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Investigation, Modeling, and Reconstruction of the Tendon-to-Bone Insertion

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Introduction and Background

Current Knowledge

- Tendon-bone insertion tissue is structurally and functionally graded to alleviate stress concentration from soft tendon to hard bone
- Gradation in microstructure is not recreated in a healing insertion as in a native tissue

Current Limitations

- There is insufficient understanding of tissue microstructure and the property governing regeneration and repair post injury
- There is no comprehensive, three-dimensional mathematical model that may be used for modeling the insertion tissue

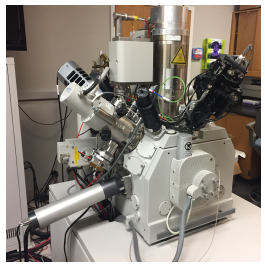
Objectives and Approaches

- Scans of tissue and density values at points within tissue obtained using micro-computed tomography
- Focused ion beam scanning electron microscope used to image microstructure of tissue at multiple depths throughout tissue
- Images obtained from scanning electron microscope pieced together in ImageJ to create 3D rendering of tissue

Methods and Results

Imaging and Scanning

- Imaging and scanning was performed using digital flexor tendon-bone units procured from the local abattoir (Nahunta Pork Center, Pikeville, NC) immediately after slaughtering.
- Tendon-to-bone connections were dissected from the two middle digits on the pigs' feet immediately after obtaining the feet. Samples were immediately fixated and critical point dried before imaging and scanning.
- Imaging was done using a focused ion beam and scanning electron microscope (FIB/SEM). A milling current and voltage of 7 nA and 30.0 kV, respectively, was used for milling, and the SEM beam voltage used was 5.00 kV (**Fig. 1**)
- Scanning was done using Micro-CT (Bruker SkyScan 1174, Billerica, MA). Scanning was done with a source voltage of 50 kV, source current of 755 µA, and image pixel size of 9.16 µm (**Figs. 2-3**).



FIB/SEM Specifications:

High Resolution SEM (Field Emission)
1nm resolution
Omniprobe Micromanipulator
FIB lift-out; in situ manipulation
Gas Injection System
Pt Deposition
Enhanced Carbon etch
Protochips *in-situ* Heating & Electrical Stage

Fig.1: FIB/SEM used for Scans

Fig. 2: Micro-CT Scanner

Micro-CT Specifications:
- 50kV Tungsten X-ray source
- 6µm 3D spatial resolution
- 2D/ 3D image analysis and realistic visualization

Fig. 3: Micro-CT Sample Mount

Results and Discussion

Density

- Density values at locations within cross-sections of the scanned tissue were obtained with DataViewer postprocessing software (Bruker, Billerica, MA).
- Density values, coupled with visual evidence, clearly indicate the location of the insertion, as well as provide a baseline for further analysis using FEM software (**Figs. 4-5**).

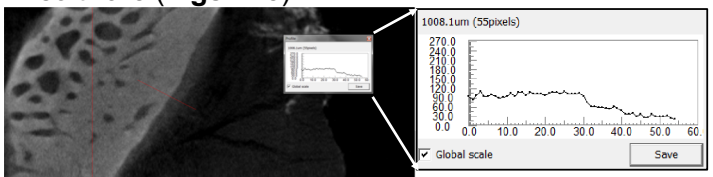


Fig. 5: Density Plot Along Insertion

Fig. 4: Cross-Section of Insertion Scan

Microstructure

- The microstructure of the tissue is seen via SEM images obtained using the FIB/SEM (**Fig. 6**).

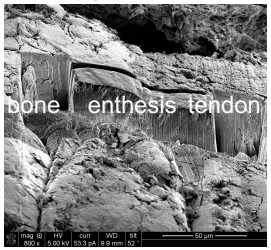


Fig. 6: FIB Milling Setup

Collagen fibers are clearly shown in **Figure 7**. Image reconstruction was performed in ImageJ (**Fig. 8**), with the end result being a 3D model that is to be import into ANSYS for further examination.

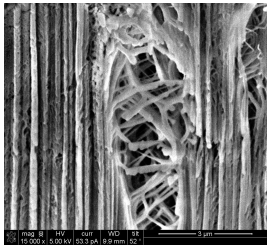


Fig. 7: Tissue Microstructure

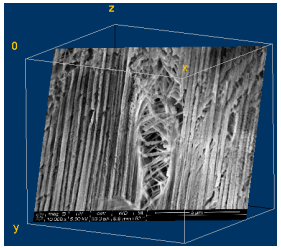


Fig. 8: 3D Image Reconstruction in ImageJ

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