Flexor tendon injury and repair: The influence of synovial environment on the early healing response in a canine mode

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Flexor Tendon Injury and Repair
The Influence of Synovial Environment on the Early Healing Response in a Canine Model

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Background: Environmental conditions strongly influence the healing capacity of connective tissues. Well-vascularized extrasynovial tendons typically undergo a robust wound-healing process following transection and repair. In contrast, avascular intrasynovial tendons do not mount an effective repair response. The current study tests the hypothesis that flexor tendons, as a function of their synovial environment, exhibit unique inflammatory, angiogenic, and metabolic responses to injury and repair.

Methods: Flexor tendons present a distinct opportunity to test the study hypothesis, as they have proximal regions that are extrasynovial and distal regions that are intrasynovial. In an internally controlled study design, the second and fifth forepaw flexor tendons were transected and repaired in either the extrasynovial or the intrasynovial anatomical region. Histological, gene expression, and proteomics analyses were performed at 3 and 7 days to define the early biological events that drive synovial environment-dependent healing responses.

Results: Uninjured intrasynovial tendons were avascular, contained high levels of proteoglycans, and expressed inflammatory factors, complement proteins, and glycolytic enzymes. In contrast, extrasynovial tendons were well vascularized, contained low levels of proteoglycans, and were enriched in inflammation inhibitors and oxidative phosphorylation enzymes. The response to injury and repair was markedly different between the 2 tendon regions. Extrasynovial tendons displayed a robust and rapid neovascularization response, increased expression levels of complement proteins, and an acute shift in metabolism to glycolysis, whereas intrasynovial tendons showed minimal vascularity and muted inflammatory and metabolic responses.

Conclusions: The regional molecular profiles of intact and healing flexor tendons revealed extensive early differences in innate immune response, metabolism, vascularization, and expression of extracellular matrix as a function of the synovial environment. These differences reveal mechanisms through which extrasynovial tendons heal more effectively than do intrasynovial tendons.

Clinical Relevance: To improve outcomes after operative repair, future treatment strategies should promote features of extrasynovial healing, such as enhanced vascularization and modulation of the complement system and/or glucose metabolism.

The healing of connective tissues, such as tendons and ligaments, is heavily influenced by intrinsic and extrinsic factors. It has been established that well-vascularized extrasynovial tissues, such as the Achilles tendon, the medial collateral ligament, and the flexor tendons of the wrist and forearm, exhibit a robust scar-mediated healing response following injury and repair1-3. In contrast, intrasynovial tissues, such as the anterior cruciate ligament and the digital flexor tendons, do not mount an effective repair response1-3. Prior studies report improved functional outcomes, lower complication rates, and decreased

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requirements for secondary reconstructive surgery for extra-
svonovial compared with intrasynovial flexor tendon repair.
Extrsvonovial tendon repair in the palm, wrist, and forearm
(zones III, IV, and V) achieves an almost uniformly successful
outcome, with a high incidence of good to excellent recovery
(85% to 100% of normal digital motion), a low incidence of
tendon rupture, and a rare necessity for secondary tenolysis
surgery. In contrast, the results of intrasynovial tendon repair
are variable and inferior to those of extrasynovial
 tendon repair with regard to the recovery of digital motion
and to the frequency of adhesion formation, which often
requires additional surgery. These clinical and animal
study observations have implied that factors such as vascularity,
the cellular phenotype, and the synovial fluid environment may
influence healing. However, because of differences between anat-
omical sites, loading environments, and surrounding tissues, it
has been difficult to define the mechanisms that drive region-
specific healing responses. This lack of understanding has hin-
dered the development of treatment strategies motivated by the
different healing patterns.

The current study takes advantage of a unique mor-
phological characteristic of flexor tendons. An individual flexor
tendon has a proximal region (i.e., in the proximal aspect of the
palm, wrist, and forearm) that is extrasynovial and a distal
region (i.e., in the distal aspect of the palm and finger) that is
intrasynovial. Using a clinically relevant animal model, we
transected and repaired digital (intrasynovial) and proximal
metacarpal (extrasynovial) flexor tendons in canine forepaws
in order to explore the biological mechanisms that drive ten-
don healing. We tested the hypothesis that extrasynovial and
intrasynovial tendons are highly adapted to their environments
and exhibit distinct inflammatory, angiogenic, and metabolic
responses to tendon injury and repair. Specifically, we hypothe-
sized that avascular intrasynovial tendon will exhibit higher basal
inflammatory potency and lower efficiency in energy produc-
tion leading to an inadequate response to injury; in contrast,
well-vascularized extrasynovial tendon will exhibit lower basal
inflammatory potency and higher capacity for energy pro-
duction leading to a robust vascular, cellular, and healing
response to injury. Further, we sought to determine if previ-
ously reported variations in clinical outcomes are attributable
to the specific biological mechanisms that drive extrasynovial
compared with intrasynovial tendon healing.

Materials and Methods

Study Design

This study was approved by the Institutional Animal Care
and Use Committee. A total of 22 adult female mongrel
canines (20 to 25 kg) were included. Under isoflurane anes-
thesia, animals were subjected to flexor digitorum profundus
(FDP) tendon transection in the second and fifth digits of the
right forepaw at the level of the proximal interphalangeal joint
(intrasynovial) in 1 digit and at the level of the metacarpal
proximal to the digital sheath (extrasynovial) in the other digit
(Figs. 1-A and 1-B). The transected tendons were repaired
using an 8-strand Winters-Gelberman technique, immobi-

lized using a cast, and subjected to passive motion beginning 24
hours after repair. Animals that had undergone repair were
randomly divided into 2 groups and euthanized 3 days or 7 days
after repair. Tendon healing responses were assessed using
quantitative real time RT-PCR (reverse transcription-polymerase
chain reaction; n = 6/group/time point), proteomics analysis (n =
4/group/time point), and histological assessment (n = 4/group/
time point). The intact tendons from corresponding left digits
were used as healthy controls. The number of animals was min-
imized through the use of the above-described paired study design
and a power analysis. The power analysis was conducted based on
the data from previously published studies. In our previous
work, there were no apparent differences in histological, bio-
chemical, and biomechanical outcomes between male and female
animals. However, we found that female animals were gener-
amly more compliant than males with postoperative controlled
motion rehabilitation. Accordingly, female canines were used in
this study. The study focused on early responses to flexor tendon
injury and repair. Three and 7-day time points were chosen on the
basis of findings in previous studies, which showed clear in-
flammatory and proliferative tendon responses at these time
points after injury and repair.

Histology

FDP tendons were fixed in 4% paraformaldehyde in phosphate-
buffered saline solution overnight, embedded in paraffin,
sectioned at 5 μm, and stained with a pentachrome reagent
(American MasterTech). Pentachrome staining highlights
mature collagen in bright orange to red and proteoglycan in
blue to green.

Gene Expression Analysis

A 2-cm tendon fragment spanning the repair site was dissec-
ted and subjected to RNA isolation, cDNA synthesis, and quanti-
tative TaqMan RT-PCR (BioMark™ HD System) with primers
and probes described previously. IPO8 and RPS9 were used
as reference genes. The relative abundances of genes in each
sample were determined with the ΔΔCt (delta-delta-cycle
threshold) method and expressed as the fold-change with
respect to intact controls. For genes whose expression levels
in intact tendons were near or below the detection limit, the
results are shown as the relative mRNA abundance (2−ΔΔCt).

Proteomics Analysis

Proteomics analysis was performed by the Washington Uni-
versity Proteomics Shared Resource (WU-PSR) using a tandem
mass-tag-based assay. Protein samples were digested with endoprotease LysC and trypsin, labeled with tandem mass tag
reagents (TMT10; Thermo Fisher Scientific), and subjected to
nanoscale liquid chromatography coupled to tandem mass
spectrometry (nano-LC-MS/MS) analysis. Raw data were con-
verted to peak lists using Proteome Discoverer (version 2.1.0.81;
Thermo Fisher Scientific). Proteins were identified with a Mascot
search engine (version 2.6.2; Matrix Science) and compared
against a SwissProt database of Canis lupus familiaris (August 2016
version; 29,541 entries) and common contaminant proteins
Quantification of protein relative abundance was performed with proteoQ (version 1.0.0.0; https://github.com/qzhang503/proteoQ) under R (R Core Team, R Foundation for Statistical Computing; https://www.R-project.org/) and RStudio (R Studio Team; http://www.rstudio.com/); proteoQ is a tool developed with the tidyverse approach (tidyverse; https://CRAN.R-project.org/package=tidyverse) under R and RStudio. Principal component analysis of protein log2 ratios was performed with the base R function stats:prcomp.

Statistical Analysis

For gene expression, a paired t test or Wilcoxon matched-pairs signed-rank test was used to compare paired groups. Two-way analysis of variance (ANOVA) followed by a Bonferroni multiple-comparison test was used to evaluate the factors of time and anatomical location. Significance was set at p < 0.05. For proteomics, linear modeling was performed using the contrast fit approach in limma to assess the significance of protein abundance differences between indicated groups of contrasts. Adjustments of p values for multiple comparisons in the proteomics analysis were performed with a Benjamini-Hochberg correction.

Results

Intrinsic Differences Between Intact Intrasynovial and Extrasynovial Tendons

The intrasynovial region of the flexor tendon differed markedly from the extrasynovial region in terms of extracellular matrix (ECM) makeup, vascularity, inflammatory markers, and metabolic enzymes (Tables I and II, Figs. 1 and 2; see also Appendix Table S1 and Fig. S1). Pentachrome staining revealed enriched levels of proteoglycans (green staining, magnified inset) and a lack of vasculature in the intrasynovial tendon (Fig. 1-C). The extrasynovial tendon (Fig. 1-D) was made up primarily of collagen and was well vascularized (purple staining, magnified inset). The scale bar in the inset of Figure 1-C is 50 μm and applies to the insets in Figures 1-C and 1-D. The scale bar in the main portion of Figure 1-C is 1 mm and applies to Figures 1-C and 1-D.

(cRAP version 1.0; January 1, 2012; 116 entries). Pentachrome staining revealed enriched proteoglycan levels and a lack of vasculature in the intrasynovial tendon; in contrast, the extrasynovial tendon was made up primarily of collagen and was well vascularized (Fig. 1-D).

Consistent with the histological appearance, the expression levels of cartilage-related genes, including SOX9 and ACAN, were considerably higher in intrasynovial tendons, while the expression levels of tenogenic genes, such as TNMD, were higher in extrasynovial tendons (Fig. 2-B). Proteomics analysis revealed 21 ECM proteins that were differentially expressed between the 2 regions of the tendon (see Appendix Table S1A). Among these, proteins related to cartilage anabolism were higher in the intrasynovial tendon and proteins related to cartilage catabolism were higher in the extrasynovial tendon (see Appendix Table S1A). When examining genes related to vascularity, the HIF1A gene (for hypoxia-inducible factor) and its downstream target gene VEGFA were higher in the intrasynovial region of the tendon than in the extrasynovial region (Fig. 2-B). In line with the concept that hypoxia can induce inflammation, the levels of inflammatory genes CD86, IL6, and NOS2 were also higher in the intrasynovial region than in the extrasynovial region (Fig. 2-B).

Proteomics analysis revealed 25 inflammation-related proteins that were differentially expressed between the 2 regions of the tendon, including proteins related to the complement system, which were higher in the intrasynovial
tendon (see Appendix Table S1C). When examining metabolic proteins, glycolytic enzymes were prevalent in the intrasynovial region and oxidative phosphorylation enzymes were prevalent in the extrasynovial region (see Appendix Table S1D).

### Healing Responses of Intrasynovial and Extrasynovial Tendons

There was a substantial difference in the healing responses of the intrasynovial compared with the extrasynovial regions of flexor tendons. The main differences included:

**Histology**
- **Surface**
  - Peritendinous tissue: Synovial sheath
  - Vascularity: Low
  - Matrix: Proteoglycan-rich
- **Interior**
  - Cell shape: Rounded fibrochondrocytes
  - Vascularity: Avascular
  - Matrix: Collagen, proteoglycan

**Molecular Contents**
- **mRNA**
  - Tendon genes: Higher level of COL3A1
  - Cartilage genes: Higher levels of ACAN, COL2A1, SOX9, and MMP3
  - Angiogenesis genes: Higher levels of HIF1A and VEGFA
  - Inflammation genes: Higher levels of CD86, PTGS1, NOS2, and TNF

**Protein**
- **ECM proteins**
  - Enriched in proteins involved in cartilage anabolism
- **Cytoskeleton proteins**
  - Enriched in type-III intermediate filament
- **Inflammation proteins**
  - Enriched in complement proteins
- **Metabolic enzyme proteins**
  - Enriched in glycolytic enzymes
- **Regulatory proteins**
  - Enriched in proteins involved in transcription initiation, post-translational modification, transportation, and secretion

### Table I: Summary of Proteins Differentially Expressed Between Intact and Repaired Intramuscular and Extrasynovial Flexor Tendons as Identified by Proteomics Analysis*

<table>
<thead>
<tr>
<th>Category</th>
<th>Intact</th>
<th>Repaired, 3 Days</th>
<th>Repaired, 7 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM</td>
<td>21</td>
<td>4 (3)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>14</td>
<td>9 (5)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Inflammation/acute/innate immune response</td>
<td>25</td>
<td>17 (7)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Regulatory</td>
<td>27</td>
<td>27 (8)</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Metabolic enzymes</td>
<td>21</td>
<td>12 (5)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>7 (1)</td>
<td>4 (0)</td>
</tr>
<tr>
<td>Uncharacterized</td>
<td>14</td>
<td>9 (5)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>85 (34)</td>
<td>43 (23)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the counts of same proteins also differentially accumulated in intact tendons. ECM = extracellular matrix.
the repaired tendons at both 3 and 7 days (Tables I and III; see also Appendix Tables S2 and S3). Histologically, extrasynovial tendons showed rapid and vigorous vascular and cellular responses, while intrasynovial tendons were largely inactive (Fig. 3; see also Appendix Fig. S2). At 3 days, the intrasynovial epitendon layer was thickened, demonstrating increased cellularity and vascularity at this time point (see Appendix Fig. S2 A). There were no vessels within the intrasynovial tendon substance (see Appendix Fig. S2 B and C). At 7 days, sparse epitendon vessel formation was noted, extending into the superficial layers of the repair site. A few small vessels were noted within the intrasynovial tendon proximal to the repair site (Figs. 3-G and 3-H; see also Appendix Fig. S2 G). In contrast, at 3 days, extrasynovial tendons had numerous small vessels within an enlarged paratenon extending along the tendon surface and within the proximal and distal tendon stumps (Figs. 3-D, 3-E, and 3-F; see also Appendix Fig. S2 D and F). At 7 days, surface vessels within the paratenon and within both tendon stumps were larger and more abundant (Figs. 3-J, 3-K, and 3-L; see also Appendix Figs. S2 J, K, and L) and profuse intratendinous vascularization was observed adjacent to and within the repair site (Fig. 3-L; see also Appendix Fig. S2 L). The more robust cellular and vascular responses in extrasynovial tendons were coupled with a greater amount of collagen deposition compared with intrasynovial tendons (orange to red in Fig. 3).

Consistent with this histological appearance, gene expression of VEGFA and HIF1A was increased in extrasynovial compared with intrasynovial tendons at 3 days (Fig. 4). For ECM-related genes, there was increased expression of COL3A1, TGFB1, SOX9, and ACAN in extrasynovial compared with intrasynovial tendons. The more robust extrasynovial healing response was also apparent when examining genes associated with inflammation and remodeling (e.g., CD86 and TGFB1), which were increased in extrasynovial compared with intrasynovial tendons. Proteomics analysis revealed a clear separation between intact and repaired tendons (orange and teal, respectively, in Fig. 5-A), between repaired tendons at 3 and 7 days (open and closed symbols, respectively, in Fig. 5-A), and between intrasynovial and extrasynovial tendons (triangle and circle symbols, respectively, in Fig. 5-A).

There were 85 and 43 proteins that were differentially expressed between the 2 tendon regions at 3 days (Fig. 5-B; see also Appendix Table S2) and 7 days (Fig. 5-C; see also Appendix Table S3), respectively. When examining ECM, cartilage and
Discussion

The marked difference in clinical results following flexor tendon repair in zone II of the hand compared with the palm, wrist, and forearm has been attributed to the unique morphological features and nutrient pathways of tendon in different anatomical regions. Previous investigations reported that intrasynovial and extrasynovial flexor tendons have different biochemical compositions and cellular activities in vitro and in vivo. Ultrastructural studies described rounded chondrocyte-like cells and irregularly organized collagen fibers with large avascular zones within intrasynovial tendons. In contrast, extrasynovial tendons are well vascularized and have elongated fibroblasts located between parallel, well-organized collagen fibers. Prior studies have noted that intrasynovial flexor tendons have higher proteoglycan concentrations and synthesize less collagen than do extrasynovial tendons. Further, previous experiments demonstrated that intrasynovial and extrasynovial tendon segments differ in their ability to synthesize ECM in vitro, indicating that intrasynovial flexor tendons are specially adapted to their avascular and synovial-fluid nutritional milieu.

The current experiment takes advantage of the opportunity to provide side-by-side histological and molecular comparisons of intrasynovial and extrasynovial tendon healing in the same animal in a clinically relevant repair model. The differences in the repair responses in the early stages of healing were striking. At the same time points following surgical repair, extrasynovial tendons demonstrated substantially greater vascular and cellular responses than did intrasynovial tendons. While intrasynovial transients increase in glycolysis in extrasynovial tendons. Appendix Table S3D), suggesting that tendon injury caused a modest enrichment of enzymes involved in glycolysis, while the abundance of components in the Krebs cycle was reduced (see Appendix Table S2D). By 7 days after repair, however, most of the above-described changes were undetectable (see Appendix Table S3D), suggesting that tendon injury caused a transient increase in glycolysis in extrasynovial tendons.

### TABLE III Summary of Healing Responses in Repaired Intrasynovial and Extrasynovial Flexor Tendons*

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Intrasynovial Tendon</th>
<th>Extrasynovial Tendon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology</td>
<td>Surface</td>
<td>Minimal deposition of non-collagen fibers (fibrin?)</td>
</tr>
<tr>
<td></td>
<td>Surrounding tissues</td>
<td>Collagen ↓ ↓</td>
</tr>
<tr>
<td>Vascularity</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Cellularity</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Matrix</td>
<td>Collagen ↓ ↓</td>
<td>Collagen ↓</td>
</tr>
<tr>
<td>Interior</td>
<td>Cellularity</td>
<td>Total ↑, chondrocyte-like cells ↑, inflammatory cells ↑, RBC ↑</td>
</tr>
<tr>
<td>Vascularity</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Cellularity</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Matrix</td>
<td>Collagen ↓ ↓</td>
<td>Collagen ↓</td>
</tr>
<tr>
<td>Molecular contents</td>
<td>mRNA</td>
<td>Tendon COL1A1 ↓, MMP1 ↑</td>
</tr>
<tr>
<td>Chondrogenesis</td>
<td>SOX9 ↓, ACAN ↓, COL2A1 ↓</td>
<td>COL2A1 ↓ ↓</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>HFIAA ↔, VEGFA ↔</td>
<td>HIFA1 ↑, VEGFA ↑</td>
</tr>
<tr>
<td>Inflammation</td>
<td>NOS2 ↑ ↑, IL6 ↔</td>
<td>CD86 ↑ ↑, IL6 ↑, CD163 ↑ ↑, IL10 ↑ ↑</td>
</tr>
<tr>
<td>Protein ECM</td>
<td>Microtubule component and actin polymerization ↑</td>
<td></td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>Complement component ↑, negative regulator of NF-κB signaling ↑</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>Relatively higher levels of enzymes involved in oxidative phosphorylation</td>
<td></td>
</tr>
<tr>
<td>Metabolic enzymes</td>
<td>Relatively higher levels of enzymes involved in glycolysis</td>
<td></td>
</tr>
<tr>
<td>Regulatory</td>
<td>Positive regulators for cell survival, growth, and transformation ↑</td>
<td></td>
</tr>
<tr>
<td>Regulatory</td>
<td>Relatively higher levels of molecules involved in protein modification and trafficking</td>
<td></td>
</tr>
</tbody>
</table>

*ECM = extracellular matrix, RBC = red blood cells, and MMP = matrix metalloproteinase. Horizontal arrows indicate no change, and vertical arrows indicate a change, with two arrows indicating a larger change.
tendons showed progressive but very limited neovascularization at 3 and 7 days, extrasynovial tendons demonstrated intense early neovascularization processes within the hypertrophied paratenon and within both tendon stumps. By 7 days, the proximal and distal stumps of intrasynovial tendons were largely avascular, while extrasynovial tendon stumps and repair sites showed extensive new vessel formation. Similar contrasts were noted in the expression of genes and proteins related to inflammation and metabolism in both intact and healing tendons. Intact intrasynovial tendons had significantly higher expression levels of complement and inflammation proteins than did extrasynovial tendons. Intact intrasynovial tendons were selectively enriched in glycolytic enzymes, whereas extrasynovial tendons contained higher levels of oxidative phosphorylation enzymes. Interestingly, the patterns of genes and proteins related to inflammation and metabolism that were seen in normal tendons reversed in response to injury, where levels of inflammation were noted to be higher and glycolytic enzymes predominated in healing extrasynovial tendons compared with healing intrasynovial tendons. These novel findings are consistent with clinical observations of a robust healing response in extrasynovial compared with intrasynovial connective tissues. At baseline, intact intrasynovial tendons maintain a low level of metabolic activity and an avascular environment that favors

![Fig. 3](https://example.com/figure3)

Intrasynovial (Figs. 3-A, 3-B, 3-C, 3-G, 3-H, and 3-I) and extrasynovial (Figs. 3-D, 3-E, 3-F, 3-J, 3-K, and 3-L) healing responses at 3 (Figs. 3-A through 3-F) and 7 (Figs. 3-G through 3-L) days. Extrasynovial tendons showed rapid and robust vascular and cellular responses, while intrasynovial tendons were largely inactive. The responses in extrasynovial tendons were coupled with more collagen deposition compared with intrasynovial tendons (orange to red stain). The scale bar in Figure 3-A is 1 mm and applies to Figures 3-A, 3-D, 3-G, and 3-J. The scale bar in Figure 3-B is 50 μm and applies to Figures 3-B, 3-C, 3-E, 3-F, 3-H, 3-I, 3-K, and 3-L.
Gene expression levels of tendon-related genes (COL1A1, COL3A1), cartilage-related genes (SOX9, ACAN, COL2A1), extracellular matrix remodeling-related genes (MMP1, MMP3, TGFB1), inflammatory or inflammatory regulatory genes (CD86, IL6, TNF, CD163, IL10), and vascularity-related genes (VEGFA, HIF1A, MCAM) in healing tendons (black: intrasynovial; blue: extrasynovial). P < 0.05 for healing intrasynovial versus healing extrasynovial, *P < 0.05 for healing tendon relative to intact tendon, line over groups indicates p < 0.05.
chondrogenesis and proteoglycan production. These baseline characteristics, necessary for establishing an effective gliding surface, result in a slow and ineffective healing response following injury and repair. In contrast, extrasynovial tendons, which possess an ample blood supply and an enriched pool of scaffold and regulatory proteins and metabolic enzymes necessary for an anabolic cell response, demonstrate a more rapid response to injury marked by a robust inflammatory response and a dramatic increase in cellularity and vascularization.

Metabolism plays a major role in the differentiation of mesenchymal stem cells and in tendon responses to injury. Prior in vitro studies have shown that oxygen tension can influence cell metabolism and drive the differentiation of mesenchymal stem cells, with hypoxic conditions promoting chondrogenesis and normoxic conditions promoting osteogenesis. Inhibition of glycolysis has been shown to strongly favor tenogenesis over chondrogenesis in vitro. Modulation of metabolism after tendon injury has shown potential to improve healing. Zhang et al. showed in a mouse model that glycolysis and lactic acid synthesis increased dramatically after tendon injury, and that inhibition of lactate synthesis was beneficial for healing. The results of the current study showed a predominance of glycolytic enzymes in intact extrasynovial tendons (indicative of a more chondrogenic phenotype) with no apparent change in the early phases after repair. In contrast, similar to the findings of Zhang et al., we noted a predominance of oxidative phosphorylation in intact extrasynovial tendons (indicative of a more tenogenic phenotype) with a brief shift to glycolysis following repair. While the modulation of glucose metabolism and lactate synthesis has been shown to improve the repair of extrasynovial tendons, it is uncertain whether or not a similar approach (e.g., administration of dichloroacetate) would be effective following intrasynovial tendon repair.

Data from the current experiment indicate that the complement system leads to proteolytic cascades, which initiate an inflammatory response through the production of proinflammatory molecules. Activation of this system can be either beneficial or detrimental to musculoskeletal tissues, depending on the context. The complement system is also important in tissue regeneration; e.g., complement C3a signaling is critical for skeletal muscle regeneration. The current study, proteomics analysis of intrasynovial and extrasynovial tendons showed a difference in expression patterns for complement system-related proteins. In intact tendons, proteins related to the complement system were more abundant in intrasynovial tendons and an inhibitor of complement C1 was more abundant in extrasynovial tendons. This finding demonstrates that a low level of baseline complement activity is present in intact intrasynovial tendons relative to extrasynovial tendons. To our knowledge, such a contrast has not previously been reported for tendons, and it may have implications for the propensity of certain tendons to develop tendinopathy. During tendon healing, complement C5 was increased and inhibitors of the complement system were decreased in extrasynovial relative to intrasynovial tendons. This finding indicates that robust activation of the complement system takes place after injury in extrasynovial tendons, and is likely a key contributor to the improved healing response relative to intrasynovial tendons.

This study has several limitations. Quadrupedal canines produce inherently different flexor tendon loading patterns than do bipeds. In previous experiments, the canine was found to be the most appropriate animal model for flexor tendon repair because of its anatomical similarity to humans, because its tendons are sufficiently large to allow for operative techniques identical to those used in humans, and because it is amenable to a postoperative rehabilitation protocol similar to that applied to patients. A second limitation is the use of only female animals. Historically, we have used both male and female animals and observed no differences in histological, biochemical, and biomechanical outcomes according to sex. However, we found that female animals were more compliant than males with postoperative controlled motion rehabilitation, and we therefore used female animals in the current study. A third limitation is the inclusion of only early time points. In prior experiments, we found that early healing time points...

Fig. 5-A Principal component analysis of proteomics data for intact and healing intrasynovial and extrasynovial tendons. Figs. 5-B and 5-C Volcano plots demonstrating proteins that were differentially expressed between healing intrasynovial and extrasynovial tendons.
were predictive of later healing outcomes. Nonetheless, it is important to determine if the specific variations in histological, gene expression, and proteomics outcomes noted in this study are predictive of structural and functional outcomes at later intervals of repair.

The experimental findings reported in this study, based on a direct contemporaneous comparison of intrasynovial and extrasynovial tendon healing in the same experimental animal, document far more extensive differences in innate immune response, metabolism, and expression of extracellular matrix for tendons as a function of synovial environment than has been described previously. Furthermore, the differences noted in neovascularization and expression levels of complement, inflammation, and glycolytic enzymes help to explain previously reported variations in clinical outcomes following tendon repair. Future treatment strategies, such as a modulation of vascularization, complement, and glucose metabolism following intrasynovial tendon injury, can utilize these findings for the improvement of tendon repair.

Appendix

Supporting material provided by the authors is posted with the online version of this article as a data supplement at jbs.org (http://links.lww.com/JBJS/G308).

References


Update

This article was updated on May 13, 2021, because of a previous error. On page e36(1), the title that had read “Flexor Tendon Injury and Repair. The Influence of Synovial Environment on the Early Healing Response in a Canine Mode” now reads “Flexor Tendon Injury and Repair. The Influence of Synovial Environment on the Early Healing Response in a Canine Model.”

An erratum has been published: J Bone Joint Surg Am. 2021 June 16;103(12):e50.