In Vitro Repetitive Straining of Electrospun Polycaprolactone (PCL) Constructs for Tendon Restoration

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INTRODUCTION: To optimize the force transmission from skeletal muscle to bone, mechano-responsive tendons and their resident cells orient themselves in uniaxial matrices of primarily collagen type I (Col1) fibers that align in the direction of external loading [1]. Abnormal loading of tendon tissues can perturb homeostatic functions, and poor intrinsic healing mechanisms, which are attributed to hypocellularity and lack of vasculature, lead to suboptimal repair, risk of re-rupture, and altered tendon function. Polycaprolactone (PCL) is a viscoelastic polymer that can be electrospun into a provisional matrix capable of mimicking the Col1 fibril network in healthy tendons [2]. Though often used in soft tissue engineering applications, the effects of repetitive straining and variability of straining protocols on PCL scaffolds have yet to be thoroughly investigated. The objective of this study is to investigate how cyclic straining affects the structure and mechanical properties of PCL matrices in a bioreactor. We hypothesize that higher density scaffolds will have superior retention of viscoelastic properties and fibril matrix structure following physiological strain loading compared to lower density scaffolds. Further, it is hypothesized that straining cell-seeded PCL scaffolds will result in an increased metabolic activity, cellular alignment, and deposition of extracellular matrix (ECM) proteins.

METHODS: 33%, 50%, and 66% (w/v) PCL with dimethylformamide and dichloromethane solutions were electrospun at 500 RPM onto a spinning mandrel to create fibrous, aligned scaffolds of varying densities. Scaffolds were placed into a bioreactor and loaded at physiological strain rate of 5% at a frequency of 1 Hz for 4 hours. The control scaffolds were unstrained. Scanning electron microscopy (SEM) images were taken and fiber diameter and orientation were determined utilizing ImageJ. Scaffolds were mechanically tested at a tensile straining rate of 0.3% per second until failure on an Instron to determine the ultimate tensile strengths (UTS) and elastic moduli (E). Control scaffolds were incubated in culture media for 7 days following sterilization before being subjected to the mechanical testing protocol to evaluate the effect of degradation on mechanical properties as preliminary work. Lastly, cellular viability studies were conducted on control samples using NIH3T3 fibroblasts seeded at $1.2x10^4$ cells/scaffold. Metabolic activity of the cells was assessed over time as an indirect measure of cellular viability. Phalloidin and Trichrome staining will also be utilized to visualize ECM deposition and cellular morphology on the scaffolds. ANOVA statistical analyses were conducted in GraphPad with significance set at $p < 0.05$.

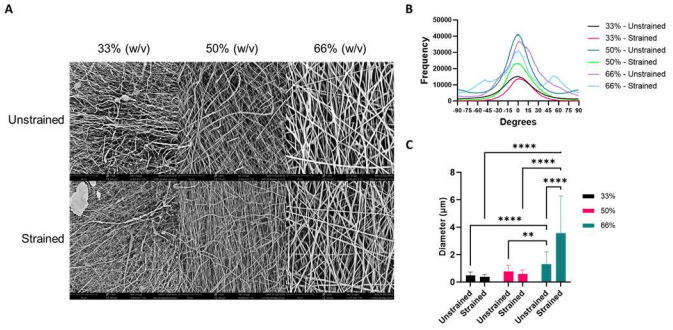

Fig. 1. (A) SEM images of the scaffold were analyzed to compare the effect of straining on (B) fiber orientation and (C) fiber diameter of varying density scaffolds. Straining had an effect on the orientation and thickness of the varying PCL scaffolds. (*p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.0005, ****p ≤ 0.0001)

RESULTS: SEM revealed a positive correlation between fiber density and diameter with 66% - Strained scaffolds being significantly thicker than others (**Fig. 1C**). Further SEM image processing suggested that straining lead to a more uniform matrix orientation (**Fig. 1B**). Though scaffolds in the bioreactor did not display any statistical differences for UTS, the 66% - Unstrained scaffolds exhibited the largest elastic modulus and was significantly higher than other unstrained scaffolds (**Fig 2B**). Further, the statistical difference between strained and unstrained groups for 33% and 50% scaffolds suggests that straining leads to an increased modulus. Mechanical testing of scaffolds subjected to sterilization and culture conditions confirmed prior results that higher density scaffolds are correlated to an increased modulus (**Fig. 2D**). It was also demonstrated that the modulus was significantly lower on Day 7 for 50% and 66% scaffolds. The 50% scaffolds appeared to be least affected by sterilization and culture conditions, indicated by the consistent observations for mechanical properties. Lastly, culturing cells on the 33% and 66% scaffolds lead to a significant increase in viable cells on Day 3 compared to Day 1 (**Fig. 3**).

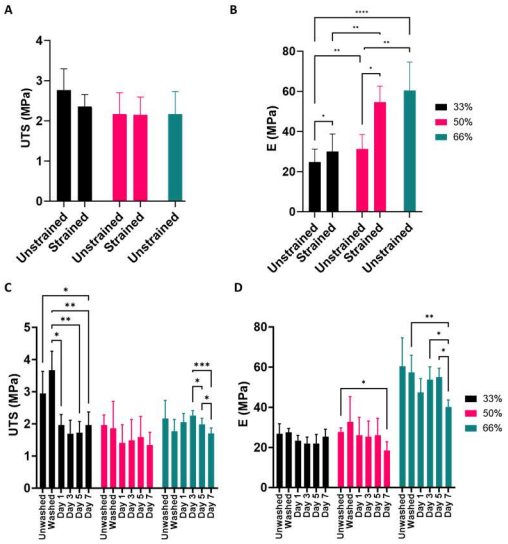

Fig. 2. (A) UTS and (B) Youngs Modulus of the strained and control scaffolds of varying polymer densities. (*66% - Strained scaffolds were omitted due to testing failure).* (C) UTS and (D) Youngs modulus of the scaffolds following sterilization and incubation *33% scaffolds did not receive a PBS wash during the sterilization process.* Strain and incubation both had an effect on the mechanical properties of the scaffolds. (*p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.0005, ****p ≤ 0.0001)

DISCUSSION: This study investigates how cyclic straining affects the mechanical properties and fibril structure of PCL scaffolds. Overall, it can be concluded that repetitive stain does influence these properties, specifically the modulus. This increase in elastic modulus can likely be attributed to the orientation of the fibril matrices and intrinsic viscoelasticity of PCL. Additionally, fibril orientation appears to become more aligned following straining; however, additional work and increased sample size is required to confirm these findings. The varying density dependent characteristics of the scaffolds in response to strain alludes to an optimal fabrication density that replicates the viscoelastic properties of tendons. Though it would be expected for uniaxial straining to elongate fibers and reduce thickness, this was not presented for 66% - Strained scaffolds. This may be due to excess gold-palladium particulates during SEM preparation and will need to be further evaluated in sequential studies. It is accepted in literature that cells may exhibit abnormal expression on substrates of varying stiffnesses [3]; therefore, it is imperative for PCL scaffolds to preserve their mechanical properties when subjected to in vitro conditions. The investigation of unstrained PCL scaffolds in cell media and incubator serves as preliminary work for evaluating the effects of cyclic loading in culture conditions and assists in developing initial studies to evaluate the genetic and protein expression of fibroblasts seeded on PCL scaffolds. It is apparent that higher density scaffolds begin to lose their mechanical properties on Day 5, which will be used as a time point in future studies. The increased cellular viability for 33% and 66% scaffolds from Day 1 to Day 3 supports the preliminary hypothesis regarding the ability of the scaffolds to promote cellular viability. Future work will investigate how varying the strain rate will affect the cellular behavior and phenotype.

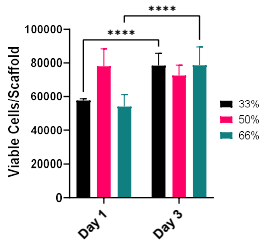

Fig. 3. Metabolic activity was compiled to draw comparisons between cell densities. (*p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.0005, ****p ≤ 0.0001)

SIGNIFICANCE: This study evaluates the structural and mechanical response of PCL fibril scaffolds to repetitive physiological straining and provides insight towards tailoring restorative, provisional therapeutics for tendinous applications.

REFERENCES: [1] Wang et al, *J. Hand Therapy*, 2012, [2] Lim et al, *Intl. J. Envir. Res. & Pub. Health*, 2021, [3] Yeung et al, *Cell Motil Cyto*, 2005

ACKNOWLEDGEMENTS: This work was supported in part by the University of Florida Provost's Office of Research, the Alliance for Regenerative Rehabilitation Research and Training (AR^3T) pilot grant, and NSF GRFP to H. Broadaway (DGE-2236414).