Placing Growth Factor Expression in Context: Protein Expression Profiles Correlated to Structure and Function in the Healing Rat Supraspinatus Tendon

INTRODUCTION:
As achieving adequate rotator cuff tendon healing continues to be an elusive clinical goal, a deeper understanding of the basic molecular mechanisms of tendon healing may be required, as well as how these mechanisms regulate tissue modeling/remodeling and the resultant functional/clinical outcome. Recent studies have used immuno-histochemistry (IHC) to analyze temporal growth factor expression profiles at various stages of supraspinatus tendon (SSP) healing in the rat [1, 3]. While some data exist regarding the remodeling of tendon tissue in the healing mouse Achilles tendon [4], there is a lack of similar data for the SSP that can be used to place growth factor and extra-cellular matrix (ECM) protein expression profiles within a structural/functional context.

The objective of this study was to relate known cellular synthesis characteristics to tissue ultrastructural remodeling and ultimately, the recovery of tendon function. We hypothesized that known growth factor expression profiles [3] could be more clearly interpreted after establishing a parallel profile of ECM protein expression (using semi-quantitative IHC), tissue ultrastructural architecture (using Transmission Electron Microscopy (TEM)), and biomechanical function (as documented in the literature [4]).

METHODS:
Male Sprague Dawley rats (n=36) were used in this study as approved by the relevant Swiss authorities. Thirty (30) rats underwent bilateral SSP tendon detachment and repair as previously described [1, 2, 3]. Animals were sacrificed at 1, 2, 4, 6, 8 and 16 weeks post injury and repair (n=5 each, yielding 10 shoulders per group). Six rats underwent sham surgeries without injury of the SSP and served as controls. The SSP tendon, muscle, and bone were isolated, formalin fixed and processed at each time point. IHC staining was performed on six shoulders at each time point against collagen I, collagen III, tenascin C, decorin, biglycan and fibromodulin. Both visual grading (global) and automatic (local) analysis of expression intensity was performed. Three SSP tendons at each time point were isolated, separated into thirds, fixed in glutaraldehyde, and imaged by TEM.

RESULTS:
IHC indicated relatively higher expression of all quantified ECM proteins at 1 and 4wk compared to other healing time points, and usually dropped to sub-baseline levels by 16wk (Table 1). Decorin expression did not return to baseline levels at any healing time point. In general, relative quiescence was seen after 4wk, partly consistent with the observed states of tissue remodeling (Figure 1) that indicated the tendon structure to have become relatively stable by 8wk.

DISCUSSION:
The observed ECM expression dynamics and matrix remodelling response is very similar to previously reported growth factor expression profiles in the same model of SSP injury healing (Table 2, [3]), but tends to lag these various factors by up to one week. This is consistent with the doctrine that growth factors induce cellular synthesis of ECM, and regulate its formation into an optimized architecture.

REFERENCES: