Experimental animal models of post-traumatic osteoarthritis of the knee

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Abstract

Due to the complex and dynamic nature of osteoarthritis (OA) and post-traumatic osteoarthritis (PTOA), animal models have been used to investigate the progression and pathogenesis of the disease. Researchers have used different experimental models to study OA and PTOA. With an emphasis on the knee joint, this review will compare and contrast the existing body of knowledge from anterior cruciate ligament transection models, meniscectomy models, combination models, as well as impact models in large animals to see how tissues respond to these different approaches to induce experimental OA and PTOA. The tissues discussed will include articular cartilage and the meniscus, with a focus on morphological, mechanical and histological assessments. The goal of this review is to demonstrate the progressive nature of OA by indicating the strong correlation between progressive tissue degeneration, change of mechanical properties, and loss of biochemical integrity and to highlight key differences between the most commonly used experimental animal models.

Introduction

Osteoarthritis (OA) is a joint disease that affects approximately 26.9 million Americans, with the knee being one of the most commonly affected joints.[1,2] OA can be classified as primary OA, which affects older patients due to the natural degradation of joints;[3,4] or as secondary OA, which is non-age related and affects patients who have suffered a traumatic injury to the joint. Secondary OA, or post-traumatic osteoarthritis (PTOA), accounts for 12% of all patients exhibiting OA and is associated with a financial burden projected at $3.06 billion annually.[5] The traumatic injury leading to PTOA is often caused by participation in sports or recreational activities, where a loading event causes injury to the soft tissues in the knee joint.[5,6]

Due to the complex and dynamic nature of OA and PTOA, it is difficult to study the etiological and progressive aspects in humans, as only end-stage tissue is readily available and in vivo investigation is limited to imaging modalities. Therefore, translational animal models are powerful tools that enable a more detailed investigation into the origins and progression of the disease. Animal models provide an opportunity to conduct a greater number of investigations quickly and thoroughly while providing the ability to divorce the natural effects of aging from the study population. Because of the differences between OA and PTOA, different animal models are typically employed. Induction of OA in animal models most often involves the inactivation of an existing gene by replacing or disrupting it in knock-out mice models.[7–9] These models assist in studying the role that specific genes play in the onset, progression, or prevention of OA. Conversely, induction of PTOA in animal models most often involves mechanical disturbances to various tissues, induced through open joint surgery, impact models, or some combination. The most common open joint surgery models include the anterior cruciate ligament transection model (ACLT) and the meniscectomy model. Impact models typically deliver a blunt impact to the knee joint. The goal of all of these models is to cause disturbances that alter joint stability and mechanics, triggering degenerative changes to the joint tissue.

Although OA is being recognized more frequently as a whole-joint disease, this review will focus on comparing the multiple models of injury focusing on two of the main tissues affected by PTOA: articular cartilage and meniscus. Previous results from animal models will be summarized with a focus placed on the morphological, mechanical, and biochemical changes noted across the articular cartilage and menisci. Murine models are highly useful for studying the pathophysiology of OA, and a previously published review of various mice models has outlined both the advantages and disadvantages of these models.[10] For this reason; in addition to their small size, thin cartilage, bone volume fraction and calcified meniscus, no rodent models will be presented within this review.[11–13] To highlight the progression of the disease, this review will summarize the studies within four different time frames: (1) early time points between 0-3 weeks, (2) mid-early time points between 4-7 weeks, (3) mid-late time points between 8-11 weeks and (4) late time points from 12 weeks and further.

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Our aim is to summarize the strong relationship between morphology, biomechanics, and biochemical integrity in the progression of PTOA and encourage future investigations to evaluate the knee joint tissues as comprehensively as possible.

Methods

PubMed and Google Scholar were searched for suitable articles published until December 31, 2019. Keywords used to find articles include: osteoarthritis (OA), post-traumatic osteoarthritis (PTOA), anterior cruciate ligament transection (ACLT), meniscectomy, impact model, animal models, articular cartilage, and meniscus. Of the numerous studies found, 41 matched the criteria set for this review.

Tissues

Articular Cartilage

Articular cartilage (AC) covers the ends of long bones and its primary function is to act as a smooth, lubricated lining between joint surfaces to facilitate the transmission of load. Chondrocytes are responsible for creating the dense [extracellular matrix (ECM)] found within AC; which is composed of water, collagen, proteoglycans, and other non-collagenous proteins.[14] Through negative electrostatic repulsion forces, proteoglycan molecules help retain water within the ECM, ultimately giving articular cartilage its unique response to tensile, compressive and shear forces.[15] AC is largely avascular, with a limited ability to heal and repair itself; therefore, both macroscopic damage and biochemical alteration of the tissue constituents have adverse effects on its integrity. Macroscopic damage can trigger many pathways of biochemical responses that serve as initiators for tissue disruption and functional loss.

Meniscus

The menisci are fibrocartilaginous crescent-shaped structures found in the knee joint. Similar to articular cartilage, the chondrocytes found in the menisci produce an ECM that retains water and gives the tissue its mechanical properties.[15] Menisci increase the contact area between the femur and tibia and enhance load transmission through the joint.[16] They also aid in maintaining joint congruity and stability and help protect the underlying articular cartilage. Therefore, meniscal damage alters load transmission through the joint and can lead to expedited degradation of the underlying cartilage and subsequent degradative effects to subchondral bone.

Models

Anterior Cruciate Ligament Transection (ACLT)

The transection of the anterior cruciate ligament (ACL) in the ACLT model eliminates restraint to anterior translation and consequently provokes altered joint kinematics and mechanical loading patterns leading to accelerated tissue degradation. A brief overview of the referenced articles that employed the ACLT model can be found in Table 1.

Articular Cartilage

Only morphological changes in AC were reported at time points between 0-3 weeks. Two separate studies reported moderate fibrillation, with higher frequency in the medial compartment of the knee.[17,18] One of the studies also reported a higher rate of hypertrophy at this time point compared to later time points and the control knee.[18] At time points between 4-7 weeks, studies reported mild surface fibrillations,[17,19–21] with a low percentage of mid to full-thickness tears as well as more damage to the femoral condyles than corresponding regions on the tibial plateau,[20,21] specifically in the medial compartment.[22,23] One study reported a higher rate of fibrillation on the medial side of the tibial plateau.[24] These findings matched mechanical property changes reported by two studies.[25,26] Mechanical testing of AC by Setton *et al* found that there was a significant decrease in the aggregate and shear modulus with an increase in the permeability,[25] and in a follow-up study they reported a decrease in the compressive and shear modulus.[26] Another study reported a decrease in the elastic modulus of the AC in both hemijoints but found no significant difference in the dynamic modulus.[23] Both the morphological and mechanical assessments made at this time frame matched biochemical changes reported. A greater depletion of glycosaminoglycan (GAG) content was found in the medial compartment of the knee, matching previous assessments.[19,20] One study reported equal loss of GAG cov-

Table 1. List of referenced studies that utilized the ACLT model.

Reference	Animal Model	Tissue	Assessments Recorded
Adams 1983	Canine	AC	Gross morphology
Matyas 2004	Canine	AC	Gross morphology
Yoshioka 1996	Lapine	AC	Gross morphology, hematoxylin/eosin, and Safranin-O Fast Green
Saito 2012	Lapine	AC	Gross morphology, hematoxylin/eosin, and Safranin-O Fast Green stain
Batiste 2004	Lapine	AC	MRI grading scale
Kaderli 2005	Lapine	AC	OARSI histopathology grading, Safranin-O Fast Green stain
Florea 2015	Lapine	AC	Equilibrium modulus, dynamic modulus, Safranin-O stain
Wachsmuth 2003	Lapine	AC	Gross morphology, hematoxylin/eosin, and Safranin-O Fast Green stain
Setton 1994	Canine	AC	Permeability, aggregate modulus, shear modulus, Safranin-O, and Masson trichrome stain
Setton 1995	Canine	AC	Equilibrium shear modulus, dynamic shear modulus, compressive modulus
Turumen 2013	Lapine	AC	Safranin-O Stain
Tochigi 2011	Lapine	AC	Mankin score
Sniekers 2008	Canine	AC	Gross morphology, Safranin-O Fast Green iron hematoxylin stain, alcian blue stain
Sah 1997	Lapine	AC	India ink, compression modulus, permeability, proteoglycan dry weight
Guilak 1994	Canine	AC	Gross morphology, tensile modulus, 1,9-dimethylmethylene blue dye stain
Adams 1983	Canine	Meniscus	India ink, glycosaminoglycan dry weight
Sandy 1984	Canine	Meniscus	Gross morphology, S-sulfate isotope labeling
Le Graverand 2001	Lapine	Meniscus	Gross morphology, hematoxylin/eosin, and Safranin-O Fast Green stain
Matyas 2004	Canine	Meniscus	Gross morphology
Levillian 2015	Lapine	Meniscus	Reduced modulus, Safranin-O Fast Green stain
Sonoda 2000	Lapine	Meniscus	Gross morphology, S-sulfate isotope labeling
Wachsmuth 2003	Lapine	Meniscus	Gross morphology
Killian 2010	Lapine	Meniscus	Gross morphology, Safranin-O Fast Green stain

erage in the femoral condyles[23] however, another study reported no significant GAG loss in the femoral condyles at this time frame.[22] Turunen *et al* were the only group to examine the AC harvested from the patella following ACLT and reported a decrease in GAG content.[27]

Studies reported that as the severity of OA increased, surface fibrillation became more severe and full depth tears more numerous between weeks 8-11.[17,20,21] Greater damage was reported in the medial compartments of both the femur[28] and tibia,[21,24] while one study reported equal damage in both hemijoints of the femoral condyles and tibial plateau.[29] Sah *et al* were the only group to conduct mechanical testing on the AC and reported a significant decrease in the compressive moduli but found no significant difference in the permeability.[30] Similar to earlier time points, both morphological and mechanical changes matched biochemical changes.[30] Depletion of GAG was reported to be greater in the medial compartment of the femoral condyles[19] as well as both hemijoints of the tibial plateau.[24,29] At 12 weeks and later time points, a majority of studies reported further degeneration and erosion of the AC, when compared to earlier time points and control limbs.[18,28,29] One study did report a decrease in surface fibrillation, despite an increase in severity of other morphological parameters.[19] Both studies by Setton found a significant decrease in the mechanical properties of the tissue compared to the control, but no significant difference when compared to an earlier time point.[25,26] Guilak *et al* reported a significant decrease in the tensile modulus.[31] Further decrease in GAG content was also reported at this time point in both the medial femoral condyle[20] and both hemijoints of the tibial plateau.[29] The results from the different studies on AC following ACLT indicate that there is a strong correlation between changes in the morphology, mechanical properties, and GAG content of the tissue as PTOA progressed in the knee joint. As the progression of fissuring and fibrillation occurred in the tissue across different time points, GAG content gradually depleted resulting in a significant decrease in the mechanical properties of the tissue over time. It can also be noted that significant changes to the medial hemijoint of both the femoral condyle and tibial plateaue were consistently observed across all times, when compared to the lateral hemijoint.

Meniscus

At early time points between 0-3 weeks, fibrillation was observed on the superior surface of the lateral meniscus[17,32,33] while fibrillation and both longitudinal and bucket handle tears were observed on the medial meniscus.[18,33] The morphological changes matched studies looking at the GAG content, which reported a reduction in dry weight[17] as well as less intense staining in the inner portions of the meniscus near the sites of fissures.[33] However, one study did report an increase in GAG synthesis in the medial meniscus.[32] To the author's knowledge, no study performed mechanical testing on meniscal tissue post ACLT at this time frame.

An increase in severity of tears on the medial meniscus were reported[33] as well as damage to the lateral meniscus[32] between weeks 4-7. Levillain *et al* were the only group to conduct mechanical testing on meniscal tissue post ACLT. They found that the modulus decreased by half in menisci from the operated knee.[34] The changes in GAG coverage at this time point were inconsistent with one study reporting a reduction in coverage[33] while two studies reported an increase in staining when compared to control tissue.[32,34]

Between 8-11 weeks, more damage was observed in the medial meniscus when compared to the lateral meniscus.[24,33,35] Fibrillation, swelling, splitting, and tearing of medial meniscus predominantly in the posterior region[35] as well as the progression of bucket tears to full tears were reported.[24,33] During this time frame, only superficial damage to the lateral meniscus was observed.[33] These changes matched the decrease in GAG content reported in the tissue. GAG coverage continued to diminish near the site of tears, with staining reported to be sporadic in both location and intensity.[33]

At later time points of 12 weeks and on, displaced and bucket handle tears were reported in both menisci,[32,36] with one study reporting a higher rate of occurrence in the medial meniscus.[18] Similar to a six week time point, Sandy *et al* reported damage to the lateral menisci as well as an increase in GAG synthesis similar to those found at a six week time point.(32) However, Le Graverand *et al* reported a significant decrease in GAG content, specifically near the sites of fissures.[33]

Even though the only study to conduct mechanical testing on meniscal tissue after ACLT reported a decrease in mechanical properties and an increase in GAG coverage, the majority of studies on meniscal tissue imply that there is a strong correlation in gross morphological changes and GAG content. It is important to note that damage to the medial menisci was reported at earlier time points and progressed over time, at later time points, equal damage to both lateral and medial menisci were reported. Despite one study reporting an increase in GAG synthesis at two different time points, as the size and number of tears in the menisci increased with time post-surgery, there was a decrease in GAG content, specifically in the areas surrounding the tears. Table 1 summarizes the animal model used, the tissues of focus, and the parameters evaluated by the study used in this review.

Meniscectomy

The meniscectomy model involves removal of portions or the entirety of the meniscus, limiting its ability to distribute load and increasing pressure on the underlying articular cartilage. Meniscectomy models are less frequently used compared to ACLT models as they expose the underlying articular cartilage to more contact and generally a more rapid onset of OA symptoms. A brief overview of the referenced articles that employed the meniscectomy model can be found in Table 2.

Table 2. List of referenced studies that utilized the meniscectomy model. Table 2 summarizes the animal model used, the tissues of focus, and the parameters evaluated by the study used in this review.

Reference	Animal Model	Tissue	Assessments Recorded
Cruz 2015	Porcine	AC	Gross morphology, OARSI score, hematoxylin/eosin, and Safranin-O stain
Lindhorst 2005	Lapine	AC	Gross morphology, hematoxylin/eosin, and Safranin-O stain
Wachsmuth 2003	Lapine	AC	Gross morphology, hematoxylin/eosin, and Safranin-O stain
Arunakul 2013	Lapine	AC	Gross morphology, Mankin score
LeRoux 2000	Canine	AC	Gross morphology, equilibrium moduli, shear moduli, dynamic shear modulus, Safranin-O stain

Articular Cartilage

In early time points between 0-3 weeks, studies reported surface fibrillation and lesions,[37] with two studies reporting greater damage in the medial hemijoint.[24,38] Gross morphological changes observed by each study matched a reported decrease in GAG coverage. Both Wachsmuth *et al* and Lindhorst *et al* reported a greater significant decrease in GAG coverage in the medial hemijoint,[24,38] while Cruz *et al* reported a significant decrease in both the femoral condyles and tibial plateau.[37]

Between 4-7 weeks, only one study investigated the morphological and biochemical integrity of the tissue. Lindhorst et al. reported further fibrillation of the tissue as well as focal erosions in the medial hemijoint. These morphological changes matched their histological findings, where they reported a significant decrease in the GAG content in the medial hemijoint.[38]

Further degeneration of the tissue was found in the medial hemijoint, between weeks 8-11.[24,38,39] A progression of surface fibrillation, focal erosions, and focal lesions were reported.[24,38] Biochemical changes matched morphological changes, with greater GAG loss reported in the medial hemijoint as well.[24,38]

At a 12-week time point, the progression of surface fibrillation and focal erosions were observed.[38] LeRoux *et al* were the only group to conduct both mechanical and histological assessments at this time point. They reported a significant decrease in both the equilibrium and shear modulus along with a significant decrease in GAG concentrations in the articular cartilage previously covered by the meniscus.[40] These changes matched their reported observations on the morphological changes in the tissue, greater damage was observed in the previously covered articular cartilage.[40]

Although there is a lack of studies that documented changes in the mechanical and biochemical integrity of the tissue across different time points, relationships between morphological, mechanical and histological can be drawn. At 12-week time point, a significant decrease in GAG content, as well as the equilibrium and shear modulus, is consistent with the visual progression of surface fibrillation and focal erosions in the medial hemijoint of the knee joint. Morphological, mechanical, and histological changes in the tissue were observed across time points, demonstrating the dependency between the three outcome measures and the overall health of the tissue. Due to the partial or full removal of the medial meniscus in this surgical procedure, there were more significant changes reported in articular cartilage in the medial hemijoint of the knee across all time points, when compared to the lateral hemijoint.

Meniscus

Due to the nature of the meniscectomy model, there was inadequate data for meniscal changes in this model. To the author's knowledge, no studies performed morphological, mechanical or histological tests on any remaining meniscal tissue.

Combination

The combination models in this review refer to models that implemented damage to two or more tissues, most often combining an ACLT and meniscal injury. A brief overview of the referenced articles that employed a combination model can be found in Table 3.

Articular Cartilage

Hulth et al. reported damage in the superficial layer of the AC in both the tibia and femur after transection of the ACL, posterior cruciate ligament (PCL), medial collateral ligament (MCL), lateral collateral ligament (LCL) and a full meniscectomy of the medial meniscus, at an early time point.[41] To the author's knowledge, no studies investigated mechanical or histological changes in the AC during this time frame. Between 4-7 weeks, Fischenich et al. reported equal damage across both hemijoints post-surgery in an ACLT and meniscectomy combination model. These changes were accompanied by a decrease in the matrix modulus in the lateral tibial articular cartilage and a significant increase in the permeability of the tissue in the lateral hemijoint. No significant differences in GAG content were reported at this time frame.[42]

Damage to both hemijoints continued to progress in the tissue, between weeks 8-11. Similar to earlier time points, the reported damage was also accompanied by a decrease in the matrix modulus in the lateral tibial articular cartilage and a significant increase in the permeability of the tissue in the lateral hemijoint. No significant differences in GAG content were reported at this time frame.[42] At later time points of 12 weeks and longer, the progression of damage continued in the knee joint.[42] Two studies reported greater damage in the medial tibial plateau when compared to the femoral condyles.[43,44] Intema *et al* reported an increase in fissuring in the lateral femoral condyle.[43] Similar to earlier time points, Fischenich *et al* reported equal progression of damage in both hemijoints along with cartilage fibrillation, erosion, and subchondral bone exposure.[42] Morphological changes were matched by the continued decrease in the fiber modulus of the cartilage found in the lateral femoral condyle as well as a significant decrease in GAG coverage in the tissue in both hemijoints. A further increase in permeability was also reported at this time point.[42] Due to the different methodologies employed to create these combination models, it is difficult to draw major conclusions between the different studies and the reported assessments. However, focusing on the study by Fischenich *et al*, it can be noted that at a 12 week time point the relationship between biochemical integrity and mechanical prop-

Table 3. List of referenced studies that utilized a combination model. Table 3 summarizes the animal model used, the transected or removed tissues, the tissues of focus, and the parameters evaluated by the study used in this review.

Reference	Animal Model	Transection/Removal	Tissue	Assessments Recorded
Hulth 1970	Lapine	ACL, PCL, MCL, LCL, meniscus	AC	Mayer's hematoxylin stain
Fischenich 2016	Lapine	ACL, meniscus	AC	Gross morphology, permeability, matrix modulus, fiber modulus, hematoxylin, and Safranin-O Fast Green stain
Intema 2010	Canine	ACL, meniscus	AC	Safranin-O Fast Green and iron hematoxylin stain, alcian blue stain
Beveridge 2013	Ovine	ACL, meniscus	AC	Gross morphology
Fischenich 2016	Lapine	ACL, meniscus	Meniscus	Gross morphology, permeability, equilibrium modulus, instantaneous modulus, hematoxylin, and Safranin-O Fast Green stain
Fischenich 2014	Lapine	ACL, meniscus	Meniscus	Gross morphology, instantaneous hematoxylin, and Safranin-O Fast Green stain

erty degradation can be made, specifically in the femoral lateral hemijoint. As fissuring increased in this region, there was a significant decrease in GAG coverage which ultimately led to a decrease in the fiber modulus and an increase in permeability.

Meniscus

To the author's knowledge, no studies that employed a combination model investigated meniscal tissue at time points earlier than 4 weeks. Fischenich *et al* reported equal damage to both menisci on the operated knee four weeks following the surgical procedure. The damage matched mechanical testing, which showed a decrease in the equilibrium and instantaneous moduli of both menisci, with a higher frequency in the medial meniscus. No significant difference in GAG content was found at this time point.[42]

At a later time point of 8 weeks, the same study reported that the damage progressed in the tissue and was equal in both menisci. The mechanical properties, equilibrium, and instantaneous moduli continued to decrease and were matched with a significant decrease in GAG content in both menisci.[42]

In later time points, one study reported equal damage to both menisci,[42] while another study reported tissue maceration in the central and posterior regions of the lateral meniscus as well as the central region of the medial meniscus.[45] Similar to earlier time points, there was a significant decrease in the equilibrium and instantaneous moduli of both menisci.[42] Both the morphological and mechanical changes were accompanied by a decrease in GAG coverage in both menisci.[42,45]

Although there was no association between changes in morphology, material properties and GAG coverage at a 4 week time point, there was an association at 8 and 12 weeks post-surgery. As GAG coverage decreased over time in both menisci, there was an associated decrease in the equilibrium and instantaneous modulus at both time points, with greater damage being reported as well.

Impact

In recent years, impact models have become a more common practice to study the origins and progression of PTOA. Impact models typically deliver a blunt impact to either the patellofemoral (PF) or tibiofemoral (TF) joints. It is important to note, however, that there are two ways the traumatic impact is delivered to the joint. One version of the impact model delivers the impact to the joint while the knee capsule is surgically exposed and the other delivers the impact while the joint is closed. In this review, Fischenich *et al* were the only group that employed the closed-joint impact model. A brief overview of the referenced articles that employed the impact model can be found in Table 4.

Articular Cartilage

Studies reported surface damage along with fissuring and fibrillation on femoral,[46] tibial,[47] and patellar cartilage[48,49] at time points between 0-3 weeks. A study by Milentijevic *et al* found that an hour post-impact, articular cartilage on the patella was rougher and contained a higher number of deep fissures when compared to AC harvested at a 3-week time point.[50] Most of the studies reported that the morphological changes in the AC were accompanied by a decrease in GAG coverage;[46,48,50] however, Donohue *et al* found an increase in GAG content in calcified cartilage of the patella.[51] Borrelli *et al* reported that the total creep strain had a significant increase immediately following impact.[52]

At time points between 4-7 weeks, one

Table 4. List of referenced studies that utilized the impact model. Table 4 summarizes the animal model used, the tissues of focus, and the parameters evaluated by the study used in this review.

Reference	Animal Model	Tissue	Assessments Recorded
Oegema 1993	Canine	AC	Gross morphology, Safranin-O Fast Green stain
Radin 1984	Lapine	AC	Gross morphology
Thompson 1991	Canine	AC	Gross morphology, Safranin-O Fast Green stain
Newberry 1997	Lapine	AC	Gross morphology, instantaneous stiffness, Safranin-O Fast Green stain
Milentijevic 2005	Lapine	AC	Gross morphology, Safranin-O stain
Donohue 1983	Canine	AC	Gross morphology, Safranin-O stain, proteoglycan dry weight
Borreli 2010	Lapine	AC	Creep Strain
Ewers 2000	Lapine	AC	Gross morphology, instantaneous shear modulus, relaxed modulus, Safranin-O Fast Green stain
Ewers 2000	Lapine	AC	Gross morphology, instantaneous modulus, relaxed modulus
Fischenich 2016	Lapine	AC	Gross morphology, permeability, matrix modulus, fiber modulus, hematoxylin and Safranin-O Fast Green stain
Borrelli 2010	Lapine	AC	Hematoxylin and eosin stain
Fischenich 2015	Lapine	AC	Gross morphology, modified Mankin score, permeability, matrix modulus, fiber modulus, hematoxylin, and Safranin-O Fast Green stain
Ewers 2002	Lapine	AC	Gross morphology, thickness direction modulus, in-plane modulus, shear modulus, Safranin-O Fast Green stain
Isaac 2010	Lapine	AC	Matrix modulus, fiber modulus, permeability, Safranin-O Fast Green stain
Fischenich 2016	Lapine	Meniscus	Gross morphology, permeability, equilibrium modulus, instantaneous modulus, hematoxylin, and Safranin-O Fast Green stain
Fischenich 2015	Lapine	Meniscus	Gross morphology, modified Mankin score, permeability, instantaneous modulus, equilibrium modulus, hematoxylin, and Safranin-O Fast Green stain
Fischenich 2013	Lapine	Meniscus	MRI, gross morphology, instantaneous modulus, equilibrium modulus
Killian 2010	Lapine	Meniscus	Gross morphology, Safranin-O Fast Green stain

study reported a loss of sheen and intracartilaginous cysts in the AC harvested from the tibial plateau,[47] while another study reported more damage in the medial hemijoint.[42] Similar to a 3 week time point, Oegema *et al* reported no significant differences in the morphology of the tissue when compared to controls, however, they did report a decrease in GAG coverage in calcified cartilage.[46] Mechanical property tests by Fischenich *et al* reported a significant increase in the permeability, which was matched with a decrease in GAG coverage.[42]

Fischenich *et al* were the only group to conduct testing on the AC following a TF impact, between 8-11 weeks. An increase in surface fissuring was reported which was accompanied by a decrease in GAG coverage close to the sites of the fissures, in both hemijoints. Similar to an earlier time point, the permeability was found to significantly decrease when compared to controls.[42]

At a 12 week and later time point, fissures were observed on the patella[48,49,53,54] and the femoral condyles.[42,55] One study reported more significant damage to the femoral condyles when compared to the tibial plateau,[42] while another study reported greater damage to the tibial plateau, including cartilage erosion.[56] Multiple studies conducted mechanical testing at this time frame. In two separate studies, Ewers *et al* reported a decrease in the instantaneous and relaxed modulus[53] as well as in the in-plane and thickness direction modulus.[57] Fischenich *et al* reported a decrease in the matrix modulus[56] but found no significant difference in the fiber modulus at this or earlier time frame and in a follow up study reported an increase in the permeability.[42] An increase in creep strain, which led to longer creep recovery times[52] and a decrease in the instantaneous stiffness, was also reported.[49] Decreasing mechanical properties were accompanied by a decrease in GAG coverage. Studies reported a decrease in coverage on the femoral condyles,[42,56] the tibial plateau,[58] and the areas surrounding clefts.[48] In a long term study, Oegema *et al* reported decreasing loss of GAG coverage at 3 and 6 months, but a restoration of GAG coverage to normal levels 1-year post-impact.[46]

Table 5. Summary of key changes reported in the articular cartilage across the four different animal models over time.

Time Point	ACLT	Meniscectomy	Combination	Impact
Early	Higher occurrence of degradation in medial hemijoint	Greater damage observed in medial hemijoint accompanied by a decrease in GAG coverage	Damage to superficial layer of tissue in both hemijoints	Surface damage observed in tissue along with a decrease in GAG coverage and mechanical properties
Mid-Early	Significant morphological, mechanical and histological changes in medial hemijoint	Degeneration of tissue integrity in medial hemijoint	Equal damage to both hemijoints, significant decrease in mechanical properties in lateral hemijoint medial hemijoint	Increase in tissue permeability and decrease in GAG content
Mid-Late	Continued degradation of tissue integrity, mechanical properties and GAG content in medial hemijoint	Continued damage and loss of GAG in medial hemijoint	Continued tissue degeneration across both hemijoints and decrease in mechanical properties in lateral hemijoint	Damage to tissue accompanied by an increase in permeability and loss of GAG coverage
Late	Further degradation of all three parameters in medial hemijoint. Significant morphological changes observed in lateral hemijoint	Damage observed across both hemijoints, with greater loss of tissue integrity from tissue previously covered by the meniscus.	Further degeneration across both hemijoints, decrease in mechanical properties in lateral hemijoint and loss of GAG coverage in both hemijoints	Further tissue degradation accompanied by decrease in modulus and an increase in permeability as well as loss of GAG content

Table 6. Summary of key changes reported in the menisci across the four different animal models over time.

Time Point	ACLT	Meniscectomy	Combination	Impact
Early	Fibrillation observed in both hemjoints, with tears being more common in the medial meniscus. Decrease in GAG	No data	No data	No data
Mid-Early	Greater changes in morphology in medial meniscus and significant changes in mechanical properties	No data	Damage to both menisci accompanied by a decrease in mechanical properties	Damage and complex tears to both menisci
Mid-Late	Progression of tears in medial meniscus, more commonly in posterior region, and decrease in GAG staining	No data	Damage progressed in both menisci accompanied by decrease in mechanical properties and GAG content	Damage and complex tears to both menisci
Late	Severe tears in both menisci	No data	Further damage to both menisci and decrease in mechanical properties and GAG content	Damage to both menisci accompanied by a decrease in mechanical properties and GAG coverage

Across different time points, it can be noted that the morphological, mechanical and histological changes are correlated to one another. Through these studies, it can also be observed that a decrease in GAG content was strongly associated with an increase in the permeability of articular cartilage. This is further supported by a study where it was found that the arrangement of the collagen fiber network and GAG chains played an important role in directing fluid flow to optimize tissue function.[59] With a decrease in the number of GAG chains, the fluid is able to move more freely throughout the tissue, thus increasing the permeability found.

Meniscus

To the authors' knowledge, no studies documented the integrity of meniscal tissue after impact via morphological, mechanical or histological testing between 0 and 3 weeks. At 4 and 8 week time points, Fischenich *et al* reported damage to the menisci with complex longitudinal tears being the most common. No significant changes in the mechanical properties or biochemical integrity of the tissue were reported at these time points.[42]

At a 12-week time point, damage was observed in both menisci, with one study reporting more severe damage and tearing in the medial meniscus[60] while another study reported tears in the lateral meniscus.[36] Both the equilibrium and instantaneous modulus significantly decreased in both menisci,[42,60] which was matched with a decrease in GAG coverage in all regions of the menisci;[42,56] however, Killian *et al* reported inconsistent changes in coverage in the central region of the lateral meniscus.[36]

Although no significant changes were found in the mechanical and biochemical integrity of the tissue at early time points, there were changes at the 12-week time point. As the progression of morphological changes occurred in the tissue, there was both a decrease in the mechanical properties and a decrease in GAG coverage.

Additional Considerations

All of the models discussed in this review have proven to be effective in generating chronic joint changes consistent with PTOA. The ACLT model, along with other surgically invasive models, have been used for many years to alter joint stability and mechanics initiating degenerative changes to the joint tissues in an effort to study the onset and progression of the disease. However, there are limitations to these models that should be taken into consideration before implementation. Surgical models fail to address occult damage to other structures including; cartilage, meniscus, subchondral, and trabecular bone. For example, in the acute clinical setting, approximately 50% of patients with ACL tears have associated meniscal injuries[61] and 80% of ACL tears have associated bone bruising.[62] By simply surgically transecting the ACL, the impact-induced bone bruises that are seen clinically are not replicated in the model. Failing to address the damage experienced in other knee tissues could result in skewed pathological findings. It is also likely that these surgical models influence synovial swelling, up-regulation of inflammation, and pain[63] all of which can affect study findings.

Both open and closed-joint impact models are able to elicit damage to the ACL and other knee tissues in the joint, making it a more comparable model to what is observed in humans in the clinical setting. The impact models performed by the studies included in this review are typically faster and easier to perform than the invasive surgical models, which often require specialized veterinarians and tools to consistently produce the desired injury. It is important to highlight that the closed-joint impact models have the benefit of reducing compounding effects from opening the knee joint. There are, however, also limitations to the impact model. Differences in equipment, varying animal size, and animal position makes the impact model more variable than a controlled surgical transection. Similar to surgical expertise and training, the elaborate systems used in the impact model must first be rigorously tested to ensure the same injury is observed in all of the joints subjected to the impact. Guaranteed damage to a tissue of interest in the surgical model is an advantage over the impact model.

As previously mentioned, most ACL tears and traumatic loading to the human knee are accompanied by meniscal damage. This review found that numerous studies investigated the effects these models had on articular cartilage, while very few studies documented the effects on meniscal tissue and even fewer documented both tissues. Of the few studies that investigated the onset and progression of PTOA in both articular cartilage and the meniscus, it is apparent that the pathology should be considered as a whole joint disease, where disturbances and responses from various tissues elicit and propagate degenerative responses in the surrounding tissues. It would also be of great value for studies to assess cartilage from both the femoral condyles and the tibial plateau to note the progression of the disease in these tissues as a function of location. Previous studies have tried to correlate some combination of morphological damage, changes in mechanical properties, and biochemical integrity. This review showed that there is a relationship between these outcome measures, especially in the studies where all three measures were assessed. Therefore, future studies on the progression of PTOA in knee joint tissues should consider evaluating morphological, mechanical, and histological changes in numerous tissues to truly encapsulate the progression of the disease in the knee joint.

Conclusions

Animal models provide invaluable insight into understanding the origin and progression of PTOA. Based on the studies used in this review, the pathology of the disease should be considered as a whole joint system. Furthermore, from this review is it evident that damage patterns for all tissues are somewhat injury mechanism dependent (Tables 5 and 6). Consequently, the various PTOA models investigated in this review may be more or less advantageous for exploring and explaining certain damage patterns and progressions. The onset of PTOA may occur through different mechanisms dependent on initial trauma experienced by the knee joint. Proper consideration of this is key to ensuring the applicability of the data collected and the translation of the information back to the human aspect of the disease. Although this review has found that these models are successful in simulating knee joint damage, the model best suited to be used in a study ultimately lies in the research question, the logistics of the model, and the resources available.

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