The Development of a Functional Enthesis is Driven by Stress Concentrations at the Tendon-to-Bone Interface

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SIGNIFICANCE: Repairs to orthopaedic hard-soft tissue interfaces (i.e., bone-tendon and bone-ligament) often fail because transitional tissues are not recreated. Improved understanding of the developmental patterns of these tissues may inspire novel repair strategies.

INTRODUCTION: Mechanical cues guide the development of musculoskeletal tissues and are integral to bone homeostasis. The hard-soft tissue interface (enthesis) presents an interesting developmental challenge because stress concentrations at the interface must be limited. A feature of soft tissue-bone interfaces that likely confers a mechanical advantage is a mineral gradient (I). The mechanism by which bioapatite accumulates on a collagen template during bone development is of special importance to the attachment of tendon to bone. At early postnatal time points while this attachment is developing, mechanical loads are very low compared to those needed for bone homeostasis in later life. The question then arises, how does the developing tendon-to-bone insertion site maintain physiologic levels of bioapatite? We characterized the morphology of the rotator cuff tendon-to-bone insertion site in a mouse model at time points throughout postnatal development and explored the mechanical consequences that this morphology might have in elevating stresses at the mineralization front.

METHODS: Animal model. The use of animals was approved by the Division of Comparative Medicine at Washington University. 45 C57Bl/6J mice were used for this study. Mice were sacrificed at postnatal days P7, P10, P14, P28, and P56. Raman spectroscopy: Fresh frozen unmineralized tissue sections were analyzed using a Raman microprobe (2), and fixed and stained with von Kossa and Toluidine Blue (N=5 per time point). Relative spectral intensities were mapped on to images of the stained sections. Transmission Electron Microscopy (TEM). Suprannusin tendon insertions were dissected, fixed with 2.5% glutaraldehyde and 4% paraformaldehyde (PFA), post-fixed with 2% osmium tetroxide, embedded in Epon, and sectioned to 80nm on an ultramicrotome. Micro computed tomography: A SCANCO μCT 40 scanner was used to measure muscle volume and length, humeral head volume, and tendon cross-sectional area from suprannusin muscle-tendon-bone specimens (N=3 per group). Applied stress calculation: Suprannusin muscle force was estimated using published results combined with our morphometric data (3). Histology: Specimens were fixed with 4% PFA, dehydrated, embedded in paraffin, sectioned to 4μm, and stained with toluidine blue (N=3 per group). Cell area relative to extracellular matrix were determined using imaging software.  

Modeling: The mechanical ramifications of the observed morphology were characterized through a series of idealized, multi-scale mathematical models. At the scale of tens of micrometers, stress concentrations were estimated at the “equators” of hypertrophic chondrocytes and the moduli of partially mineralized tissues were estimated. At the scale of hundreds of microns, an axisymmetric idealization (Fig. 2) was employed to estimate average mechanical stress fields acting on the growth plate, using techniques described elsewhere (4). These were combined to estimate peak stresses at the leading edge of the mineral gradient.

RESULTS: A mineral gradient of constant absolute size was present throughout post-natal development near the tendon-bone interface. While humeral head size increased, the width of the mineral gradient region maintained a constant length (Table 1) from time points immediately post-natal through adulthood. At P7 and P10, the gradient region coincided with the mineralization front of the secondary ossification center. Morphological parameters, i.e. muscle volume, indicate that applied stress increased linearly with age (Table 1). The mineral gradient acted to elevate stresses at its leading edge during early time points (Fig. 2). Cell-to-matrix ratio decreased with age in mineralized fibrocartilage (Fig. 3A); again resulting in elevated stresses at early time points (data not shown). When combined, these suggest that peak stresses at the equators of chondrocytes are elevated at P7, and then drop rapidly to approximately adult levels by P10 (Fig. 3B).

DISCUSSION: Mineral gradients likely contribute to a mechanically superior tendon-bone interface by reducing stress concentrations in the mature enthesis. However, this same gradient plays a markedly different role during development.

ACKNOWLEDGEMENTS: NIH AR055580. REFERENCES  

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Table 1: Morphological properties of postnatal mouse shoulders.

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Fig. 1: Mineral gradients are present in the murine tendon insertion by postnatal day 7. A) Raman microprobe map of a P7 shoulder, color indicates relative mineral content; scale is 10μm. B) TEM image of a P14 mouse shoulder, scale is 2μm.

Fig. 2: A) The rotator cuff tendon insertion is approximately axisymmetric. B) Model layer thickness was varied according to morphological parameters. C) The radial stress concentration factor at the bone-tendon interface is elevated early in development.

Fig. 3: A) Area fraction of the mineralized or un-mineralized side of the insertion that is matrix vs. cells. B) Normalized peak local radial stress at the mineral interface between cell equators.