MORPHO-MECHANICS OF THE SHEEP CALCANEAL ENTHESIS AS A RELEVANT ANIMAL MODEL FOR TISSUE ENGINEERING

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Introduction

Enthesis lesions are among the most critical tendon-related injuries [1]. At the enthesis a complex interplay between the hierarchical structure of the extracellular matrix (ECM) and a progressive gradient of mineralization/mechanics coexists. The sheep calcaneal tendon (i.e. tendon of triceps surae muscle or TTSM) is an excellent animal model due to its similarity with human tissue. However, no studies have comprehensively described both the TTSM structure and mechanics. Here, we investigated the structure of sheep TTSM enthesis via scanning electron microscopy (SEM) and histology. Quantitative backscattered electron imaging (qBEI) was used to quantify the local mineral content, combined with nanoindentation (nIND) to measure the local stiffness and hardness at the enthesis. Mechanical tensile tests with digital image correlation (DIC) and cyclic tests were used to clarify the tensile gradients of mechanical properties.

Methods

19 right TTSM-enthesis-calcaneal bone segments were extracted from sheep from local abattoirs. For SEM analysis, samples (n=2) were fixed and decellularized [2]. The orientation of collagen fibrils was calculated via the Directionality plugin of ImageJ with a consolidated method [3]. Histology was performed with Safranin-O (for GAGs) and fast green (for collagen) staining on fixed and decalcified (n=2) samples via a previously published protocol [4]. To perform qBEI and nIND, samples (n=3) were dehydrated and embedded in polymethylmethacrylate (PMMA) and their surface was ground and polished. qBEI was applied using a Field Emission-SEM calibrated with an established method [5]. Signals from the embedding resin/non-mineralized tissues were excluded (threshold = 5.2 Ca wt%). The elastic modulus and hardness were measured using nIND (Berkovich tip, in displacement control, with a maximum penetration depth of 200 nm) on the mineralized part of enthesis. Tensile tests were carried out in a testing machine (Instron) with a 10 kN load cell. A 3D-DIC (Dantec) measured the principal strains, using a white speckle pattern onto samples stained with methylene blue. Before tests, samples were hydrated in saline. Samples (n=6) were monotonically loaded to failure with a strain rate of 1% s^{-1}. Two images of unloaded samples were acquired for zero-strain analysis. Other samples (n=6) were cyclically loaded (100 cycles) in displacement control at 5% of strain (frequency = 1 Hz). The peaks of load were acquired.

Results & Discussion

TTSM SEM morphology revealed a collagen fibril orientation dependent on the investigated region (random at the enthesis and aligned in the tendon). Histology allowed to visualize the fibrocartilaginous transition between tendon and bone at the enthesis region and a progressive cartilaginous evolution of epitenon close to the bone. qBEI revealed peaks around 27 wt% of calcium content at the mineralized fibrocartilage. nIND highlighted a steep gradient of indentation modulus and hardness at the tendon-bone interface, occurring in a 10-15 µm width of transition region. Tensile tests confirmed a nonlinear behavior of TTSM. Maximum principal strains (ε_{P1}) measured VIA DIC showed peaks up to +40% in the tendon body. Minimum principal strains (ε_{P2}) showed positive (up to +15% at the enthesis sides and in the tendon tissue) and negative values (down to -40% in the enthesis center). Load peaks showed a progressive reduction of 20% of the initial mean value over 100 cycles.

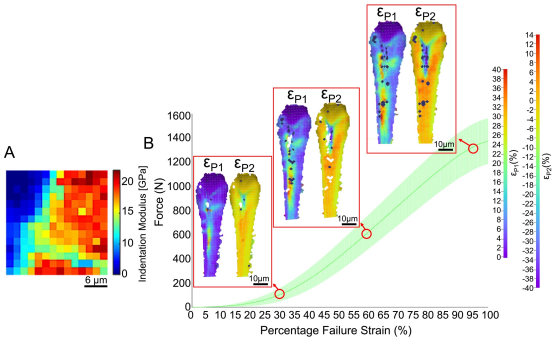

Figure: A) Typical nIND 14x14 matrix with a spacing of 2µm between each indent in the enthesis; B) Mean and standard deviation of force-strain (as % of failure strain) curves and typical DIC ε_{P1},ε_{P2} *maps.*

Our study helped to elucidate the structure/mechanics of the sheep TTSM, paving the way to produce biomimetic scaffolds for enthesis tissue regeneration.

References

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Acknowledgements

Horizon Europe Marie Skłodowska Curie Postdoctoral Fellowship 3NTHESES (n.101061826) is acknowledged for funding the study.