INTRODUCTION: Surgical repair of tendon to bone injuries is often followed by a rehabilitation protocol that consists of a period of immobilization and/or a period of exercise, with the belief that the mechanical environment plays a role in the healing process. Current repair protocols provide a less than desired outcome and often result in re-injury and disability. Using animal models, previous studies have evaluated the effect of loading on normal tendons and ligaments, as well as on healing and repair tissue [e.g., 1, 2]. Few studies to date have focused explicitly on the important region of the healing insertion site in a tendon to bone attachment model. Therefore, the purpose of the current study is to investigate the healing process of the tendon to bone insertion site subjected to a range of postoperative activity levels. We hypothesized that immobilization would result in detrimental changes to the structural, compositional, and biomechanical properties.

MATERIAL AND METHODS: Studies were approved by the UPenn IACUC. Seventy six Sprague-Dawley rats (452±60gm) were operated upon bilaterally to detach and repair the supraspinatus tendon at its insertion site [2]. Post-operative activity level was controlled in three groups: cast immobilization (IM), cage activity (CA), and exercise (EX) (10m/min, 1hr/d, 5d/wk) [2]. Animals were sacrificed at 2, 8, or 16 week timepoints. A separate group of 15 uninjured animals (CTL) served as normal, un-operated controls. Histologic sections from 5 animals in each group were stained for structural, in situ hybridization, or histologic analysis. For structural analysis, sections were stained with 0.1% picrosirius red and viewed under polarized light. Digital images were taken and analyzed to determine the distribution of collagen fiber orientations [3], entropy (H) and the second angular moment of momentum (M2) for each specimen [4]. Fiber distributions were compared using the Kolmogorov-Smirnov test, and H and M2 were compared using a MANOVA followed by an LSD post-hoc test. Geometric and mechanical data were statistically compared between groups using a MANOVA followed by an LSD post-hoc test. A stress relaxation test was performed (to curve fit parameters to the experimental data). To determine properties of the insertion site, the Quasilinear Viscoelastic Model (QLV) [6] was used to extract viscous and elastic parameters from stress relaxation experiments. This model has been used successfully to model a variety of soft tissues, but it has never been used to model the normal stress relaxation experiments. This model was then used to curve fit parameters to the experimental data. The remaining animals from the 8 week timepoints (IM: n=10, CA: n=11, EX: n=10) were geometrically quantified and biomechanically tested. For cross-sectional area, thickness and width were measured as described previously [2]. For biomechanical testing, specimens were immersed in a 39°C PBS bath and preconditioned. A stress relaxation test was performed (to determine the localized expression of a variety of extracellular matrix genes [5]. Finally, sections were stained with 2% toluidine blue for histologic analysis.

RESULTS: Histologically, increases in cellularity and cell shape were seen in the healing tissue compared to the uninjured control. Subtle improvements were seen in the injured tissue with decreasing activity over time. Structurally (collagen distribution, H, and M2), the injured tissue was significantly less organized than the uninjured control. The IM group showed properties closest to normal and the fastest improvement over time (e.g., Fig. 1). The composition of the healing tissue (as measured qualitatively by in situ hybridization) was closest to normal in the IM group and furthest from normal in the EX group (e.g., Fig. 2). The ratio of type I to type III collagen expression was highest in the IM group compared to the EX group. Expression of aggrecan, biglycan, and type XII collagen was also highest in the IM group. Type X collagen was lower than normal in all injured groups.

DISCUSSION: This study presents a new tendon to bone injury and repair animal model and evaluates the effect of activity level on the healing tissue. Most properties did not return to normal after 8 weeks of healing in any injury group. The immobilization group had properties closest to normal. Contrary to our initial study hypotheses, A, B, and τ2 decreased and the area increased with increasing activity level (i.e., immobilization compared to exercise). Therefore, the peak and equilibrium stress response decreased with increasing activity. Equilibrium stress was also achieved more quickly for the immobilized group (as indicated by an increased τ2).

These data suggest that the healing tissue began to approach the properties of normal uninjured tissue by 8 weeks post-injury if the tissue was immobilized. Increased activity was effective in stimulating matrix synthesis, but ineffective in improving the properties measured. More material of lesser quality was produced when activity level was increased. This finding was supported by the structural studies (increased activity caused a decrease in the organization) and by the compositional studies (e.g., type I to type III collagen decreased with increasing activity). It is important to note that our results are applicable only to the specific tissue, injury model, and activity protocols chosen. It is possible that increased loading applied through other mechanisms (e.g., passive motion) may be beneficial. Additionally, true immobilization of the insertion may be detrimental. A more complex modeling approach may also be necessary to determine true intrinsic properties of the tissue.

FUTURE STUDIES will evaluate healing at longer timepoints, the effect of remobilization, and the cellular mechanistic response to activity level. A more complex modeling approach may also be necessary to determine true intrinsic properties of the tissue.


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