

# Progressive Mineralization Modulates Collagen Fibril Mechanics at the Ligament-to-Bone Insertion: A Molecular Dynamics Study

Afif Gouissem<sup>1</sup>, Fadi Alkhatib<sup>1</sup>, Malek Adouni<sup>2</sup>

<sup>1</sup>Australian University, Mechanical Engineering Department, Kuwait; <sup>2</sup>Abdullah Al Salem University, Biomedical and Instrumentation Engineering Program, Kuwait.

**Disclosures:** A. Gouissem: None; F. Alkhatib: None; M. Adouni: None.

**INTRODUCTION** The ligament-to-bone interface, or enthesis, is a distinct tissue interface that is critical for adequate load transfer from soft ligamentous tissue to hard mineralized bone. It is paramount to joint stability, alignment, and movement regulation, reducing excessive or abnormal motion during physical activity[1]. The insertion achieves this mechanical function via a changing mineral gradient from unmineralized ligament to fully mineralized bone. The mineral volume fractions (MVFs) increase from 9.4% on the ligament side to 65.2% on the bone side, and create such a mechanically graded interface that, as a result, stress concentration was minimized and distributed more evenly [1-2]. Although the importance of the interface of such tissue is recognized, there remains incomplete knowledge about how the natural mineral gradient influences the mechanical properties of collagen fibrils—the fundamental building blocks of connective tissue. Some previous studies using experimental testing and computational modeling investigated mineralized collagenous tissues, but generally assumed either uniform mineral distribution, or a simplified distribution. An increasing understanding of the mineral distribution and its impact on the fibril properties cannot take place until the complexities of the gradual transition from intrafibrillar (crystals embedded in the collagen fibril structure) mineral to extrafibrillar (crystals surrounding the fibril surface) mineral have been modeled [3]. Although the mechanism by which minerals influence tissue behavior is currently unknown, additional mineralization may affect tissue mechanical behavior. This limitation mitigates our capability to accurately characterize the mechanical environment at the insertion point and limits our understanding of the behavior of the tissue under physiological and pathological conditions. To understand the mechanical role of progressive mineralization with respect to mechanical behavior, requires knowledge of both the healthy functional tissue as well as the changes that occur with aging and disease, such as ectopic mineralization and calcific tendinopathy, in which alteration in the natural mineral gradient can result in mechanical failure [1].

**METHODS:** A mesoscopic molecular dynamics (MD) model (fig.1) of type I collagen fibrils was constructed to study the effect of progressive mineralization on mechanical properties [4-5]. The model included both intrafibrillar minerals (ellipsoidal crystals in gap regions and platelets within the hexagonal collagenous structure) and extrafibrillar minerals (cylindrical shell blocks encapsulating the fibril). Five equidistant locations along the insertion were simulated that corresponded to MVF values of 9.4%, 22.3%, 37.3%, 51.3%, and 65.2%. Randomness in the size and distribution of mineral clusters was sampled to represent biological variability. MD simulations were performed with LAMMPS software with tensile loading applied at physiological strain rates, and to capture the stochastic variation of the mineral clusters, the entire analysis was repeated five independent times at each MVF, and average mechanical properties were computed. Stress-strain curves were analyzed to extract Young's modulus, yield stress, yield strain, ultimate tensile strength, and ultimate tensile strain.

**RESULTS:** The results showed that Young's modulus was almost unchanged across all MVF levels (4.33 to 4.57 GPa; 3% total change (maximum)), suggesting that elastic stiffness is mostly mineral-independent. Ultimate tensile strain also showed insignificant change (40 to 43%) with mineralization. Ultimate tensile strength increased substantially from 1003 MPa (MVF=9.4%) to 2378 MPa (MVF=65.2%), or an increase of 137%. Yield strain increased non-linearly from 14.7% to 27.4%, while yield stress increased from 619 MPa to 1267 MPa. At higher mineral contents (MVF>37%), molecular sliding was considerably suppressed, and in most simulations, the fibril transitioned to strain hardening before yield. These changes in mechanical properties are due to increased interatomic bonds between the hydroxyapatite and collagen beads that restrict the sliding of collagen molecules within the fibril.

**DISCUSSION:** The progressive mineral content creates a mechanically graded interface that allows for smooth load transfer from soft ligament to stiff bone, thereby reducing stress concentrations. Our results show that although stiffness is unchanged, the load-bearing capacity and yield behavior are dramatically improved with mineralization through lateral molecular constraint, which is particularly important to consider when thinking through the insertion mechanics during both normal physiologic and pathologic states. Disruption of the natural mineral gradient through aging, injury, or disease (e.g., ectopic mineralization) can thus lead to compromised mechanical integrity and increased propensity for tissue failure. The depth-dependent variation in mechanical properties that we observe in this study also helps to explain the reported susceptibility of insertions to degenerative changes and reconstructive failures [3].

**SIGNIFICANCE:** This study introduces a new mesoscopic MD framework that uses realistic mineral gradients to analyze ligament insertion mechanics. The results show how progressive mineralization helps distribute load and reduce stress at tissue interfaces. Clinically, this work helps explain how changes in mineral gradients—like those in calcific tendinopathy or aging—impact tissue mechanics and injury risk. These insights can inform tissue engineering and surgical techniques to restore proper mineral gradients and improve ligament and tendon repair outcomes.

**REFERENCES:** [1] Ricard-Blum S, (2011) Cold Spring a004978. [2] Svensson RB et al., (2010). Biophys J ;99(12):4020–7. [3] Niu Y, et al.(2024) Mater Des.Mar;239:112830. [4] Gouissem, et al., (2022), Bioengineering. Apr 28;9(5):193. [5] Buehler et al. (2008). J Mech Beh Biom Mat. 2008 Jan;1(1):59–67.

**Fig. 1:** Blue) Sections through the collagen fibril along the (0001) plane. Purple) Snapshots of collagen fibrils. Red) Collagen fibril at different mineral volume fractions response.

