## Full Field 3D Measurement of Micromechanics – Microstructure Relationship at Mineralized Fibrocartilage Zone of Achilles Tendon to Bone Enthesis

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Introduction: Fibrocartilaginous enthesis is an anisotropic insertion where tendons and ligaments connect to bone epiphyses and apophyses. Mineralized fibrocartilage (MFC) and unmineralized fibrocartilage (UFC) zones allow the tendon or ligament to adapt to the varying angles of the bone during joint movement enabling load transmission from the tendon to bone to avoid stress concentration [1]. UFC and MFC plays a crucial role in anchoring the tendon to the bone. The interface between these two zones is often regarded as the weakest link in transmitting forces from the tendon to the bone [2]. Understanding mechanics and the structure relationship of the UFC as an anchoring site is essential for the development of implantable biomaterials and for the surgical reconstruction of the enthesis after rupture. The hypothesis is that the orientation and size of the lacunae in the enthesis is associated with the level and distribution of strain. In this study, the full field local 3D mechanics of the microstructure mineralized fibrocartilage was evaluated using a combination of high resolution *in-situ* micro computed tomography (µCT) imaging and digital volume correlation (DVC) to acquire 3D microstructure and deformation of loaded and unloaded interface.

Method: Samples were dissected from 8 months old male mice legs (Achilles tendon to calcaneus) under University of Portsmouth ethical approval (TETHIC-2022-104588). In-situ  $\mu$ CT (Versa 610, Zeiss) tensile testing was performed with 4X optical magnification, 1.35  $\mu$ m pixel size, and 1601 projection number. First, two consecutive tomograms were acquired for DVC error analysis. Then samples were scanned once in unloaded condition and then at 1.5N uniaxial load corresponding to physiological range of force-displacement curve (Deben CT500, Deben Ltd, UK). Images were rigidly registered (Avizo, USA) and the region of interest was selected. The 1st principle (Ep1), 3rd principle (Ep3), Von Mises (Evm), and maximum shear strain ( $\gamma$ ) were computed using DVC with a multi-step processing scheme from 84 to 24 voxels/subvolume, 0% overlap, and removed body rigid movement (LaVision, UK). Data (n=3) was expressed as mean  $\pm$  standard deviation (SD) and p value < 0.05 was considered as statistically significant.

Result:  $\mu$ CT 2D images showed the lacunae shape and size changes throughout the samples allowing for the determination of the volume and thickness of MFC at each plane. DVC results demonstrated the mechanics of MFC zone including  $\epsilon$ p1,  $\epsilon$ p3,  $\epsilon$ vm, and  $\gamma$  in global and local area.  $\epsilon$ p1 strain distribution of sagittal plane showed the highest values at the centre of the samples MFC (2.5%) the lowest at the periphery (0.56%) at the edge. The load transmission was not homogenous and at the centre,  $\epsilon$ p3 reach the value of -0.8%,  $\epsilon$ vm 1.6%, and  $\epsilon$  2.75%. The strain vector field showed a lateral direction at the MFC zone under a uniaxial tensile.

**Discussion:**  $\mu$ CT tomograms combined with DVC computations produced a full 3D internal strain map including sagittal, transverse, and coronal planes at MFC over a displacement of 1.5 mm. The data determined the local mechanics, e.g., strain and displacement in the MFC and adjacent bone. The strain response is non-linear, with certain regions such as MFC center, exhibiting higher strain. With uniaxial tensile testing this central strain decreased gradually to the periphery of the enthesis. It was shown from the  $\mu$ CT tomograms that lacunae alignment varied throughout the enthesis and becomes more aligned with depth and with larger volume at the centre of the enthesis in comparison to sides and this is related to the increase in the level of strain. This could be due to the number and orientation of the chondrocytes which reduces the localised resilience in comparison to the underlying zone of osteoblasts.

Significance: This study revealed the 3D local strain distribution of mineralized fibrocartilage and its relation to lacunae alignment, thickness, and volume where fibro chondrocytes are present to secure the anchoring.

References: [1] Rossetti et al., Nature Materials, v.16, 664-670 (2017). [2] Tits et al., Acta Biomaterialia, v. 166, 409-418 (2023).

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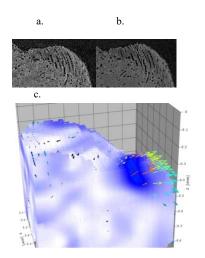


Figure 1. a. unloaded 2D tomogram, b. loaded 2D tomogram, c. 3D vector field.

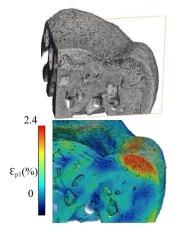


Figure 2. 3D representation of calcaneus Enthesis and global 3D Ep1 strain map.

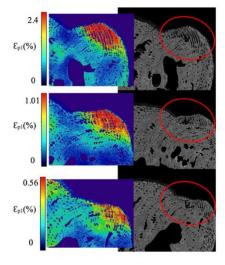


Figure 3. Sagittal planes of 2D tomograms of loaded sample and their Ep1 strain distribution.