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.3 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, translation- al 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.7,8 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.9 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.10–12

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.

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.

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.
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. 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. 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. Menisci increase the contact area between the femur and tibia and enhance load transmission through the joint. 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 degenerative effects to subchondral bone.

Models

Anterior Cruciate Ligament Transection (ACLT)

The transection of the anterior cruciate ligament (ACLT) 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.

One of the studies also reported a higher rate of hypertonpsy at this time point compared to later time points and the control knee. At time points between 4-7 weeks, studies reported mild surface fibrillations, 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, specifically in the medial compartment. One study reported a higher rate of fibrillation on the medial side of the tibial plateau. These findings matched mechanical property changes reported by two studies. 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, and in a follow-up study they reported a decrease in the compressive and shear modulus. Another study reported a decrease in the elastic modulus of the AC in both hemijoints but found no significant difference in the dynamic modulus. 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.

One study reported equal loss of GAG co-

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 |
| Baliste 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 |
| Gulak 1994| Canine       | AC     | Gross morphology, tensile modulus, 1,9-dimethylmethylen 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     |
| Kilian 2010| Lapine       | Meniscus| Gross morphology, Safranin-O Fast Green stain |
age in the femoral condyles\textsuperscript{23} however, another study reported no significant GAG loss in the femoral condyles at this time frame.\textsuperscript{22} Turunen \textit{et al} were the only group to examine the AC harvested from the patel-
la following ACLT and reported a decrease in GAG content.\textsuperscript{20}

Studies reported that as the severity of OA increased, surface fibrillation became more severe and full depth tears more numerous between weeks 8-11.\textsuperscript{17,20,21} Greater damage was reported in the medial compartments of both the femur\textsuperscript{24} and tibia,\textsuperscript{21,24} while one study reported equal damage in both hemi-
joints of the femoral condyles and tibial plateau.\textsuperscript{25,26} Sah \textit{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.\textsuperscript{30} Similar to earlier time points, both morphological and mechanical changes matched biochemical changes.\textsuperscript{30} Depletion of GAG was reported to be greater in the medial compartment of the femoral condyles\textsuperscript{24} as well as both hemi-
joints of the tibial plateau.\textsuperscript{24,25} 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.\textsuperscript{18,28,29} One study did report a decrease in surface fibrillation, despite an increase in severity of other mor-
phological parameters.\textsuperscript{19} Both studies by Setton found a significant decrease in the mechanical properties of the tissue com-
pared to the control, but no significant differ-
ence when compared to an earlier time point.\textsuperscript{25,26} Guilak \textit{et al} reported a significant decrease in the tensile modulus.\textsuperscript{31} Further
decrease in GAG content was also reported at this time point in both the medial femoral
condyle\textsuperscript{24} and both hemi-joints of the tibial plateau.\textsuperscript{24} 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
tissue properties at the medial meniscus are observed in both the femoral condyle and
meniscus of the tibia.\textsuperscript{24,25} These studies by Setton found a significant
decrease in GAG synthesis as well as damage to the medial meniscus.\textsuperscript{31} The
morphological changes matched studies looking at the AC content, which reported a reduction in
dry weight as well as less intense staining in the inner portions of the meniscus near the
time point.\textsuperscript{18,33} However, one study did report an increase in GAG
synthesis in the medial meniscus.\textsuperscript{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\textsuperscript{33} as well as damage to the lateral meniscus\textsuperscript{22} between
weeks 4-7. Levillain \textit{et al} were the only group to conduct mechanical testing on
meniscal tissue post ACLT. They found that the
modulus decreased by half in meniscus from the operated knee.\textsuperscript{34} The changes in
GAG coverage at this time point were inconsistent with one study reporting a
reduction in coverage\textsuperscript{35} while two studies reported an increase in staining when com-
pared to control tissue.\textsuperscript{32,34}

Between 8-11 weeks, more damage was
observed in the medial meniscus when compared to the lateral meniscus.\textsuperscript{24,33,35} Fibri-
lillation, swelling, splitting, and tearing of medial meniscus predominantly in the
posterior region\textsuperscript{18} as well as the progression
of bucket tears to full tears were reported.
\textsuperscript{24,34} During this time frame, only superfi-
cial damage to the lateral meniscus was observed.\textsuperscript{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.\textsuperscript{33}

At later time points of 12 weeks and on,
discharged and bucket handle tears were
reported in both menisci,\textsuperscript{32,36} with one study
reporting a higher rate of occurrence in the
medial meniscus.\textsuperscript{34} Similar to a six week
time point, Sandy \textit{et al} reported damage to
the lateral meniscus as well as an increase in
GAG synthesis similar to those found at a
six week time point.\textsuperscript{(32)} However, Le
Graverand \textit{et al} reported a significant
decrease in GAG content, specifically near
the sites of fissures.\textsuperscript{31}

Even though the only study to conduct mechanical testing on meniscal tissue after
ACLCT 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 meniscus was reported at earlier
time points and progressed over time, at
later time points, equal damage to both lat-
eral 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 menis-
cus 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 tis-
ues 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 underly-
ing articular cartilage. Meniscectomy models
are less frequently used compared to
ACLCT models as they expose the underly-
ing articular cartilage to more contact and
generally a more rapid onset of OA symp-
toms. A brief overview of the referenced
articles that employed the meniscectomy
model can be found in Table 2.

| 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 |

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Articular Cartilage

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

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.

Further degeneration of the tissue was found in the medial hemijoint, between weeks 8-11. Biochemical changes matched morphological changes, with greater GAG loss reported in the medial hemijoint as well.

At a 12-week time point, the progression of surface fibrillation and focal erosions were observed. 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. These changes matched their reported observations on the morphological changes in the tissue, greater damage was observed in the previously covered articular cartilage.

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 changes 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 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.

### Table 3. List of referenced studies that utilized a combination model.

| 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 | Oxine       | 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 |

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.

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. At later time points of 12 weeks and longer, the progression of damage continued in the knee joint. Two studies reported greater damage in the medial tibial plateau when compared to the femoral condyles. Intema et al. reported an increase in fissuring in the lateral femoral condyle. 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. 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. 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-
equality degradation can be made, specifically in the femoral lateral hemi-joint. 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,43 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.

In later time points, one study reported equal damage to both menisci,43 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

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### 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 cartilage48,49 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

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**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 |
| Borrelli 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, while another study reported more damage in the medial hemi-joint. 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. Mechanical property tests by Fischenich et al reported a significant increase in the permeability, which was matched with a decrease in GAG coverage.

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 hemi-joints. Similar to an earlier time point, the permeability was found to significantly decrease when compared to controls.

At a 12 week and later time point, fissures were observed on the patella and the femoral condyles. One study reported more significant damage to the femoral condyles when compared to the tibial plateau, while another study reported greater damage to the tibial plateau, including cartilage erosion. 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 as well as in the in-plane and thickness direction modulus. Fischenich et al reported a decrease in the matrix modulus 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. An increase in creep strain, which led to longer creep recovery times and a decrease in the instantaneous stiffness, was also reported. Decreasing mechanical properties were accompanied by a decrease in GAG coverage. Studies reported a decrease in coverage on the femoral condyles, the tibial plateau, and the areas surrounding clefts. 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.

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

| Time Point | ACL/ Meniscectomy | Combination | Impact |
|------------|-------------------|-------------|--------|
| Early      | Higher occurrence of degradation in medial hemi-joint | Greater damage observed in medial hemi-joint accompanied by a decrease in GAG coverage | Damage to superficial layer of tissue in both hemi-joints | 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 hemi-joint | Degeneration of tissue integrity in medial hemi-joint | Equal damage to both hemi-joints, significant decrease in mechanical properties in lateral hemi-joint | Increase in tissue permeability and decrease in GAG content |
| Mid-Late   | Continued degradation of tissue integrity, mechanical properties and GAG content in medial hemi-joint | Continued damage and loss of GAG in medial hemi-joint | Continued tissue degeneration across both hemi-joints and decrease in mechanical properties in lateral hemi-joint | Damage to tissue accompanied by an increase in permeability and loss of GAG coverage |
| Late       | Further degradation of all three parameters in medial hemi-joint. Significant morphological changes observed in lateral hemi-joint | Damage observed across both hemi-joints, with greater loss of tissue integrity from tissue previously covered by the meniscus. | Further degeneration across both hemi-joints, decrease in mechanical properties in lateral hemi-joint and loss of GAG coverage in both hemi-joints | 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 | ACL/ Meniscectomy | Combination | Impact |
|------------|-------------------|-------------|--------|
| Early      | Fibrillation observed in both hemi-joints, with tears being more common in the medial meniscus. Decrease in GAG | 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 |

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 |

Late | Severe tears in both menisci | No data | 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. 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 meniscus46 while another study reported tears in the lateral meniscus.47 Both the equilibrium and instantaneous modulus significantly decreased in both menisci,42,46 which was matched with a decrease in GAG coverage in all regions of the menisci;42,46 however, Killian et al reported inconsistent changes in coverage in the central region of the lateral meniscus.46

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 injuries41 and 80% of ACL tears have associated bone bruising.48 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 pain49 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 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 it is 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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