Quantification of Radial Tie Fibers in the Knee Meniscus

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Introduction

- The menisci are crescent shaped fibrocartilaginous structures in the knee that function in load distribution, shock absorption, and lubrication. These tissues are composed predominantly of type I collagen fiber bundles with a primary population oriented in the circumferential direction along with radial “tie” fibers that interdigitate.
- Radially oriented “tie” fibers (RFTs) are thought to exist as sheets that originate from the perimeniscal region and branch and extend into the inner zone of the meniscus [1].
- Prior work showed that meniscal mechanical properties vary as a function of location, with for example the posterior horn having a greater stiffness than the anterior horns or body regions [2].
- Our ongoing research supports the hypothesis that radial tie fibers contribute to strain reconstitution surrounding a radial tear. Further, this research displays differences in strain reconstitution between injured anterior and posterior horn specimens.
- We hypothesized that meniscal RTF density and structure would vary as a function of location (anterior, body, and posterior) and region (inner margin and peripheral margin) and that a greater fiber density and thickness would be found in the posterior horn and outer zone of the bovine meniscus compared to other regions and zones.

Materials and Methods

Sectioning, Staining & Imaging:

- Medial menisci were harvested from 6 juvenile cows (~6 months of age).
- 6µm thick serial sections from each location and zone were cryo-sectioned and stained with Picrosirius Red to identify collagen.
- Six regions of interest (1.6x1.75mm) within each zone of each section were imaged using polarized light microscopy (Fig. 1).

Analysis (Fig. 2):

- To determine RTF density, each section was thresholded and analyzed to determine the area fraction of RTF within a region of interest.
- To determine RTF thickness, images were thresholded and binarized and a thickness map was created using the BoneJ plug-in in ImageJ. Fiber diameter was extracted from the thickness map.
- Means were compared via t-tests to determine statistically significant results.

Results

Density

- A total of 251 regions of interest (Fig. 1.a.) were analyzed across 6 medial meniscus samples.
- All menisci were comparable in width (~1.8cm) and length from posterior horn to anterior horn (~4cm).
- Statistical analysis showed lower mean fiber density in the posterior horn as compared to the anterior horn, \(t(9)=6.89, p=0.000\); body-a section, \(t(10)=8.75, p=0.000\); and body-b section \(t(7)=7.86, p=0.000\) (Fig. 3.a.).
- Additionally, the inner zone was found to have a greater fiber density than the outer zone (Fig 3.b.).

Thickness

- Average and maximum fiber thickness was also analyzed (data not shown).
- Across all locations and zones, fiber thickness ranged from ~9µm to ~19µm.
- Maximum fiber thicknesses were greater in the inner zones (ranging from 53.96µm to 60.69µm) than the outer zones (ranging from 50.56µm to 53.79µm).

Discussion

- In this study, we quantified RTF density and thickness by location and zone.
- Contrary to prior results using adult menisci [1], we found that RTF density was greatest in the anterior horn and inner zones of the meniscus.
- Though thickness results were not statistically different, in general the thickest fibers were found in the body and inner regions of the meniscus.
- This study represents the first time differences in RTF density and thickness have been quantitatively compared by location and zone.
- Ongoing research will quantify density and thickness throughout development and aging by quantifying fetal and adult RTFs.

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