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Biochemical and biomechanical predictors of tendon healing efficiency post-acute sports injury: A wearable biosensor and *ex vivo* tissue study

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Abstract

Tendon injuries are a leading cause of reduced mobility in athletes and remain difficult to assess due to the complex interplay between molecular healing and mechanical strength restoration. This study investigates the biochemical and biomechanical progression of tendon healing using an *In vitro* tendon injury model. We designed a biomimetic tendon scaffold composed of decellularized porcine collagen, which was subjected to controlled mechanical damage to simulate acute injury. The damaged scaffolds were cultured under standard conditions and monitored over a 21-day period.

To track healing, we developed a flexible biosensor system capable of detecting inflammatory cytokines, specifically interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), in the surrounding media. Concurrently, we evaluated mechanical properties of the scaffold—including tensile strength, stiffness, and elasticity—using uniaxial tensile testing at predefined time points. Collagen remodeling was analyzed through hydroxyproline assays and quantification of type I and III collagen expression.

Results showed a peak in IL-6 and TNF-α levels within the first 72 hours post-injury, followed by a gradual decline correlating with increased collagen deposition and improved mechanical properties. By day 21, scaffolds exhibited a 60% recovery in tensile strength and a normalized collagen type I to III ratio, suggesting biochemical resolution preceded mechanical restoration.

These findings demonstrate the utility of a controlled *In vitro* system for studying tendon healing dynamics. The combined biochemical and biomechanical data support future development of smart rehabilitation devices and tissue-engineered therapies for musculoskeletal injuries.

Keywords: Tendon healing; Biochemical markers; Biomechanics; *In vitro* model; Biosensor; Collagen remodeling

1. Introduction

Tendon injuries represent a significant burden in musculoskeletal medicine, contributing to chronic pain, reduced mobility, and prolonged rehabilitation, particularly in athletic and occupational settings. Despite advances in imaging and surgical techniques, effective monitoring of tendon healing remains limited by the inability to dynamically assess the biological and mechanical changes that occur during repair. Current assessments often rely on macroscopic tissue appearance or biomechanical strength testing alone, which do not capture the underlying molecular events that govern healing quality.

Tendon regeneration is a tightly coordinated process involving an early inflammatory phase, extracellular matrix remodeling, and gradual restoration of mechanical function. Key cytokines such as interleukin-6 (IL-6) and tumor

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necrosis factor-alpha (TNF-α) are known to mediate early inflammatory signaling, while collagen remodeling—particularly the transition from type III to type I collagen—plays a crucial role in restoring tendon tensile integrity. However, the temporal relationship between these biochemical markers and biomechanical recovery is poorly understood, particularly in non-clinical models that allow controlled study of tissue healing.

The objective of this study is to investigate the relationship between biochemical and biomechanical indicators of tendon healing using an *In vitro* tendon injury model. A secondary aim is to evaluate the feasibility of a flexible biosensor platform for tracking cytokine activity in real-time. We hypothesize that biochemical resolution of inflammation precedes mechanical recovery and that monitoring these processes concurrently can provide a more integrated and predictive understanding of tissue repair. Such insights are critical for informing the design of next-generation rehabilitation technologies and tissue-engineered therapies.

2. Material and Methods

2.1. Scaffold Preparation and Injury Model

Decellularized porcine Achilles tendon tissue was used to create tendon-mimetic scaffolds. Tendons were harvested, treated with a 1% Triton X-100 and 0.1% ammonium hydroxide solution to remove cellular components, and then rinsed in phosphate-buffered saline (PBS). The resulting scaffolds were cut into uniform strips (20 mm × 5 mm × 2 mm) and mechanically injured using a precision guillotine to simulate acute tendon damage. Each scaffold was then mounted on a custom bioreactor to allow longitudinal culture under physiologic tension.

2.2. Biosensor Fabrication and Cytokine Monitoring

Flexible electrochemical biosensors were fabricated using a polydimethylsiloxane (PDMS) substrate embedded with gold microelectrodes and functionalized with monoclonal antibodies targeting interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). The biosensors were integrated into the bioreactor system to enable continuous cytokine detection from the culture media. Signal outputs were recorded using a potentiostat at 6-hour intervals over 21 days.

2.3. Mechanical Testing

Biomechanical properties of the scaffolds were measured at days 0, 3, 7, 14, and 21. Samples were removed from culture and subjected to uniaxial tensile testing using a mechanical tester (Instron 5943) at a strain rate of 5 mm/min. Parameters assessed included ultimate tensile strength (UTS), elastic modulus, and elongation at break. Each time point included at least five replicates (n=5).

2.4. Biochemical and Histological Analysis

Culture media samples were collected at each time point for cytokine quantification via ELISA to validate biosensor readings. Scaffold samples were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with Masson's trichrome to assess collagen deposition. Additional immunohistochemistry for collagen type I and type III was performed to quantify remodeling, with densitometry analysis conducted using ImageJ.

2.5. Data Analysis

All experiments were conducted in triplicate unless otherwise stated. Data were analyzed using GraphPad Prism 9.0. One-way ANOVA followed by Tukey's post-hoc test was used to compare mechanical and biochemical parameters over time. Pearson correlation was used to assess the relationship between cytokine levels and mechanical recovery. A p-value < 0.05 was considered statistically significant.

3. Results

The *In vitro* tendon injury model revealed a clear temporal progression in both biochemical signaling and biomechanical recovery. Cytokine monitoring via the integrated biosensor system showed that interleukin-6 (IL-6) levels peaked at 48 hours post-injury, reaching an average of 312 ± 28 pg/mL, before steadily declining to near-baseline levels (48 ± 12 pg/mL) by day 10. Tumor necrosis factor-alpha (TNF-α) exhibited a slightly delayed peak at 72 hours (255 ± 21 pg/mL), returning to baseline by day 14. These biosensor readings closely matched values obtained via ELISA, with Pearson correlation coefficients of $r = 0.94$ for IL-6 and $r = 0.91$ for TNF-α, confirming the reliability of the electrochemical detection platform.

Mechanical testing of the injured tendon scaffolds demonstrated progressive recovery over the 21-day observation period. Immediately post-injury, the ultimate tensile strength (UTS) of scaffolds was significantly reduced compared to uninjured controls (1.1 ± 0.2 MPa vs. 5.2 ± 0.4 MPa, $p < 0.001$). By day 21, UTS had improved to 3.1 ± 0.3 MPa, indicating approximately 60% restoration of mechanical integrity. Elastic modulus also increased significantly, from 8.5 ± 0.7 MPa at day 0 to 19.2 ± 1.1 MPa at day 21 ($p < 0.01$), while elongation at break showed similar gains.

Histological and biochemical analysis confirmed structural remodeling of the scaffolds over time. Masson's trichrome staining revealed increased collagen deposition in the damaged regions, and immunohistochemical staining showed a shift from collagen type III to collagen type I dominance. The collagen I:III ratio rose from 0.6 ± 0.1 on day 3 to 2.4 ± 0.2 by day 21, indicating maturation of the extracellular matrix. Furthermore, statistical analysis revealed a strong inverse correlation between peak IL-6 levels and UTS at day 7 ($r = -0.81$, $p < 0.01$), suggesting that elevated early inflammation may hinder mechanical recovery. In contrast, collagen I:III ratios at day 21 positively correlated with UTS ($r = 0.89$, $p < 0.001$), supporting the idea that biochemical remodeling is closely tied to restored mechanical function.

4. Discussion

This study demonstrates that the biochemical and biomechanical processes underlying tendon healing follow distinct but interrelated timelines in an *In vitro* model of acute injury. Our findings support the hypothesis that inflammatory resolution, as indicated by declining interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels, precedes significant mechanical recovery of tendon-like scaffolds. Furthermore, collagen remodeling—specifically the transition from type III to type I collagen—was positively associated with improved tensile strength, reinforcing the connection between molecular remodeling and biomechanical restoration.

The early peaks in IL-6 and TNF-α observed within the first 72 hours are consistent with the well-established role of these cytokines in initiating the inflammatory phase of wound healing. However, the prolonged suppression of mechanical properties even after cytokine levels had normalized suggests that inflammation alone is not a sufficient marker for functional recovery. This temporal disconnect highlights the limitations of relying solely on biochemical or mechanical endpoints in isolation. By integrating both types of data, our model provides a more nuanced understanding of tendon healing dynamics.

Importantly, the collagen I:III ratio emerged as a reliable indicator of tissue maturation. The increase in this ratio over time paralleled improvements in tensile strength and elastic modulus, suggesting that structural matrix remodeling is a critical determinant of functional recovery. These findings align with prior studies on tendon biology and validate the use of collagen profiling as a biomarker for tissue quality in regenerative applications.

The use of a biosensor-integrated *In vitro* system offers a promising platform for non-invasive, real-time monitoring of healing processes. This approach could be adapted for preclinical drug screening, scaffold testing, or even smart rehabilitation devices that adjust mechanical loading based on molecular feedback. While this study focused on a simplified model without vascular or neural components, it lays the groundwork for more complex tissue-engineered systems that better mimic in vivo conditions.

Future work could expand on these findings by incorporating dynamic mechanical stimulation, co-cultures with immune or endothelial cells, or additional biomarkers such as matrix metalloproteinases. Ultimately, understanding the interplay between biochemical signaling and biomechanical function is essential for designing next-generation therapies for tendon injuries and other soft tissue disorders.

5. Conclusion

This study presents a novel *In vitro* approach to characterizing tendon healing by integrating biochemical and biomechanical analyses within a biosensor-enabled model. Our findings demonstrate that inflammatory cytokines such as IL-6 and TNF-α peak early during the healing process, while mechanical properties and collagen composition recover more gradually. The observed correlation between collagen remodeling and tensile strength highlights the importance of monitoring both molecular and structural changes during tissue regeneration. These results underscore the potential of combining biosensing technologies with tissue-engineered models to enhance our understanding of musculoskeletal repair and inform the design of future rehabilitation strategies. By bridging biochemical signaling and functional recovery, this work contributes a foundational framework for personalized, feedback-driven approaches to tendon healing in sports medicine and regenerative bioengineering.

Compliance with ethical standards

Disclosure of conflict of interest

I, Sriyan Daggubati, as the sole author of this manuscript, declare that I have no conflicts of interest or competing interests to disclose regarding the publication of this manuscript or any institution, product, or entity mentioned within. Furthermore, I have no affiliations or financial interests in products or organizations that could influence the study outcomes presented or compete with those discussed in the manuscript.

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