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Chronic Changes in the Articular Cartilage and Meniscus Following Traumatic Impact to the Lapine Knee

Kristine M. Fischenich¹, Keith D. Button³, Garrett A. Coatney^{1,2}, Ryan S. Fajardo³, Kevin M. Leikert⁴, Roger C. Haut⁴, and Tammy L. Haut Donahue^{1,2}

¹Department of Mechanical Engineering, Colorado State University, Fort Collins, CO USA

²School of Biomedical Engineering, Colorado State University, Fort Collins, CO USA

³Department of Radiology, Michigan State University, East Lansing, MI USA

⁴Orthopaedic Biomechanics Laboratories, College of Osteopathic Medicine, Michigan State University, East Lansing, MI USA

Abstract

The objective of this study was to induce anterior cruciate ligament (ACL) and meniscal damage, via a single tibiofemoral compressive impact, in order to document articular cartilage and meniscal changes post impact. Tibiofemoral joints of Flemish Giant rabbits were subjected to a single blunt impact that ruptured the ACL and produced acute meniscal damage. Animals were allowed unrestricted cage activity for 12 weeks before euthanasia. India ink analysis of the articular cartilage revealed higher degrees of surface damage on the impacted tibias ($p=0.018$) and femurs ($p<0.0001$) compared to controls. Chronic meniscal damage was most prevalent in the medial central and medial posterior regions. Mechanical tests revealed an overall 19.4% increase in tibial plateau cartilage thickness ($p=0.026$), 34.8% increase in tibial plateau permeability ($p=0.054$), 40.8% increase in femoral condyle permeability ($p=0.029$), and 20.1% decrease in femoral condyle matrix modulus ($p=0.012$) in impacted joints compared to controls. Both the instantaneous and equilibrium moduli of the lateral and medial menisci were decreased compared to control ($p<0.02$). Histological analyses revealed significantly increased presence of fissures in the medial femur ($p = 0.036$). In both the meniscus and cartilage there was a significant decrease in GAG coverage for the impacted limbs. Based on these results it is clear that an unattended combined meniscal and ACL injury results in significant changes to the soft tissues in this experimental joint 12 weeks post injury. Such changes are consistent with a clinical description of mid to late stage PTOA of the knee.

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Corresponding author: Tammy Haut Donahue, 1374 Campus Delivery, Mechanical Engineering, Fort Collins, CO 80523, Tammy.Donahue@ColoState.edu, Voice (970)491-1319, Fax (970) 491-3872.

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Conflict of Interest statement

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article

Keywords

meniscus; impact; osteoarthritis; knee

1. Introduction

Osteoarthritis (OA) has been considered by the World Health Organization to be among the top 10 conditions representing a global disease burden (Lopez et al., 2006). While OA is often a result of joint degradation over time, early onset can be triggered by a number of factors (Felson et al., 2000; Lawrence et al., 2008) including joint trauma (Lohmander et al., 2007). With a high occurrence of knee injury (Hootman et al., 2002) and knee OA (Zhang et al., 2008), post-traumatic osteoarthritis (PTOA) of the knee has been of particular interest to researchers and clinicians.

It has been reported that as high as 78% of knee injuries involving ACL tears are due to “non-contact” type of injuries (Noyes et al., 1983). High compressive tibiofemoral loading, often experienced during jump landings (Boden et al., 2000; Meyer and Haut, 2005; Speer et al., 1995; Yeow et al., 2011) is one potential non-contact mode of ACL rupture. These compressive loads have been shown to cause acute damage to articular cartilage, bone, and the menisci both clinically and in a laboratory setting (Killian et al., 2010a; Levine et al., 2012; Meyer and Haut, 2005). Current models of knee PTOA have primarily used *in vivo* animal models with surgical transection of the ACL (ACLT) to study the mechanisms of damage to these joints (Batiste et al., 2004; Hellio Le Graverand et al., 2001; M Yoshioka et al., 1996). However, ACLT models do not account for occult and acute damages to the surrounding structures which are often present in ACL injuries (Isaac et al., 2010a; Rosen et al., 1991).

Menisci are often injured in conjunction with the ACL (Felson and Neogi, 2004; McDaniel and Dameron, 1980). The menisci act to distribute joint load and stabilize the knee. Their unique wedge-like shape and composition allow them to bear up to 75% of loads acting across the knee (Shrive et al., 1978). Meniscal damage can alter the contact loads acting on the adjacent articular cartilage, which may lead to further cartilage damage and the development of osteophytes (Crema et al., 2010). Additionally, over 80% of ACL injury cases note osteochondral lesions in the posterior-lateral and posterior-medial aspects of the tibia and/or anterior-lateral aspect of the femoral condyle (Atkinson et al., 2008). These lesions are associated with damage to overlying articular cartilage and chondrocytes, which may help precipitate degenerative changes to the joint (Frobell et al., 2009).

To recapitulate what is observed in non-contact ACL injury scenarios, a closed joint *in vivo* lapine tibiofemoral impact model has been utilized in the current study. Previous work with this tibiofemoral impact (ACLF) model (Isaac et al., 2010b; Killian et al., 2010a) has shown that, in addition to ACL rupture, the impact produces acute meniscal, cartilaginous, and bone damages. The objective of the current study was to assess the gross, mechanical, and histological changes to both articular cartilage and menisci 12 weeks after impact. It was hypothesized that any untreated acute damage resulting from the impact would exacerbate in

the joint and lead to chronic changes in material and biochemical properties, consistent with the clinical description of mid-to late-stage PTOA.

2. Materials and Methods

2.1 ACLF Model

This study was approved by the All-University Committees on Animal Use and Care. Six skeletally mature Flemish Giant rabbits (5.7 ± 0.2 kg) were anesthetized (2% isoflurane and oxygen) and subjected to an impact of the right tibiofemoral joint. The animals were housed in individual cages ($60 \times 60 \times 14$ in), and were euthanized twelve weeks post injury.

Impact was delivered similar to a previous study (Isaac et al., 2008). In brief, the rabbits were placed in a supine position with the right tibiofemoral joint at 90° flexion. A 1.75 kg mass with a pre-crushed aluminum honeycomb interface (Hexweb, 3.76 MPa crush strength, Stamford, CT) head was dropped from a height of 70 cm, striking the distal femur. Magnetic resonance imaging (MRI) was performed to document any acute damage. Left, non-impacted, limbs served as paired controls. Gait abnormalities were assessed on a qualitative basis by a licensed veterinary technician (JA) during the duration of the study. The rabbits favored the non-impacted limb for 3–5 days, but showed no signs of gait abnormality for the remaining duration of the study. The rabbits were monitored and buprenorphine (0.3mL/kg BW) was administered for pain every 8 hours for 72 hours following impact.

2.2 Tissue Harvesting and Morphological Assessment

Tissue was harvested immediately after euthanasia and refrigerated until mechanical tests were performed (within 18 hours). The surfaces of the plateaus and condyles were stained with India ink to highlight surface fissures and other irregularities. Blinded gross morphological assessments from three graders were made with the following grading scale (Makoto Yoshioka et al., 1996): 1 = intact cartilage with the surface appearing normal, 2 = a few surface lesions that retain ink, 3 = moderate fibrillation retaining intense black patches of ink, and 4 = full thickness erosion exposing underlying bone.

Any visible meniscal damage was morphologically scored similar to previous grading systems (Pauli et al., 2011). Scoring was performed by four blinded individuals and averaged. Blinded graders were asked to assign a score from 0–4 to three different regions (anterior, central, and posterior) (Killian et al., 2010b) for both medial and lateral menisci, with 0 = normal, 1 = surface damage, 2 = un-displaced tears, 3 = displaced tears, and 4 = tissue maceration. Grading, as well as mechanical and histomorphological meniscal assessments, were performed regionally to account for previously identified regional variations. (Killian et al., 2010b; Sweigart et al., 2004)

2.2 Mechanical Analysis

Indentation relaxation testing was performed on both tissues in a room temperature 0.9% phosphate buffered saline bath. The articular cartilage was tested at four sites to account for both covered and uncovered tibial cartilage and weight bearing and non-weight bearing femoral cartilage (Figure 1). The thickness of each indentation site was estimated by

inserting a needle at a location adjacent to each indentation site and observing when the force changed due to contact with calcified cartilage. A 1.59 mm diameter, spherical nonporous steel probe was then pressed into the cartilage to a depth of 30% of its estimated thickness for 180 seconds. After a 1200s rest time the probe was replaced with the needle and the thickness (t) was determined at the indentation site. The mechanical tests were simulated with a previously described fibril-reinforced biphasic cartilage model (Golenberg et al., 2009) and implemented in a finite element package (Abaqus v.6.3, Hibbet Karlsson & Sorensen, Inc., Pawtucket, RI, USA). The voids ratio was assumed linear and depth-dependent, increasing from 70% at the cartilage-bone interface to 85% at the superficial zone (Golenberg et al., 2009). The matrix modulus (E_m), fiber modulus (E_f), and tissue permeability (k_0) of cartilage were determined from this model with a custom-written, Gauss-Newton constrained nonlinear least square minimization procedure.

A cylindrical steel 1.59 mm diameter indenter tip was used for load relaxation testing of the menisci (Fischenich et al., 2014; Li et al., 2006; Sweigart et al., 2004). An indentation depth of 0.25 mm was applied, and held for 900 seconds to reach equilibrium. Similar to previous studies (Li et al., 2006) Hertzian contact was assumed between an elastic half space (meniscus), and a rigid sphere (indenter). A Poisson's ratio of 0.01 was assigned to the menisci (Sweigart et al., 2004). The elastic modulus and Poisson's ratio of the indenter were 210 GPa and 0.3 respectively.

2.3 Histological Analysis

All tissues were fixed in 10% formalin. The bones were then decalcified using 20% formic acid. Tibial plateaus were sectioned coronally at the midpoint in the anterior-posterior frame of reference. Each femoral condyle was sectioned sagittally at the midpoint of the condyle. Sectioned plateaus and condyles were embedded in paraffin and sectioned at 6 μm . The menisci were embedded in optimum cutting temperature medium (OCT, Pelco; Redding, CA), flash frozen using liquid nitrogen, and cut into 6 μm slices. All sections were stained using Hematoxylin, Safranin-O, and Fast Green.

Bone/cartilage sections were imaged and the articular cartilage was histologically graded in three categories, using a modified Mankin scale (Table 1). Meniscal slides were imaged and GAG staining intensity was evaluated similar to previous studies (Fischenich et al., 2014): 0 = no staining, slight staining = 1, moderate staining =2, and strong staining =3. Stain intensity evaluations were performed by 4 individuals and the data from each specimen and location were averaged across individuals. Meniscal GAG coverage was analyzed quantitatively using Image J (NIH, Bethesda, MD) with FIJI package. First, the total area of tissue was determined using the analyze particle tool in Image J. Color Deconvolution was then applied to isolate the red associated with GAG from the blue/green of the cytoplasm. Once the blue/green was removed the area of GAG coverage was determined as a percent of the total area of the tissue of interest.

2.4 Statistical Analysis

Two factor (site, group) repeated measures analysis of variance (ANOVA) with Tukey's post-hoc tests were conducted on mechanical and thickness data from the articular cartilage

test sites. The morphological grades of the articular cartilage were subjected to a two factor (site, group) ANOVA on ranks to compare overall values. Histology results of the articular cartilage and all meniscal morphology and GAG intensity were analyzed using a Wilcoxon signed-rank. Meniscal mechanics and GAG coverage data were subjected to a one way repeated measures ANOVA with post hoc Tukey tests to compare each scoring category between the impacted and contralateral control limb. Significance was taken to be $p < 0.05$ for all metrics.

3. Results

3.1 Morphology

Acutely, all impacted joints experienced ACL rupture and 8 of the 12 menisci experienced damage, as confirmed with MRI. At twelve weeks post impact all impacted joints demonstrated some level of osteoarthritic change including osteophyte formation, articular cartilage fibrillation, and synovial inflammation.

Gross morphological analyses at 12 weeks post impact indicated that the impacted tibial plateaus had a higher degree of articular surface damage than the controls ($p=0.018$). The femurs also showed a higher degree of surface damage in the impacted limbs ($p<0.001$) (Table 2). Figure 2 shows representative images of cartilage damage including full thickness erosion in 4 of 6 medial femoral condyles (2a), 1 of 6 lateral femoral condyles (2b), and 5 of 6 medial tibial plateaus (2c).

Twelve weeks post injury, statistically significant increases ($p<0.05$) in damage between impacted and contralateral limbs were observed for all medial meniscal regions (Figure 3). All lateral meniscal regions had an increase in damage, although not significant ($p<0.1$). Damage was most severe in the posterior meniscal region of both hemijoints and the central region of the medial meniscus. Tearing was seen chronically in all 6 medial menisci and 4 of the lateral menisci (Table 3). The medial meniscus sustained more extensive damage than the lateral (a score 2.5 in 11/18 medial regions and only 6/18 lateral regions).

3.2 Mechanical Analysis

Extensive tissue damage and maceration limited mechanical testing at numerous sites. Full thickness erosion was found in 4 of 6 medial femoral condyles, so only $n=2$ mechanical tests could be performed at this site. Despite that, there was an overall 40.8% increase in permeability ($p=0.029$) and a 20.1% decrease in E_m ($p=0.012$) of femoral articular cartilage when comparing injured to control limbs. Likewise, the tibial plateau data showed an overall increased cartilage thickness of 19.4% ($p=0.026$) and an increased tissue permeability of 34.8% ($p=0.054$) (Table 2).

The lateral anterior region of the menisci was the only full sample set of $n=6$. The remaining regions were as follows: lateral central and lateral posterior $n=5$, medial anterior $n=4$, medial central $n=2$, and medial posterior $n=1$. Because statistical analysis could not be performed on the medial central and medial posterior meniscal regions due to small sample sizes, the regional data were averaged for a given hemijoint and specimen (Figure 4a and 4b). Significant decreases ($p<0.05$) in the instantaneous and equilibrium elastic moduli compared

to control limbs were documented in the lateral menisci. The medial meniscus indicated the largest average percentage decrease from control to impacted limb in both the instantaneous and equilibrium elastic modulus, but only the data collected at equilibrium showed a significant decrease ($p < 0.05$). Gross damage to the medial central and posterior regions reduced the number of mechanically testable samples. However, of the remaining four regions (lateral anterior, central, and posterior and medial anterior) significant decreases ($p < 0.05$) were documented in the lateral central, and medial anterior both instantaneously and at equilibrium (Figure 4a and 4b).

3.3 Histological Analysis

Using a modified Mankin grading system the medial femur was found to have significant changes ($p < 0.05$) in GAG content and articular cartilage fissures as compared to the control limb. Although not significant the tidemark integrity decreased ($p < 0.1$) in both the medial femur and tibia (Table 4). No significant differences were found in the lateral hemijoint. Damage was primarily characterized by a loss of GAG in the femur and increased articular cartilage fissuring.

Average meniscal GAG coverage and stain intensity were well correlated ($R^2 = 0.85$, $n = 6$ in all regions). Qualitatively assessing stain intensity showed large percentage decreases in average grades of intensity in anterior meniscal regions, but no significant differences (Figure 5). Quantitative GAG coverage assessments, however, did significantly decrease in the anterior regions of both the lateral ($p < 0.02$) and medial ($p < 0.04$) meniscus of the impacted limb, as well as the lateral central region ($p < 0.04$). The greatest decreases were seen in the anterior regions with an average 39.3% decrease in the lateral anterior region and a 74.3% decrease in the medial anterior meniscal regions between control and impacted limbs.

4. Discussion

Using an *in vivo* closed joint traumatic impact model we were able to rupture the ACL as well as create acute articular cartilage and meniscal damage with a single tibiofemoral compressive impact. Damage was not assessed at time zero beyond MRI, as a previous impact study showed no acute change in tissue mechanical properties following impact (Ewers et al., 2002). More damage was seen at dissection in the medial hemijoint than the lateral, which is consistent with clinical studies on OA patients (Bellabarba et al., 1997; McDaniel and Dameron, 1980; Smith and Barrett, 2001). To the authors knowledge this is the first study to look at whole body meniscal mechanical properties in both healthy and impacted tissue.

The irregular changes in GAG coverage of the impacted menisci are of particular interest. Coverage was shown to decrease in anterior regions despite these being locations of less damage. Immobilization has been shown to decrease GAG content in the meniscus (Djurasovic et al., 1998), and dynamic overloading under specific conditions has been shown to up-regulate aggrecan production (Zielinska et al., 2009). In the current study it is unknown how the loading pattern changed as a result of the initial acute damage in the joint. However, in the ACL deficient knee it has been shown that there is a significant anterior

translation of the tibia. If this is the case in our model, it may suggest there was a decrease in load experienced in the anterior region of the meniscus post trauma. The unloading of the anterior region might help explain GAG loss in that region, while less of a change was observed in the posterior regions despite heavy damage. Furthermore, a previous canine ACL transection study (Adams et al., 1983) documented initial decreases in meniscal GAG at 1 week post injury with levels returning to normal or above normal after 15–18 months. This initial decrease in GAG could be due to an inflammatory response, as numerous studies (Lemke et al., 2010; McNulty et al., 2010) have demonstrated GAG degradation under inflammatory conditions. We were limited in our assessment of GAG changes, as they may be temporal and this study is presenting results from a single time point of 12 weeks.

The main tear orientation of the acutely damaged menisci in this model was longitudinal vertical tears, which have been reported as the most common in clinical injury scenarios (Lewandrowski et al., 1997; Tandogan et al., 2004). Meniscal damage in this model was mostly seen in the posterior region, which is also in agreement with clinical findings (Greis et al., 2002; Smith and Barrett, 2001). When compared to previous ACLT models (Adams et al., 1983; Brophy et al., 2012; Hellio Le Graverand et al., 2001; Smith and Barrett, 2001; M Yoshioka et al., 1996) the chronic gross meniscal damage observed in this ACLF model showed a higher incidence of tearing and more extensive tear patterns. ACLT models have documented chronic damage primarily as incomplete tears and the occasional bucket handle tear (Adams et al., 1983; Hellio Le Graverand et al., 2001; Smith and Barrett, 2001). The ACLF model produced full thickness, displaced, and complex tearing of meniscal tissue chronically. The difference between models suggests that when acute damage is present the pattern and progression of meniscal damage is affected.

In addition to gross meniscal damage, changes were also observed in the underlying cartilage. Mechanical data from indentation-relaxation tests revealed increases in cartilage permeability and decreases in matrix stiffness in the impacted versus contralateral control joints. The changes in permeability may be a result of significant increases in surface damage in both the tibia and femur. This would be consistent with a previous study by Setton et al. (Setton et al., 1993) suggesting that surface fibrillation increases tissue permeability, potentially increasing loads on the solid matrix. Increases in solid matrix stresses may also help explain the decreases in femur E_m and the trend for a decrease in E_m on the tibial plateau observed in the current study.

There were also increases in thickness of the tibial plateau cartilage. This was likely a result of tissue swelling due to damage to the network of collagen fibers, which has previously been observed in canine models (Adams and Brandt, 1991; Brandt, et al., 1991). However, these thickness changes were not associated with corresponding statistically significant changes in the matrix fiber modulus, as might be expected. One shortcoming of the current study was that the fibril-reinforced biphasic model did not account for potential changes in the water content (voids ratio) and the orientation of fibers that may be present in diseased cartilage. Future studies may need to adjust for these changes when modeling potentially damaged cartilage. Another shortcoming of the current study was the initial observation of altered gait during the first 3–5 days following impact, potentially raising concerns over using the contralateral limb as the control. Additionally, due to the qualitative nature of

assessing gait, there may have been additional subtle changes in joint loading at later time points that were not observable to the veterinary technician. Future studies may want to quantitatively evaluate gait over time, perhaps using a force plate treadmill, in order to better assess any abnormal loading.

Previous studies (Donohue et al., 1983; Isaac et al., 2010b; Milentijevic et al., 2005) have investigated articular cartilage changes following impact using biochemical analysis. Substantial loss of GAG through the depth of the impacted cartilage was observed as early as 3 weeks post injury by Milentijevic et al. (Milentijevic et al., 2005). In a longer study investigating tibial plateau changes, Issac et al. (Isaac et al., 2010b) reported significant increases in fissuring and loss of GAG at 6 and 12 months. While not all of these models included ACL failure, outcomes from this impact-induced ACL failure model are in agreement with these previous studies (Saito et al., 2012; Makoto Yoshioka et al., 1996). Significant loss of GAG and an increase of surface fissuring were documented in the medial femur. Greater GAG depletion was also observed in the medial compartment where fissuring and erosion occurred more frequently, with greater GAG depletion documented in the femoral condyle versus the tibial plateau.

Significant increases in meniscal damage and decreases in mechanical properties likely contributed to the overloading and erosion of the articular cartilage. Meniscal morphological damage, as well as the permeability of the tibial cartilage, significantly increased after traumatic loading of the joint. It is likely that the acute injury to the meniscus may have caused exacerbated chronic damage to underlying cartilage, just as a recent human cadaver study has shown damage and depression in the sub-meniscal cartilage after a combination of ACL rupture with a meniscal tear (Levine et al., 2012). The medial hemijoint served as the site of the highest degree of overall change in both the menisci and articular cartilage. Meniscal damage was most common in the central and posterior regions of the medial meniscus while histological and morphological analyses identified the proximal and distal medial femoral condyle as the most affected site for articular cartilage. Further analysis is necessary to determine the relationship between the tissues' degradation times, but it seems clear that there may exist some relationship between meniscal and articular cartilage damages in the chronic setting after blunt force trauma to the joint. An additional study involving an impact to the joint resulting in acute and occult damages in the absence of an ACL rupture would be necessary to determine if acute meniscal injury and overloading of the joint alone would cause similar damage.

Traditional ACLT models have shown that destabilization of the joint can lead to early development of OA, but they fail to address damage to surrounding structures often documented following traumatic injury. In this regard, the current ACLF model may help provide a more complete understanding of whole joint changes after blunt force trauma. This model has shown significant chronic damage to the joint, including damage to the cartilage surface and its matrix as well as significant meniscal damage. This is the first study to document changes to the menisci following a severe impact loading of the joint. It is clear from the current study that significant changes occur in the menisci, possibly correlating with articular cartilage damage. These damages should be considered in future PTOA studies on the knee joint. Thus, the current *in vivo*, small animal model for the study of

PTOA may ultimately aid in the development of future intervention strategies to help limit or mitigate joint degeneration in this trauma patient.

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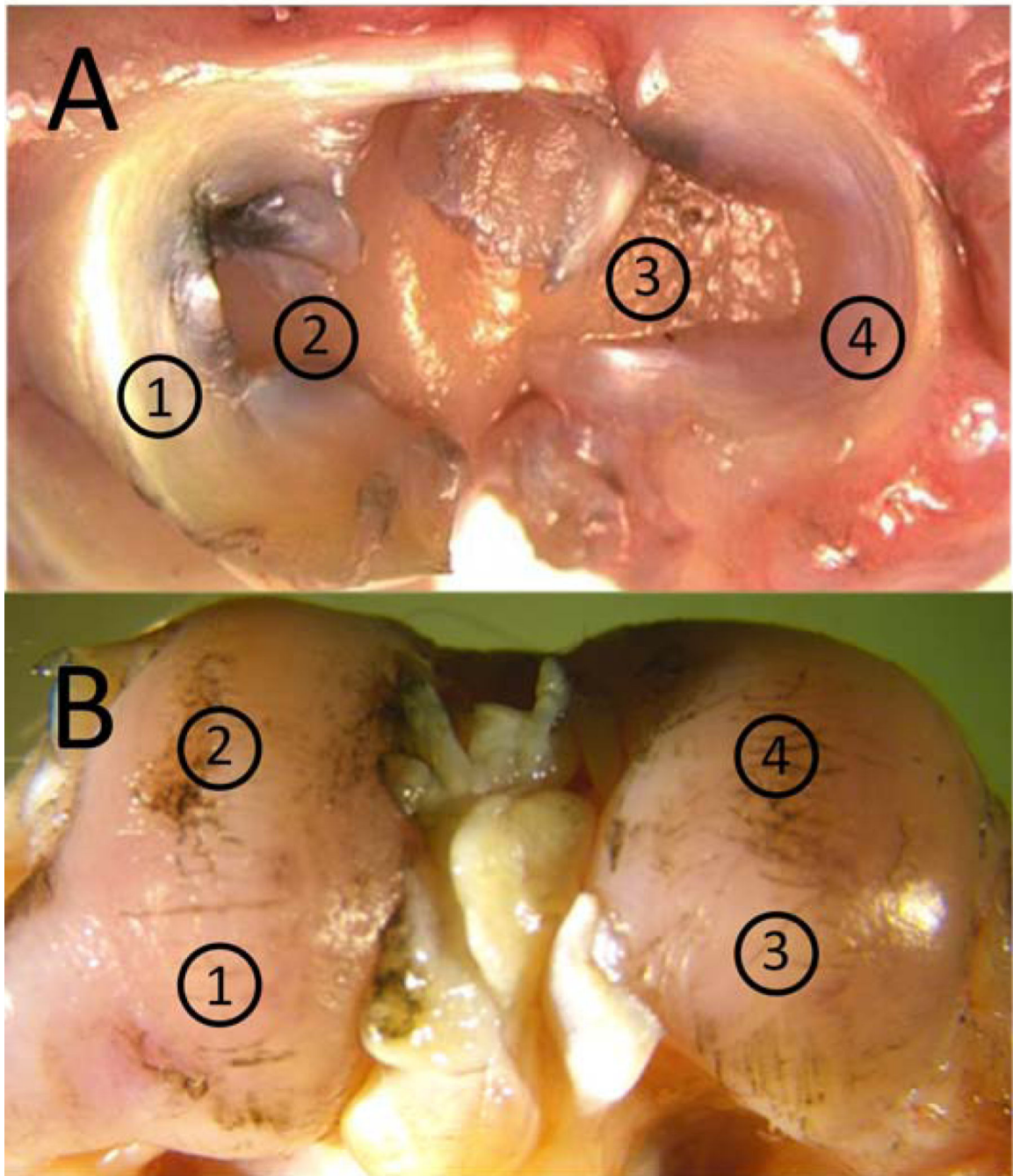


Figure 1.

Sites for indentation relaxation A) tibial sites 1 and 4 were covered by meniscus and sites 2 and 3 were uncovered. B) femoral sites 1 and 3 were sites that contacted the tibia during 90 degrees of flexion (impact orientation) and sites 2 and 4 were sites that contacted the tibia during normal gait.

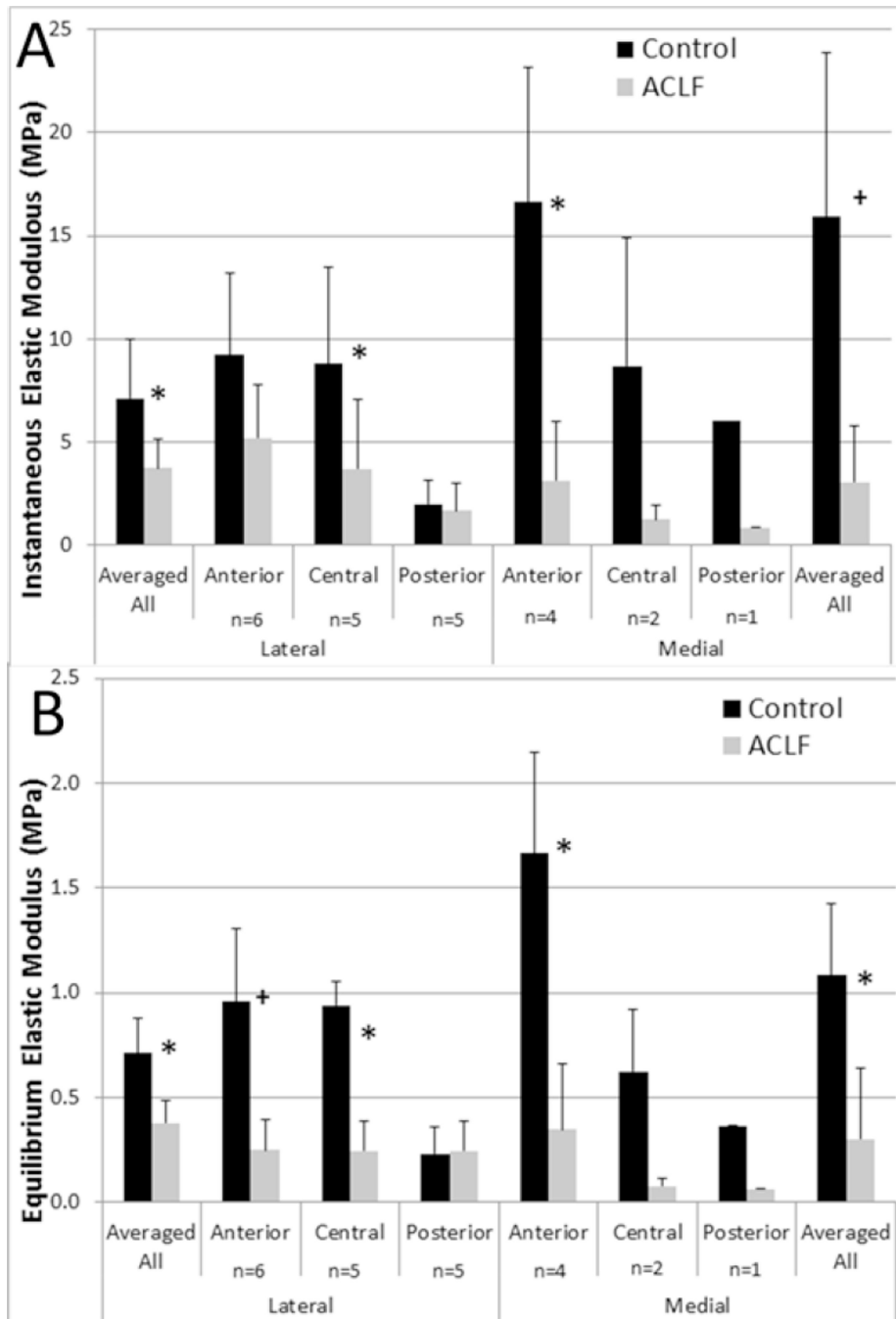


Figure 2. Full thickness cartilage erosion was noted on the medial femoral condyles (a), on the lateral femoral condyle (b), and full thickness erosion was noted in the medial-posterior aspect of the tibia (c).



Figure 3.

ACLF Menisci 12 weeks post-impact (animals 1–6 left to right and top to bottom, all specimens are oriented identical to the first image). Dashed line outlines example synovium, Solid line outlines example of meniscal tissue, displaced parrot beak tear (a), tissue maceration to central region (b), complex tearing of both the medial and lateral menisci (c), and tissue maceration of the posterior horn (d).

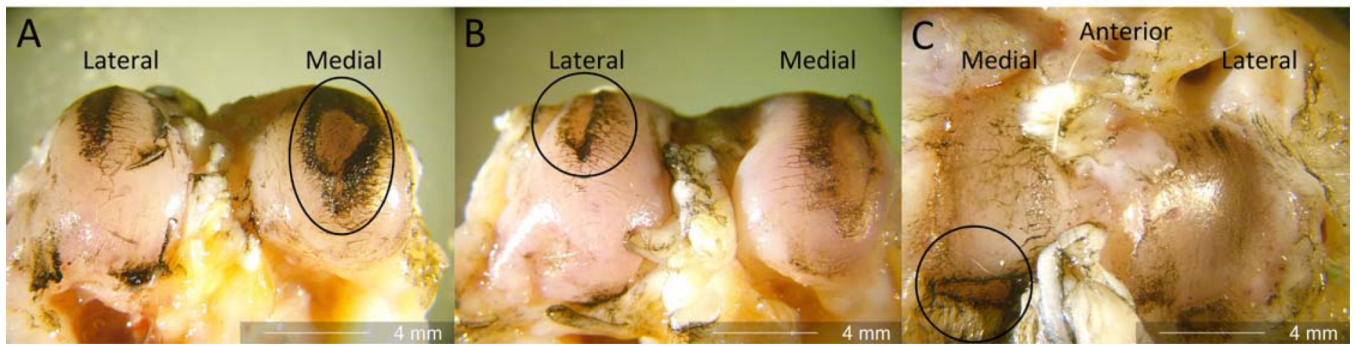


Figure 4.

A) Instantaneous elastic modulus of the B) Equilibrium elastic modulus by region of the menisci (mean with standard deviation) * denotes $p < 0.05$ and $^+$ denotes $p < 0.1$ between control and ACLF.

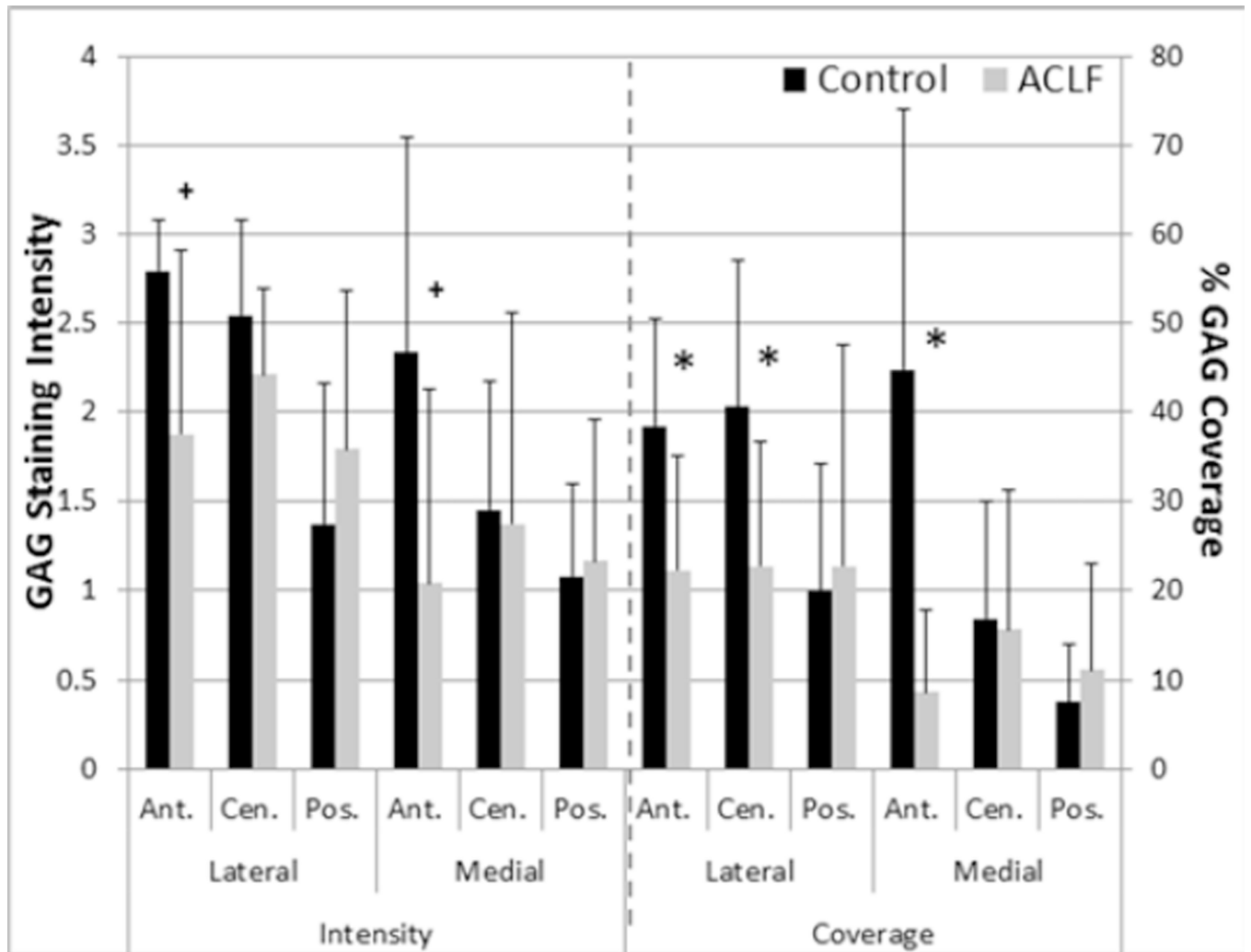


Figure 5. GAG intensity grading and percent area of GAG coverage in the menisci (mean with standard deviation) * denotes $p < 0.05$ and + denotes $p < 0.1$ between control and ACLF.

Table 1

Modified Mankin grading scale

Measure	Score	1	2	3	4	5	6
GAG Staining	0	Uniform	Loss of staining in superficial zone <50% of length of condyle or plateau	Loss of staining in upper 2/3 < 50% of length of condyle or plateau	Loss of staining in upper > 50% of length of condyle or plateau	Loss of staining in full depth of cartilage <50% of length of condyle or plateau	Loss of staining in full depth of cartilage >50% of length of condyle or plateau
			Loss of staining in superficial zone >50% of length of condyle or plateau	Loss of staining in upper 2/3 < 50% of length of condyle or plateau	Loss of staining in upper > 50% of length of condyle or plateau	Loss of staining in full depth of cartilage <50% of length of condyle or plateau	Loss of staining in full depth of cartilage >50% of length of condyle or plateau
Fissures		Surface fibrillation <50% of length of condyle or plateau	Surface fibrillation >50% of length of condyle or plateau	1–2 midzone fissures	3–5 midzone fissures	Full depth fissures or 5+ midzone	Large segments of cartilage eroded with full depth fissures
		Absent	Surface fibrillation >50% of length of condyle or plateau	1–2 midzone fissures	3–5 midzone fissures	Full depth fissures or 5+ midzone	Large segments of cartilage eroded with full depth fissures
Tidemark Integrity		Normal	Focal loss	Diffuse loss	Total loss	NA	NA
		Multiple	Focal loss	Diffuse loss	Total loss	NA	NA

Table 2

Average articular cartilage morphology and mechanics of tibia plateau and femoral condyles (mean with standard deviation)

	Femur		Tibia	
	Control	ACLF	Control	ACLF
Morphology score	1.43 (0.444)	2.32 (1.07)*	1.43 (.663)	1.96 (0.989)*
t (mm)	0.47 (0.16)	0.53 (0.14)	0.63 (0.27)	0.75 (0.31)*
Em (MPa)	1.21 (0.38)	0.97 (0.51)*	1.09 (0.81)	0.87 (0.44)
Ef (MPa)	9.35 (7.25)	13.5 (16.1)	8.30 (10.5)	11.3 (16.2)
k₀ (10⁻¹⁴ m⁴/Ns)	9.68 (6.89)	17.20 (14.36)*	19.9 (18.6)	26.7 (16.4)*

* denotes significant difference of $p < 0.05$ between control and ACLF.

Table 3

Meniscal Morphological Scores at 12 Weeks (mean with standard deviation)

Rabbit	Lateral			Medial		
	A ⁺	C ⁺	P ⁺	A [*]	C [*]	P [*]
1	0 (0)	0 (0)	2.3 (0.8)	1.3 (0.4)	3.0 (0)	3.5 (0.5)
2	0.3 (0.4)	0.3 (0.4)	0.3 (0.4)	2.3 (0.4)	3.0 (0)	3.5 (0.5)
3	0 (0)	0.3 (0.4)	0 (0)	0.3 (0.4)	3.5 (0.5)	0.3 (0.4)
4	2.0 (0)	2.8 (0.4)	3.0 (0)	1.5 (0.5)	3.0 (0)	3.3 (0.4)
5	0.5 (0.5)	3.0 (0)	3.0 (0.7)	1.0 (1.2)	3.3 (0.4)	3.0 (0.7)
6	1.8 (0.4)	3.8 (0.4)	3.8 (0.4)	1.0 (0)	2.8 (0.4)	3.5 (0.5)

* denotes p<0.05 and

+ denotes p<0.1 between control and ACLF.

All control menisci where normal.

Table 4

Average Mankin score for the measures of interest (mean and standard deviation)

	Femur						Tibia					
	Control			ACLF			Control			ACLF		
	Lateral	Medial		Lateral	Medial		Lateral	Medial		Lateral	Medial	
GAG Staining	3.71 (1.76)	1.67 (0.58)		4.46 (1.30)	4.88 (1.30)*		1.71 (0.43)	1.96 (0.71)		2.63 (1.35)	3.58 (1.49)	
Fissures	2.38 (0.56)	1.58 (1.44)		3.04 (2.08)	4.79 (1.11)*		1.54 (2.02)	2.13 (1.49)		1.25 (1.12)	2.71 (2.05)	
Tidemark Integrity	1.67 (1.51)	0.63 (0.80)		2.46 (0.99)	2.79 (1.48)+		1.04 (0.91)	0.71 (0.70)		1.29 (1.03)	1.88 (1.47)+	

* denotes p<0.05 and

+ denotes p<0.1 between control and ACLF.