INTRODUCTION: Osteoarthritis is a progressive disabling disease that is one of the most common diagnoses in the world. Many factors can cause the progression of this disease, with meniscal injuries representing the greatest single contributor. Historically, meniscus injuries in the avascular zone do not heal due to the poor vascularity of the tissue and the limited regenerative capacity of cartilage. Thus, while it is known that torn meniscal cartilage degenerates over time, the kinetics and dominant molecular-genetic factors of this process remain unknown due to the absence of a quantitative in vivo model. To this end, we have developed a rabbit model to assess the natural history of meniscal changes following injury via delayed gadolinium enhanced MRI of cartilage (dGEMRIC) imaging.

METHODS: Surgery: Under general isoflurane anesthesia, the medial meniscus of Dutch-belted rabbits was exposed via antero-medial para-patellar arthrotomy and lateral luxation of the patella. A 5mm long full-thickness tear was created in the anterior portion of the medial meniscus. Animals were euthanized at various timepoints and medial meniscus samples were sent for histological processing.

Imaging: With the rabbits under general isoflurane anesthesia to prevent motion artifacts, standard MRI and dGEMRIC scans were performed pre-operatively and at 2, 4, 8, 12, 16 weeks post-operatively. A custom apparatus was developed to hold the knee in the same orientation for every scan. The surface coil used was built to conform to the animals and was interfaced with a clinical 3 Tesla Siemens Trio MRI. A fat-suppressed, T1-weighted high-resolution scan was then performed (Sagittal T1-weighted FLASH, TR=45ms, TE=9.03ms, 256x256 pixels, 0.5mm slice thickness, flip angle=25°, 1 signal average, time: 9:32min). To assess for loss of native cartilage in the injured zone with replacement of fibrocartilage, a negatively charged gadolinium contrast agent (Magnevist, Bayer Pharmaceuticals, Wayne, NJ) was injected in the aural vein of the rabbit at a dose 0.2cc/kg. The knee was then cycled and a MRI scan was performed at 50 minutes post-injection to allow for steady state diffusion conditions. These images allow us to track the loss of proteoglycans through increased signal/contrast uptake and creation of fibrocartilage at the injury site. Normalized meniscal contrast intensity (NMCI), was calculated by dividing the signal intensity of a 2D region of interest (ROI, green traced area in Figure 2) in the medial meniscus by the signal intensity of a 2D region of interest (ROI, green traced area in Figure 2) in the synovium and synovial fluid space, signaling post-injury inflammation. The ROI to quantify the NMCI and NMCE is indicated by the green outline. The injury is also highlighted in the post-op dGEMRIC images (arrows). Note that the meniscus lateral to the injury is much brighter than the meniscus medial to the injury, suggesting proteoglycan loss is first observed in avascular zone.

RESULTS: The scanning procedures proved to be well tolerated by the rabbits, and the high resolution MR scans produced excellent images that could clearly discern the margins of the meniscus, such that interobserver tracing of the ROI was precise to 0.001 cm² (p<0.01). Analyses of the dGEMRIC images clearly demonstrated an increase in signal intensity post-injury in the meniscus, which represents presence of fluid at the site of injury and also a loss of GAG content (Figure 2), and in the synovium and synovial fluid space, signaling post-injury inflammation. These data were used to quantify the meniscal changes (Figure 3).

REFERENCE


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Figure 1. Rabbit dGEMRIC model of meniscal degeneration after injury. A representative exposure of the rabbit medial meniscus (left), and the surface coil over the knee of a rabbit in a custom knee holder (right).

Figure 2. Longitudinal assessment of meniscal degeneration after injury via standard MRI and dGEMRIC. MR and dGEMRIC 2D images of a rabbit’s knee before (pre-op) and at the indicated time after meniscus injury are show. The ROI to quantify the NMCI and NMCE is indicated by the green outline. The injury is also highlighted in the post-op dGEMRIC images (arrows). Note that the meniscus lateral to the injury is much brighter than the meniscus medial to the injury, suggesting proteoglycan loss is first observed in avascular zone.

Figure 3. 2D and 3D longitudinal quantification of meniscal degeneration via standard MRI and dGEMRIC. The NMCI (solid line) and NMCE (dotted line) of the meniscus in Figure 2 are presented over time (left). The 3D reconstructed images of the meniscus (solid line) and the dGEMRIC bright space between the lateral and medial meniscus (dotted line) we generated to quantify the cartilage and defect volumes respectively at the indicated time point.

DISSCUSSION: Despite the prevalence of meniscectomy and its known association with the initiation of OA, progress to understand the natural history of meniscus degeneration injury has been limited due to the absence of an animal model with longitudinal outcome measures. Here we present evidence in support of an appropriate rabbit model, in which cartilage changes after injury can be quantified in vivo using standard MRI and dGEMRIC. Histology and our future directions with this model will also be presented.