3D STRAIN MEASUREMENT OF THE TENDON-BONE JUNCTION USING *IN-SITU* XCT MECHANICS AND DIGITAL VOLUME CORRELATION

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Introduction

Understanding the strain concentration and distribution at the tendon to bone junction or enthesis as a part of the musculoskeletal system is essential in development of implantable biomaterials and reconstructive surgeries [1]. Digital volume correlation (DVC) enables the 3D and internal mechanical deformation of complex structures. *In-situ* tensile testing micro X-ray computed tomography (micro-XCT) generates 3D scans of loaded and unloaded tissues [2]. Since soft tissue has a low X-ray attenuation coefficient, an appropriate contrast agent is usually used that enhances visualization enabling the 3D strain to be computed at the enthesis [3]. These contrast agents can potentially affect the mechanical properties of the tissues. The purpose of this study was to evaluate an optimal contrast agent whilst maintaining the tissue mechanical properties and to measure the full internal 3D strain at tendon to bone insertion by micro-XCT and DVC.

Methods

Samples were dissected from 8 months old male mice legs (Achilles tendon to calcaneus) under University of Portsmouth ethical approval (TETHIC-2022-104588). The force-displacement curves were acquired by uniaxial tensile testing until failure for samples stained with different contrast agents including phosphotungstic acid (PTA) in water, PTA in ethanol, and mercury II chloride ($HgCl_2$) in water and compared to untreated samples. Data (n=3) was expressed as mean ± standard deviation (SD) and **p** value < 0.05 was considered as statistically significant. Samples with the optimal contrast agent was used for *in-situ* micro-XCT tensile testing with 4X optical magnification, 1.37 µm pixel size, and 1601 projection number. Samples were scanned once in unloaded condition and then at 1.5N load. Images were rigidly registered (Avizo, USA) and the 1st principle (Ɛp1), 3rd principle (Ɛp3), Von Mises (Ɛvm), and maximum shear (γ) strain were measured using local approach DVC with a multi-step processing scheme from 84 to 24 voxels/subvolume, 0% overlap, and removed body rigid movement (LaVision, UK).

Results

The ultimate load (mean ± SD) of samples treated with $HgCl_2$, PTA EtOH, PTA H_2O, and untreated sample were 9N, 4N, 3N, and 6N, respectively. As shown by the slope of the curves in the linear region, the sample stained with $HgCl_2$ had substantially higher stiffness (12 N/mm) while the sample stained with PTA either in ethanol or distilled water had no significant difference compared to the untreated sample stiffness (4 N/mm). Subsequent experiments were conducted with PTA in ethanol. DVC results demonstrated Ɛp1, Ɛp3, Ɛvm, and γ strain throughout the sample. Although the load transmission is not homogenous, it was found that the load was localized mostly at the center of the enthesis where Ɛp1 reach the value of 16%, Ɛp3 -17.5%, Ɛvm 22.5%, and γ 12%. Von Mises strain was more intense in the center of the tendon and penetrated further into the bone than the 1st principle strain.

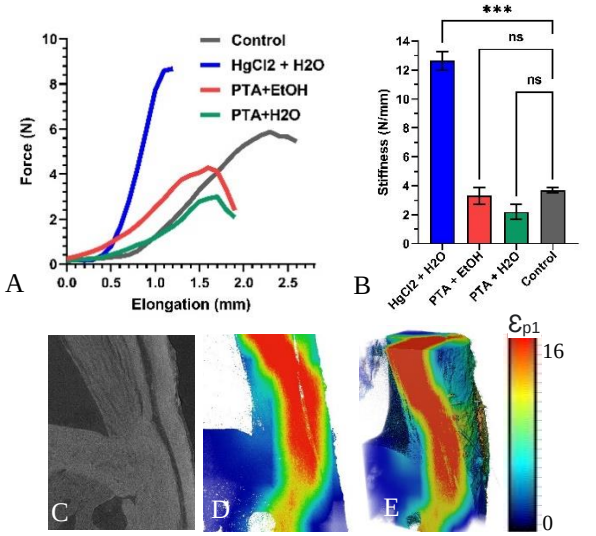

Figure 1: A) force elongation curve and B) stiffness for different contrast agents. C) unloaded 2D tomogram D) DVC 1st principle strain map on loaded 2D tomogram E) 3D 1st principle strain map.

Discussion

Compared to natural tissue, PTA in ethanol was the contrast agent that least affected the mechanical properties and can be used as contrast enhanced agent for *in-situ* micro-XCT tensile testing in elastic range. DVC results produced a full 3D internal strain map at tendon to bone insertion. This protocol can be used for 3D strain evaluation of soft tissues.

References

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