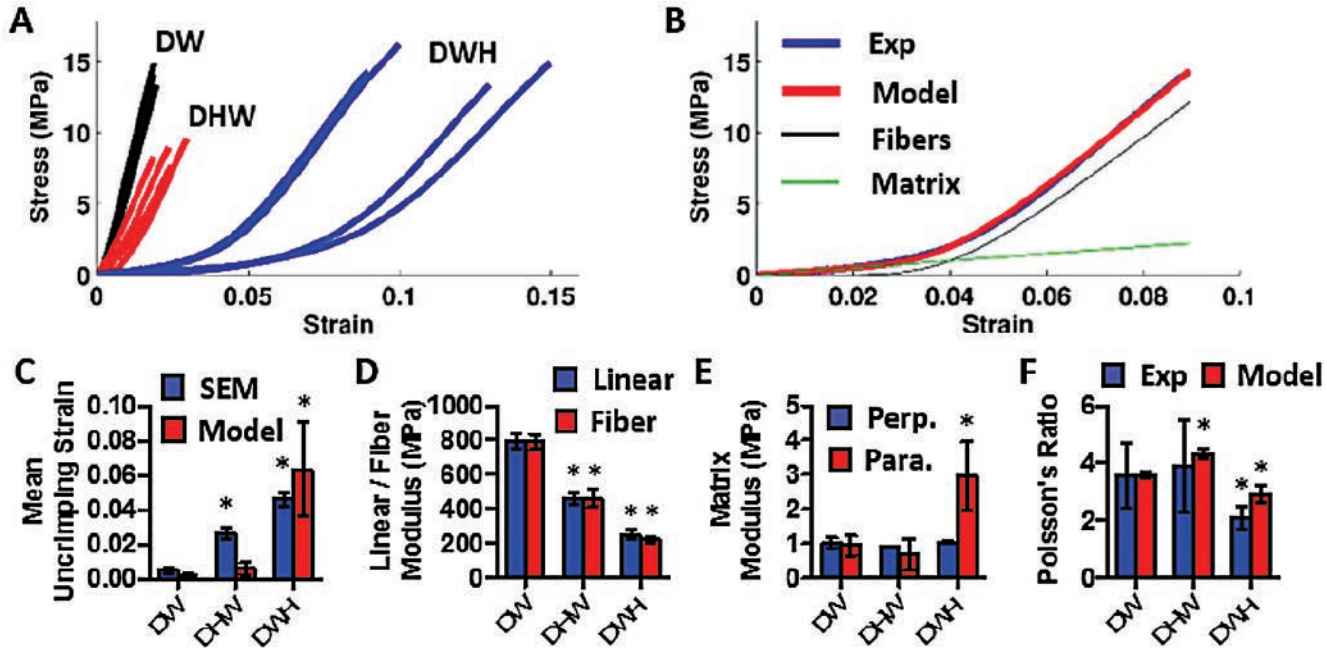

Figure 1. (A) Stress-strain response for each scaffold. (B) Representative model fit of DWH sample (R2=0.999). (C) Mean uncrimping strain increased with heat treatment. (D) Linear-region modulus and model fiber modulus decreased with treatment. (E) Testing perpendicular to fibers produced similar matrix moduli across all groups but the DWH group exhibited a higher value when tested parallel to fibers. F) Poisson's ratios were consistent between model and experiments. Mean ± SD. * $p < 0.05$ compared to DW.

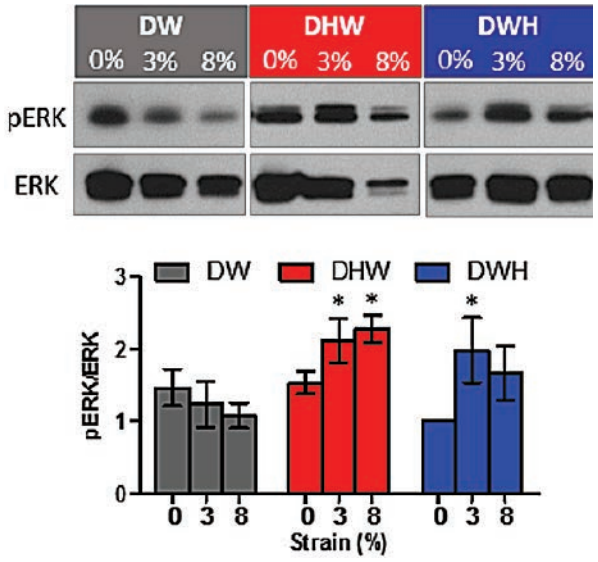

Figure 2. The level of phosphorylated ERK (normalized to total ERK) did not change with strain for the DW group. However, strain applied to both DHW and DWH scaffolds increased ERK activation. Mean ± SD. * $p < 0.05$ compared to 0%.

and altered cell mechanotransduction with applied uniaxial stretch. We found that the greatest amount of fiber crimping was generated in the DWH scaffolds, which also exhibited the greatest changes in tensile mechanics, with a large toe-region and significantly reduced fiber modulus. Interestingly, this was the only scaffold group whose mechanical behavior included resistance generated within the non-fibrous matrix (e.g., fiber-fiber interactions). In contrast, the DHW scaffolds, which also exhibited crimped fibers under SEM, had a minimal toe-region and mechanical contribution from the matrix term. This suggests that washing and then heating the scaffolds not only generates greater fiber crimping, but also more fiber-fiber interactions that influence the uncrimping process in response to load. Despite these differences in mechanics with scaffold treatment, cell mechanosensing appeared be more dependent on local fiber topology and interaction than bulk scaffold mechanics, as increased ERK activation was evident for both the DHW and DWH scaffolds in response to applied stretch. Future work will investigate additional mechanotransduction mechanisms that may lead to functional changes in scaffold maturation during culture as a result of this crimped micro-architecture.

Significance

Micron-scale crimp produced within nanofibrous electrospun scaffolds successfully reproduced the non-linear mechanics of native tissue and provided topological cues that influence cell mechanotransduction. As such, these engineered materials may provide better replication of native tissue structure and biological function.

Acknowledgments

This work was supported by the National Institutes of Health (T32 053461 and R01 EB02425).

References

1. **Rigby, et al.** *J Gen Phys* (1959)
2. **Hansen, et al.** *J Biomech Eng* (2002)
3. **Sahoo, et al.** *Biomed Mat* (2007)
4. **Chao, et al.** *Biofabrication* (2014)
5. **Szczesny, et al.** *J Biomech Eng* (2012)
6. **Nerurkar, et al.** *Spine* (2008).