Macro- and Micro-Scale Changes in Meniscus and Cartilage in a Large Animal Model of Meniscal Injury

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Introduction

The meniscus is a complex and integral load-bearing tissue in the knee[1] that is commonly injured.[2] Depending on the type of injury, the ability of the meniscus to transfer and distribute loads is reduced to varying degrees. For example, with tears of the enthesis (root tears), free meniscal excursion occurs with joint loading, the mechanical equivalent meniscus removal (meniscectomy).[3] Indeed, small animal (mouse and rat) models have used destabilization of the medial meniscus (DMM) to instigate controlled joint degeneration.[4] Even with smaller surgical excisions to treat radial or circumferential tears in the inner zone, the joint degrades over the longer term,[5,6] as evidenced by the early onset of OA in humans. While degeneration of the articular cartilage is well established in small animal models of meniscus destabilization, and in human cohorts after meniscus surgery, progression of disease in the meniscus itself is not well studied, especially in larger animal models. Thus, the purpose of this study was to investigate how meniscus injury in a large animal model instigates pathologic remodeling of the meniscus itself and the joint as a whole, at the macro- and micro-scale.

Methods

Juvenile (6 month old) Yucatan minipigs (n=12) underwent bilateral arthroscopic surgery and received two of the following four injuries to the medial meniscus—sham, DMM, a vertical longitudinal tear (1/3 arc length, red-white zone), or a radial tear (50% of meniscus width) (n=6/group). Animals were euthanized at one month and joints harvested for a series of macro- and micro-scale analyses. For macro-scale tests, intact joints were compressed to 1× body weight (400 N) at 15° of flexion using a custom rig and universal test frame. Pressure sensors (TekScan #6900-110) were inserted into the joint to measure load transfer through the medial compartment [3]. Next, tissues were imaged for macroscopic changes to the meniscus and for cartilage wear (using India ink)[7]. Subsequently, medial menisci were harvested and sectioned (16 micron thickness, vertical plane, anterior and posterior horns) for atomic force microscopy (AFM)[8]. Additional sections were subjected to histological analysis of proteoglycan (PG) content (Safranin O/Fast Green)[9]. Osteochondral cylinders were isolated from the medial tibial compartment and indented using a 2 mm spherical indenter to determine cartilage mechanical properties in the region covered by the meniscus[10]. These samples then underwent microCT scanning followed by decalcification and paraffin processing for analysis of proteoglycan content (Safranin O/Fast Green)[11]. Histological outcomes were graded using the OARSI scoring method[7] by four blinded observers.

Results

Sham operated menisci showed no macroscopic changes. Most vertical tears were no longer visible at 1 month, while radial tears were readily apparent. DMM menisci showed some healing with evidence of fibrous tissue at the anterior horn (Figure 1A). However, this scar tissue was not mechanically competent, as the mean contact pressure was significantly higher in DMM joints compared to other groups (Figure 1B). Menisci from sham limbs showed more intense staining for PGs compared to DMM menisci, with slight reductions in staining intensity radial and vertically defected menisci (Figure 2A). AFM analysis revealed an increase in the indentation modulus of radial fibers in the inner zone of the anterior horn with radial defects, as well as a decrease in circumferential fiber modulus in the outer zone of the posterior horn with DMM (Figure 2B). While no macroscopic signs of wear were visible on the tibial cartilage surface, PG staining was markedly reduced in DMM (Figure 3A) and slightly reduced in radial and vertical samples. OARSI scoring revealed changes in all groups, with significance for radial and vertical samples (Figure 3B). Cartilage indentation modulus was significantly decreased for DMM samples, and trended lower for vertical and radial samples (Figure 3C). There were no changes in the subchondral bone for any group at this time point (data not shown).

Discussion

This is the first study, to our knowledge, to investigate meniscal remodeling in a minimally invasive (arthroscopic), large animal surgical model of meniscus injury. Despite the fibrous "healing" of the DMM in this model, load transfer to the underlying cartilage remained altered at 1 month post-surgery, with increases in contact

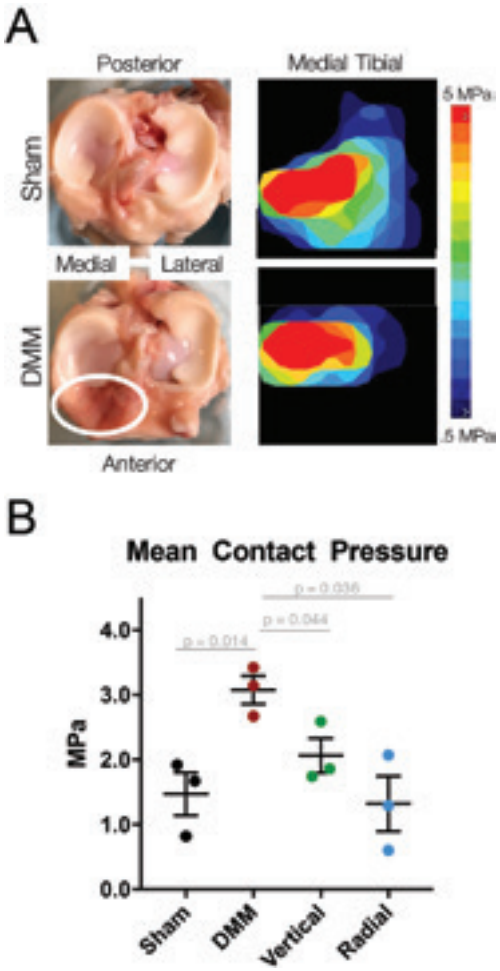

Figure 1. Macroscopic images **(A)** of sham (upper left) and DMM (lower left) operated menisci, showing fibrous attachment formation in the DMM meniscus (white circle). Representative contact pressure maps shown from peak contact load point (right). Quantification of mean contact pressure **(B)** for all operative groups.

pressure and decreases in cartilage PG staining and indentation modulus. This is consistent with outcomes from open surgical procedures in small[4,12] and large animal[13] models of DMM in the literature. Meniscus remodeling was also evident at this time point for DMM samples. Specifically, the PG content of the inner anterior horn was reduced, as was the indentation modulus of circumferential fibers in the outer posterior horn. This suggests that unloading caused by DMM results in remodeling of the meniscus. Vertical and radial tears did not alter load transmission at this time point, but both resulted in signs of cartilage degeneration (according to OARSI scoring) and radial tears increased micromechanics of the radial tie fiber network in the anterior horn of the meniscus. Future work will increase the sample size and extend the study duration to detail the temporal progression of meniscal injury-induced remodeling of the meniscus (and joint as a whole) at both the micro- and macro-scales in this large animal model. Understanding the progression of joint disease after meniscal injury in a large animal model will improve surgical decision making and inform novel repair strategies.

References

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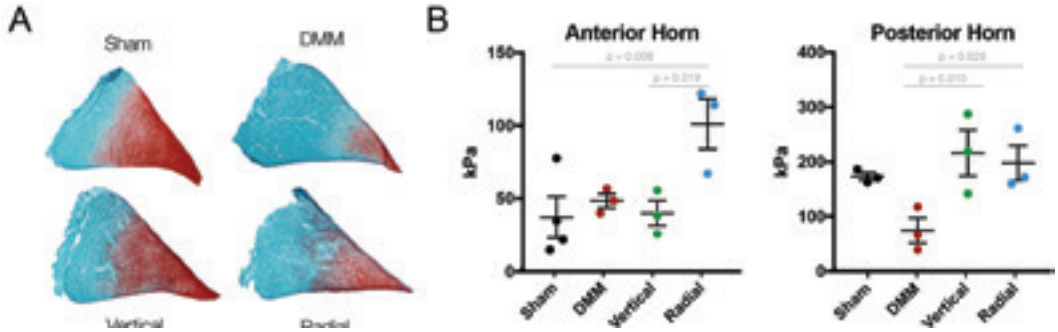

Figure 2. Histological staining **(A)** of radial vertical sections from the anterior horn of the meniscus. Staining done with Safranin O / Fast Green for proteoglycan content. AFM indentation modulus **(B)** of the Anterior Horn, inner zone radial fibers (left) and Posterior Horn, outer zone circumferential fibers (right).

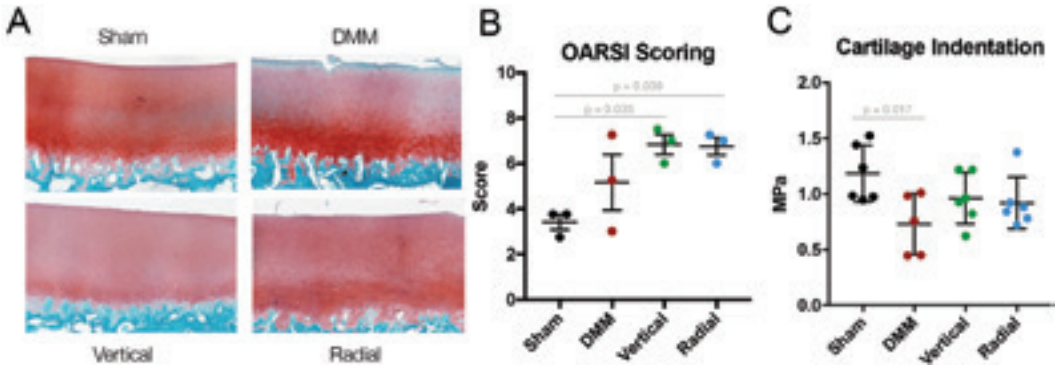

Figure 3. Histological staining **(A)** of osteochondral sections from each surgical group. Staining done with Safranin O / Fast Green for proteoglycan content, scale bar = 500 microns. Scoring **(B)** by four blinded observers using OARSI guidelines for large animals. Cartilage indentation modulus **(C)** for each group.