Sustained Structural and Functional Deficits in the Porcine Knee Six Months Following Meniscus Destabilization

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Introduction

To better understand the progression of OA pathology, and to establish a test bed for assessing interventions in the context of an OA phenotype, effective large animal, human scale models of joint injury are necessary. Our previous work developed a porcine destabilization of the medial meniscus (DMM) model, where transection of the anterior horn of the medial meniscus resulted in deleterious changes in the knee at an early time point. However, these changes resolved at later time points, as the anterior attachment scarred back into place and the knee resumed normal biomechanics.[1] In a second iteration of this model, a 6mm portion of the medial meniscus anterior horn was resected en bloc, resulting in more severe joint pathology at a six-week time point.[2] The aim of the current study was to assess the progression of this OA phenotype over a six-month period and to assess changes in gait.

Methods

Sixteen skeletally mature (12-month-old) Yucatan minipigs underwent mini-arthrotomy of the right stifle, and a 6mm portion of the medial meniscus anterior horn was resected en bloc. Animals were sacrificed at either 6 weeks or 6 months post-operatively (N = 8 for both), with contralateral limbs serving as intact controls. Four of the animals in the six-month group were used for gait analysis. Videos of walking pigs were captured at 30 fps both preoperatively and four months post-op. Using DeepLabCut markerless tracking software,[3] the hip, knee, ankle, and hind hoof of each pig were tracked across the gait cycle (Figure 3A). Landmark positions were used to compute knee flexion angles. Further analysis was conducted using the GAITFour® walkway system, six months post-operatively. The pigs walked across a sensor-embedded mat, and the contact pressure of each hoof-strike was recorded. A model which normalized by velocity and trial number was used to measure differences between left (intact) and right (DMM) limbs. After sacrifice, stifle joints were dissected, and osteochondral segments of both the medial femoral condyle and medial tibial plateau were isolated. Indentation creep tests were performed, and the resulting deformation curves fit to a model outputting compressive and tensile moduli and permeability.[4] Specimens were then scanned via microCT before and after contrast enhancement with Lugol's solution (I_2 and KI in water). Cylindrical regions of bone were defined superficial and deep to the cartilage interface, and bone volume fraction was calculated for each. For histologic analysis, osteochondral sections were stained with Safranin O/ Fast Green, and synovium sections were stained with Hematoxylin/ Eosin. Statistics were performed in Graphpad.

Results

Quantitative tissue analyses were performed at three regions per timepoint—on the medial tibial plateau in areas previously uncovered and covered by the meniscus, and on the medial femoral condyle (Figure 1). At 6 weeks, the cartilage was softer across regions of interest, with statistically significant differences in all regions, except for the uncovered tibial plateau. This mechanical weakening persisted at 6 months with statistical differences detected in the compressive modulus in the covered tibia and in the tensile modulus in the uncovered tibia region. Bone volume

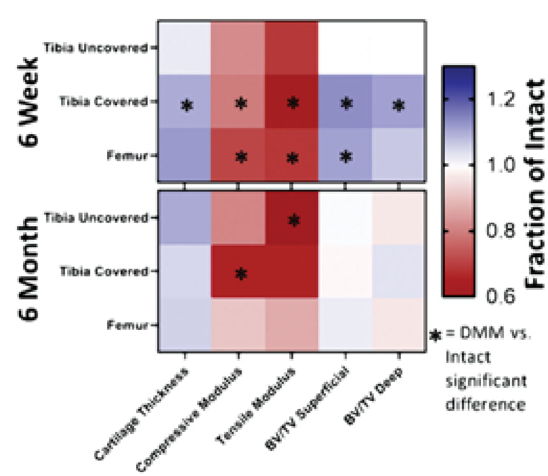

Figure 1. Tissue-scale quantitative outcomes of the operative limb after DMM for medial tibial plateau regions previously covered by the meniscus or uncovered, and for the medial femoral condyle, expressed as a fraction of intact control.

fraction increased in the covered tibia and superficial femoral condyle regions in DMM knees, but these subchondral changes were attenuated by the 6 month time point. (Figure 1). Histologically, osteochondral sections of DMM knees showed some degree of surface fibrillation and roughening at 6 weeks, and mild loss of proteoglycan by 6 months. (Figure 2A) At 6 weeks, the synovium of DMM knees showed hyperplasia and signs of fibrosis, but little difference was detectable by 6 months. Figure 2B). For gait analysis, graphical representations of post-op DMM knee flexion revealed a 'double-hump' signature during stance, which was not observed in the other groups (Figure 3B). Pigs exhibited greater maximal knee flexion in their post-op intact knee compared to pre-operative and post-op DMM knees (Figure 3C). Further, time spent in stance was shorter in both knees post-op compared to preoperatively (Figure 3D). When normalized for velocity and trial number, total pressure index percentage was significantly lower on the right hind limb when compared to the left hind limb (model adjusted effect: −0.5 , 95% CI: [−0.9, −0.1], p = 0.005; (Figure 3E) Similarly, the total scale pressure (the sum of peak pressure values recorded from each activated sensor by a hoof during mat contact) showed significantly less pressure in the right hind limb compared with the left hind limb (model adjusted effect: -1.4, 95% CI: [−2.4, −0.5], p = 0.004; (Figure 3F).

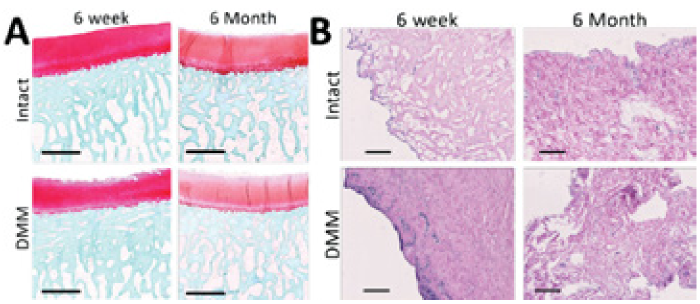

Figure 2. (A) Safranin O / Fast Green-stained osteochondral sections of the medial tibial plateau (scale =1mm) **(B)** Hematoxylin and Eosin stained synovium sections (scale = 100μm).

Discussion

This study showed that a more aggressive medial meniscus destabilization involving a partial anterior horn resection (as opposed to a simple transection) results in a durable osteoarthritic phenotype in a porcine model. We showed persistent cartilage weakening as well as histologic evidence of degenerative changes six months after surgery. Most importantly, functional assays of animal gait showed detectable abnormalities at four and six months after surgery. Animals displayed decreased range of motion during stride in the affected limb, as well as a reduction in time spent in the stance phase, indicating that animals were favoring the unoperated limb. Further, the kinematic parameters assayed during the gait analysis showed a significant and consistent decrease in weight distribution on the affected limb post-surgery. Joint pain alters normal function, particularly locomotion, and is one of the clinical signs of osteoarthritis. The alterations in gait and loadbearing in pigs 6 months postoperatively may reflect painful ambulation secondary to OA changes as a result of DMM.

Significance

This study developed a clinically relevant large animal model of OA leading to both structural and functional deficits of the knee and changes in pig gait patterns.

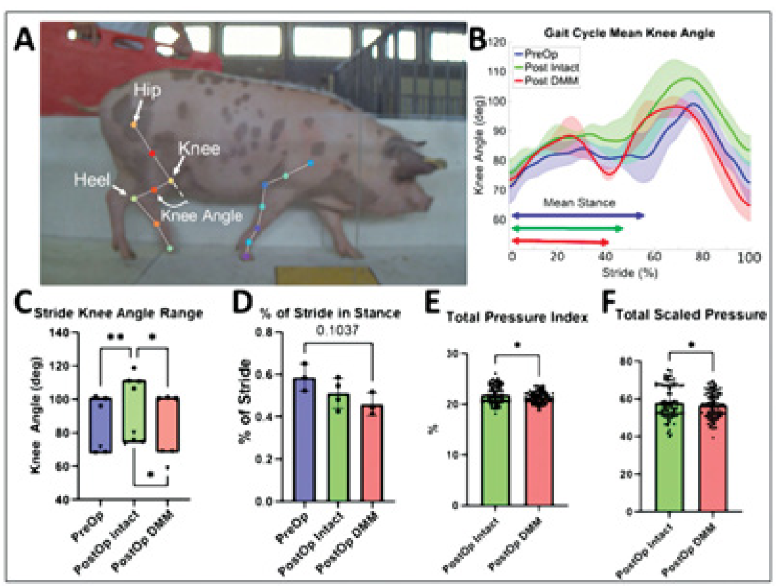

Figure 3. (A) Still from motion tracking showing tracked landmarks. **(B)** Average knee angle during gait cycle preoperatively, post-op on intact control limb, and post-op on DMM limb. **(C)** Average minimum and maximum knee angle during stride. D) Average % of each stride spent in the stance phase. **(E)** Total Pressure Index and F) Total Scaled Pressure, of each hoof strike as measured with the GAITFour system. *p<0.05, **p<0.01.

Acknowledgments

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