Impact of Cyclic Tensile Loading Duration and Recovery on Gene Expression in Tenocytes

Angelina Santos[1,2], Yuna Heo[2], Catherine Cheung[2], Su Chin Heo[2]

[1]Bucknell University, Department of Biomedical Engineering

[2]University of Pennsylvania, Department of Orthopaedic Surgery and Bioengineering

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Introduction

Tendinopathy is a prevalent musculoskeletal disorder characterized by inflammation and degeneration, which leads to impairment of the tendon. A major contributing factor is repetitive and high-intensity mechanical loading of the tendon, which leads to the accumulation of microinjuries that fail to heal properly. Such overuse can cause irreversible changes in tendon structure and mechanics, resulting in chronic pain and diminished function. Globally, approximately 32 million tendon injuries occur each year, especially with Achilles tendon pathologies affecting an estimated 6% of the US population during their lifetime. Despite its prevalence, the underlying cellular mechanisms that drive disease progression remain poorly understood [1, 2]. Furthermore, current treatments are limited to alleviating symptoms rather than addressing the fundamental causes of disease.

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Tendinopathy is associated with compromised mechanical properties, disrupted collagen architecture, and alterations in ECM composition [3]. Tenocytes, the primary resident cells within tendons, play a crucial role in ECM maintenance and turnover. Proper mechanical stimulation is critical for maintaining tendon homeostasis, as both underloading and overloading can disrupt gene expression and tissue organization, contributing to tendon degeneration [4]. Thus, understanding how tenocytes respond to varying mechanical stimuli is essential to better understanding the pathogenesis of tendinopathy.

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In this study, we investigate the effect of loading duration (1 vs. 3 days) and subsequent post-loading recovery (24 hours) on tenogenic and catabolic gene expression in tenocytes subjected to high-intensity cyclic strain. Using a short-term *in vitro* tendon overloading model, we examined transcriptional responses to different loading conditions, focusing on genes associated with tendon identity and ECM remodeling. Insights from this work may inform the development of targeted regenerative therapies and improve clinical outcomes for patients with tendon disorders.

Materials & Methods

Aligned nanofibrous polycaprolactone (PCL) scaffolds were fabricated via electrospinning and cut into two sizes: large (20 mm x 55 mm) or small (5 mm x 55 mm). Scaffolds were hydrated in ethanol and coated in fibronectin to promote cell adhesion. Bovine Achilles tenocytes (from four biological replicates) were cultured in 100mm tissue-culture plates until reaching

approximately 80% confluency, then seeded onto the scaffolds at densities of 2×10^6 (large scaffolds) or $7.5x10^5$ (small scaffolds) per scaffold (Figure 1).

Cyclic uniaxial tensile loading was applied to tenocyte-seeded scaffolds at 8% strain amplitude and 2 Hz for 4 hours per day using a custom-designed bioreactor. Large scaffolds were used to compare 1 day vs. 3 day loading durations. Small scaffolds were used to evaluate the effects of a 24-hour recovery period post-loading. Control scaffolds remained under static, free-swelling conditions in culture media throughout the 3 day experimental period without mechanical stimulation. Following the final loading or recovery time point, TRIzol was added to each scaffold, and samples were subsequently processed for RNA extraction.

RT-qPCR was used to assess expression levels of the mechanosensitive tenogenic markers type-I collagen (COL1), type-III collagen (COL3), tenascin-C (TNC), and the catabolic enzyme matrix metalloproteinase-3 (MMP3). GAPDH was used as the reference gene. Statistical significance ($p<0.05$) was determined using two-sample t-tests and ANOVA.

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Results, Conclusions & Discussion

No statistically significant differences were observed in COL1, COL3, TNC, and MMP3 expression between 1 day (1d) and 3 days (3d) of loading. However, expression trends indicated a decrease in COL1 and an increase in COL3 and TNC with prolonged loading. MMP3 levels slightly decreased, although expression levels remained low across both timepoints (Figure 2). These patterns are consistent with the expected early cellular response to overloading, characterized by a shift from strong COL1 to compliant COL3 as a result of rapid matrix remodeling [3]. Elevated TNC expression is commonly associated with tissue injury and may be indicative of potential healing [5]. Although increased MMP3 expression was anticipated due to its role in ECM degradation [6], the lack of a robust transcriptional response suggests that changes in protein levels and enzyme activity may not align with gene expression. Further investigation into protein abundance and activity, as well as the expression of other MMPs, may provide additional insights.

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When evaluating the effect of a 24-hour recovery period, statistically significant differences in gene expression were found between 1 and 3 days of loading. TNC expression increased between 1 day and 3 days of loading and remained elevated during the recovery phase, although it showed a slight decrease post-recovery (1dR or 3dR). Interestingly, TNC expression in the 1 day loading group after recovery was significantly lower than control levels. In contrast, MMP3 expression markedly increased over time, with a sustained elevation during recovery, significantly surpassing nearly all other loading conditions (Figure 3). Overall, gene expression profiles did not return to baseline following the 24-hour recovery period.

Altogether, these findings suggest the onset of a degenerative cellular response after only 1 day of high-intensity loading, which is maintained through 3 days of loading and persists even after cessation of mechanical stimulation. The continued elevation of catabolic markers and decline in tenogenic gene expression post-loading indicate that a 24-hour recovery period is insufficient to restore tenocyte homeostasis. This study provides the importance of loading duration and recovery timing in regulating tenocyte responses and provides foundational insights for developing *in vitro* degenerative tendon models. In addition, developing long-term loading models and incorporating additional molecular and protein-level analyses will be critical to informing novel therapeutic strategies for tendon repair.

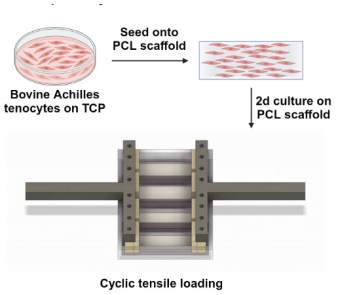

Figure 1. Schematic of experimental workflow.

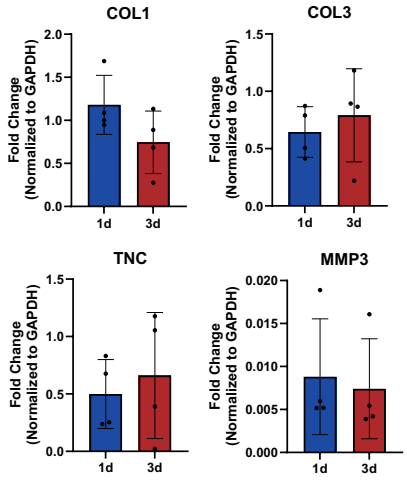

Figure 2. Gene expression of tenocytes subjected to mechanical loading for 1 day or 3 days (n=4 biological replicates, error bars: mean ± SD).

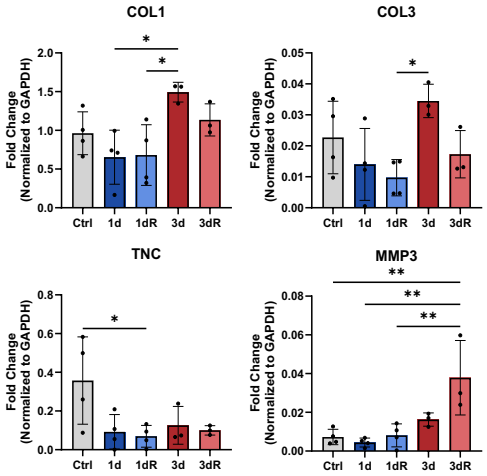

Figure 3. Gene expression of tenocytes subjected to mechanical loading for 1 day (1d), 1 day followed by 24-hour recovery (1dR), 3 days (3d), 3 days followed by 24-hour recovery (3dR) (n=4 biological replicates, error bars: mean ± SD; *p<0.05, **p<0.01).

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Acknowledgements

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