The Early Inflammatory Response After Flexor Tendon Healing: A Gene Expression and Histological Analysis

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Introduction: Flexor tendon healing after surgical repair progresses through three overlapping phases: inflammation (days 1-7), proliferation (days 3-14), and remodeling (day 10 onward) [3,4]. Previous attempts to improve flexor tendon healing have focused on the later stages of healing (i.e., proliferation and matrix synthesis). The early inflammatory phase of tendon healing, however, is not fully understood and its modulation during healing has not yet been studied. Therefore, the purpose of this work was to characterize the early inflammatory phase of flexor tendon healing with the goal of identifying inflammation-related targets for future treatments.

Methods: Canine flexor tendons (N=11) were transected and immediately repaired using techniques identical to those used clinically. Temporal changes in cell population (N=3-5 per timepoint) and gene expression (N=3 per timepoint) were examined during the first 9 post-operative days. Gene Expression: Gene expression of inflammation-related (IL-1β, COX2), matrix degradation-related (MMP1a), extracellular matrix-related (COL1), and tendon differentiation-related (SCX) factors were examined. Data was analyzed using the delta delta Ct method (compared to GAPDH) and Ct values were compared using a 2-way ANOVA (for treatment and time), followed by a Fisher’s post-hoc test. Significance was set to p < 0.05. Histology: Immune cell populations were identified by a pathologist (NH) and polarized light microscopy was used to assess matrix degradation (i.e., loss of collagen alignment).

Results: Cellularity at the repair site was significantly increased 1 day post-operatively (Table 1). The dominant cell type contributing to increased cellularity was the polymorphonuclear cells (PMNs) (Figure 1). On day 3, a shift in the immune cell population was noted (Table 1). Cells of the monocyte/macrophage lineage began to infiltrate the repair and fewer PMNs were evident (Figures 1, Table 1). By day 9, fibroblasts infiltrated the repair site between the repaired tendon stumps (data not shown). Gene expression patterns paralleled changes in repair-site cell populations. IL-1β and COX2 levels rose dramatically as early as day 1 (Figure 2). Expression of these genes subsequently decreased over time (Figure 2), coincident with changes in cell populations from immune cells to tendon fibroblasts. In contrast to the marked elevation of pro-inflammatory genes, extracellular matrix- and tendon differentiation-related genes were significantly down-regulated on day 1 (Figure 2), an interval during which immune cells were prominent at the repair site. Matrix- and differentiation-related gene expression values increased toward baseline levels (Figure 2) as the number of PMNs decreased and tendon fibroblasts began to populate the repair site (Figure 1, Table 1). Simultaneous with these early changes in gene expression, cellular proliferation, and cellular migration, MMP levels increased beginning at the time of injury (Figure 2). MMP levels increased further as macrophages cleared the wound of debris and dead tissue and as fibroblasts proliferated within the repair, initiating the remodeling phase of healing (Figure 2). The temporal increase in MMP expression coincided well with morphological changes in collagen fiber alignment (data not shown).

Discussion: The results of this study have implications for developing strategies to improve outcomes after tendon repair. Pro-inflammatory factors such as IL-1β and cytotoxic mediators secreted by immune cells may be important for attracting fibroblasts to the repair site [3,4]. However, the extraordinarily high levels of pro-inflammatory cytokines reported here likely lead to MMP-mediated catabolism of surrounding extracellular matrix and collateral tissue damage leading to impaired tendon healing. These findings suggest that modulation of the inflammatory environment may be an effective strategy to enhance tendon healing.

Significance: Over the past few decades, flexor tendon repair outcomes have been improved through advances in rehabilitation and surgical techniques. However, clinical outcomes remain variable and result in 1.5 million days lost from work per year [1,2].

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<td>Day 9</td>
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**Table 1:** Histological analysis of the early inflammatory response 1, 3, and 9 days post-operatively. The prevalence and type of immune cells at the repair site was assessed over time. A standard scoring system was used to determine the levels of each outcome: Cellularity/PMN/Mono + <50 per HPF, ++ 51-100 per HPF, +++ 101-150 per HPF, ++++ >150 per HPF. Fibroblast percentage: + = less than 5%, ++ = 5-50%, +++ = >50%. PMN = Polymorphonuclear cells, Mono = monocytes/macrophages, Fibro% = fibroblast percent compared to overall cellularity, HPF = High powered field (20x), (N=3-5).
Figure 1: Representative histologic sections of repaired tendons 1, 3, and 9 days post-operatively. The sections were stained with H&E and viewed under bright field for identification of immune cells (PMNs). PMNs (black arrows) infiltrated the repair site on day 1, but decreased over time. 200 μm scale bar, (N=3-5).
Figure 2: Fold changes in gene expression of inflammation- (IL-1β, COX2), matrix degradation- (MMP1α), extracellular matrix- (COL1), and tendon differentiation-related (SCX) factors relative to normal 1, 3, and 9 days post-operatively. * p < 0.05, † p < 0.10, 2-way ANOVA, significant effect of time, bars signify Fisher’s post-hoc comparisons, * by x-axis signifies a significant difference compared to normal tendons, (n=3).