Mouse Models of Osteoarthritis and Joint Injury

by

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ABSTRACT

Nearly 21 million Americans are affected by osteoarthritis, a complex disease characterized by degenerative lesions to the articular cartilage and subchondral bone in the joints. The complexity of the disease makes the use of human models impractical and complicated. Therefore, various animal models have been developed to study the progression of OA and possible therapeutic techniques. Of those models, mouse models play an integral part because of their cost-effectiveness, favorable logistics, and ability to be genetically manipulated. Three main mouse models were reviewed: (1) genetic deletion, (2) treadmill running, and (3) surgically induced injuries. Several strains of knockout mice have been develop in the past 10 years and they provide a great opportunity to study the evolution of OA. Up until now, treatment for OA has been pain management-related, but the development of more advanced mouse models has laid out the framework for possible OA preventing and repairing techniques.

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I would like to dedicate this thesis to my parents, who have always given me undeniable love and support. Throughout my life, they have always been there to guide me and support me in my dreams. I would also like to thank my siblings, Sergio and Gaby, and my girlfriend, Lisa, who have been present with me every day of my undergraduate career despite the distance. Thank you also to my extended family, my close friends, and every person who has had an impact on my life and in the person I am today.

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CHAPTER I

Introduction
According to a report by the Center for Disease Control (CDC) which analyzed survey data from the National Health Interview Survey for the years 2007-2009, nearly 50 million Americans have physician-diagnosed arthritis. Since then, the number is only expected to increase within the coming decades. Of those 50 million, nearly 27 million Americans are affected by osteoarthritis – the most common form, amongst more than 100, of arthritis.

Osteoarthritis is a degenerative joint disease characterized by the breakdown of the joint’s cartilage. Since it is a degenerative disease – that is, the condition does not improve over time – treatment for it mostly focuses on pain management. Osteoarthritis is joint injury, and therefore all joints in the human body are susceptible to it. However, heavily used joints such as the knee, elbow, and fingers are usually more affected because of the role of cartilage in the joints.

1.1 THE STRUCTURE OF A JOINT

Synovial joints, also known as diarthrosis, are the most common types of joint in the human body. These joints allow for the most movement and their structure is very similar. For purposes of simplification, we will take the knee joint to overview the structure of synovial joints.

The knee joint is a hinge joint composed of different tissues which allow for smooth pivoting of the knee under pressures and movement. The major tissue structures present in the knee are:

Articular bodies: In the femur, these are the lateral and medial condyles and in the tibia, there are two condyles. The patella is located in the anterior wall of the joint capsule and it
communicates with the patellar surface, which in turns is connected to the femoral condyles.

Articular capsule: The articular capsule has synovial fluid, which is responsible for the lubrication of the joint. The capsule is located behind the patella.

Bursae: These are sacs filled with fluid to help cushion the bones and reduce friction during movement.

Cartilage: The cartilage is a thin, elastic tissue which protects the bone and provides a smooth surface for sliding. There are two types of joint cartilage in the knees: the pressure-resistant fibrous cartilage and the hyaline cartilage which covers the surface of movement. Cartilage is very poor at remodeling, and thus the reason osteoarthritis is a degenerative disease. Additionally, contrary to popular belief, cartilage is too thin to provide shock absorption, instead it lubricates the joint.

Menisci: These are articular disks that allow for easy sliding of the joint surfaces. They are made up primarily of collagen, and serve to protect the bones as well as providing some shock absorption.

Ligaments: The ligaments provide stability to the knee by limiting movement and there are four of them: the anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), medial collateral ligament (MCL), and lateral collateral ligament (LCL). These ligaments, though strong, are susceptible to injury and are often injured in the general population.

To understand why a lesion to cartilage is so serious and can lead to the development of osteoarthritis, we need to consider Figure 1 below, which shows a simplified schematic of a typical synovial joint. Notice that the cartilage is located in a crucial position within the
joint. Because of its location within the joint, articular cartilage is essential to the well being of the joint.

![Figure 1. A schematic of a synovial joint.][1]

Articular cartilage lubricates the joints by using a "weeping" mechanism in which synovial fluid is squeezed out of the cartilage during compression of the joint as shown in Figure 2.

![Figure 2. The mechanism of articular cartilage for lubricating the joint.][2]
1.2 OSTEOARTHRITIS

1.2.1 CAUSES

There are a variety of causes believed to lead to osteoarthritis. However, no consensus exists amongst experts. Furthermore, there are even some disagreements within the scientific community. For example, some experts believe mechanical stress to cause osteoarthritis, yet regular exercise has not been found to increase a person’s risk of osteoarthritis.

Osteoarthritis is often thought to result from the “wear and tear” of the joints; however, there are other causes such as metabolic disorders, trauma, genetic inheritance, and congenital defects. Table 1 provides a list of factors that are believed to influence osteoarthritis.2

<table>
<thead>
<tr>
<th>Constitutional Factors</th>
<th>Environmental Factors</th>
<th>Biomechanical forces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetics</td>
<td>Nutrition</td>
<td>Joint instability</td>
</tr>
<tr>
<td>Age</td>
<td>Exercise</td>
<td>Displaced joint loads</td>
</tr>
<tr>
<td>Sex, Parity</td>
<td>Drugs</td>
<td>Joint overloading</td>
</tr>
<tr>
<td>Hormone and metabolic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social Rank</td>
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</table>

Without a healthy articular cartilage, the bone surfaces become less protected and they become exposed to injury. Additionally, muscles may also atrophy and ligaments may become less elastic. Figure 3 shows a dramatization of a knee joint affected by osteoarthritis.
1.2.2 TREATMENT

As a degenerative disease, osteoarthritis cannot be cured. In fact, if not treated, osteoarthritis will continue to get worse over time. Treatment ranges from lifestyle changes to medication to even surgery. However, surgery is often looked as a last resort, and most treatment focuses on managing pain and avoiding surgery.

Lifestyle changes such as ridding bad eating habits and following a healthy diet can go a long way in treating osteoarthritis. Additionally, exercise such as swimming and other low-impact activities can help manage the symptoms. Getting rest to the joints is also very important, especially if the affected joints are the knees and fingers. ³

Medications for osteoarthritis are readily available. The most common are NSAIDs, though they may have some side effects. Acetaminophen (Tylenol) is often preferred to NSAIDs because it has fewer side effects. Other drugs such as Nimesulide and Synvisc

Figure 3. Comparison of a healthy knee and an osteoarthritic knee. Notice the deterioration of the bone tissue. ³
require prescription but are also effective in treating osteoarthritis' symptoms. Newer drugs, such as Tanezumab™, are still being reviewed by the FDA as they have been found to present some serious side effects.

1.2.3 ANIMAL MODELS

The prevalence of osteoarthritis makes the need for newer drugs and treatments paramount. Two main approaches in dealing with osteoarthritis are currently being researched. The more common one involves the development of newer drugs which allows the affected to carry more active and pain-free lifestyles. The other approach is the relative young field of regenerative medicine. This is concerned with being able to effectively regenerate lesions in articular cartilage, the cornerstone in osteoarthritis.

In both approaches, there exists a great need to find appropriate models to study osteoarthritis. However, finding appropriate models is a difficult task even nowadays where human models are basically impossible and in some case, even unethical. Therefore, animal models present a viable alternative to study arthritis; but even within animal models, some present more difficulties than others.

Mouse models are currently preferred amongst the scientific community because it allows for genetic manipulation and a rapid gestation period in mice. There are currently three main techniques that are used in mouse models; however, the literature on these techniques is fairly recent and difficult to find because of a lack of condensation. This thesis aims to review and condense the three main techniques in mouse models: (1) genetic manipulation, (2) treadmill running, and (3) mechanical stimulation/lesion. It is our hope that this thesis will provide a basic foundation to build upon for future research to be more easily conducted and reviewed.
CHAPTER II

Animal Models for Osteoarthritis
Animal models are common in the study of osteoarthritis as they allow for the study of cartilage degeneration and to evaluate potential drugs for treatment. According to Kenneth Pritzker, an animal model “can be defined as a homogenous set of animals which have an inherited, naturally acquired, or experimentally induced biological process, amenable to scientific investigation, that in one or more respects resembles the disease in humans.”

However, animal models are not the only models used in studying osteoarthritis. Biochemical, cell or organ culture models can provide insight into the mechanisms affecting the cartilage, bone, or selected cells within these tissues. Nevertheless, these ex vivo models are most useful in understanding short-term effects and cannot account for structural changes that occur over months to years. Therefore, animal models are best suited to study these structural changes as they allow the study of their progress over time and the effect different therapeutic treatments might have on the affected structures, such as the cartilage and bone.

The development of animal models for osteoarthritis involves several stages. This is important because each stage requires it to be very well thought out and analyzed. For example, when determining the availability of an animal model, logistical factors must be defined; a population of dogs or horses can only be housed in special and limited facilities. Table 2 shows four main stages in the development of animal models.

<table>
<thead>
<tr>
<th>TABLE 2 Development stages for animal models of OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Disease identification or induction in a set of animals</td>
</tr>
<tr>
<td>2. Availability of sufficient animals under experimentally controlled conditions</td>
</tr>
<tr>
<td>3. Characterization of functional and structural similarities and differences between the model and human osteoarthritis</td>
</tr>
<tr>
<td>4. Testing hypotheses related to osteoarthritis aetiology, pathogenesis or therapy</td>
</tr>
</tbody>
</table>
2.1 CLASSIFICATION OF ANIMAL MODELS

Many different animal models for osteoarthritis exist, each with its own relevance to disease progression, joint damage, and factors in the development of the disease. In general, osteoarthritis animal models can be classified into two main types: (1) spontaneous models and (2) induced models (surgical instability or genetic manipulation.)

While there is no one animal model of osteoarthritis which can be used as a gold standard, two animals models have been used extensively. A very studied example of spontaneous osteoarthritis involves the use of Hartley albino guinea pigs. Similarly, the transection of the anterior cruciate ligament (ACL) in dogs is one of the most frequently used induced models.7

2.1.1 SPONTANEOUS MODEL: HARTLEY ALBINO GUINEA PIGS

In guinea pigs, the spontaneous osteoarthritis occurs in the medial compartment of the knee joint, usually when the pigs are older than 3 months. According to a review by A.M. Bendele at the University of Colorado – Boulder, the pathogenesis of naturally occurring osteoarthritis is not completely understood, but it is believed that body mass plays an important factor, similar to the cases in humans. This could be attributed to guinea pigs and human beings heavily loading the medial aspect of the knee; in fact, 75% of the load passes through the medial aspect of the human knee.7

Guinea pig models have allowed for progress to be made studying different matrix metalloproteinase (MMP) such as collagenase 1 and 3, which are expressed by guinea pigs and human beings alike.7 Therefore, this model can be used to study the pathogenesis of
osteoarthritis and potential treatments. Figure 4 shows the progress of spontaneous osteoarthritis in Hartley guinea pigs.

Figure 4. Progressive loss of cartilage in the tibial plateau of Hartley guinea pigs. OA in this model closely resembles progression in humans. Cartilage is colored in red. 23

2.1.2 INDUCED MODEL: DOG ACL TRANSECTION

The transection of the ACL in dogs attempts to mimic the lesions naturally occurring in dogs or humans following a traumatic injury to the knee joint. 7 These lesions typically progress into osteoarthritis, providing an opportunity to study the disease as it evolves through time. Additionally, an important feature of this model is “the presence of numerous large osteophytes on the outside surfaces of the patellar grooves.” 7 This model allows for
the study of the presence of cytokines such as TNF-α and the opportunity to “obtain reasonable quantities of cartilage for RNA analysis.” However, as mentioned before, dog animal models present special logistical challenges since the dogs can only be housed in specialized facilities. This model is also known as the Pond-Nuki dog model.

2.2 ANIMAL MODELS IN OSTEOARTHRITIS

The prevalence and heterogeneity of osteoarthritis allow for multiple animal models to be used, including canine, murine, lapine, porcine, and equine models, each with its own advantages and disadvantages. Two factors important to determine the utility of an animal model are: (1) the size of the joint and (2) the thickness of the cartilage. Table 3 shows various cartilage thicknesses in common animal models. Similarly, Figure 5 provides a visual comparison of distal femurs.

<table>
<thead>
<tr>
<th>Species</th>
<th>Murine</th>
<th>Lapine</th>
<th>Canine</th>
<th>Porcine</th>
<th>Caprine</th>
<th>Equine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC cartilage thickness (mm)</td>
<td>0.1</td>
<td>0.3</td>
<td>0.95</td>
<td>1.5</td>
<td>1.1</td>
<td>1.75</td>
<td>2.35</td>
</tr>
</tbody>
</table>

**Figure 5.** Comparison of distal femurs from a rat (left), a goat (middle), and a human (right). A U.S. dime and quarter are inserted for visual reference.
2.2.1 **RODENTS: MICE AND RATS**

According to Chu et al., rodent models are a cost effective way to provide proof-of-concept data and "to serve as a bridge between *in vitro* experiments and more costly large animal preclinical studies." However, rodents' cartilage is small, as shown in Table 3 above. Mice offer the ability for various mechanistic *in vivo* studies due to the existence of transgenic, athymic, and knockout strains. Nevertheless, the small size of the joint in mice makes it impractical to study the effects of surgical implants.

Rats offer similar advantages to mice in the economic side, but they also increase the feasibility of the creation of cartilage defects. Additionally, this ability to create defects allows for the study of potential repair of xenogenic cells within the diarthroidal environment. Yet, the intrinsic repair of rats, the small size of their joints, and the potential to damage the thin cartilage still prove to be limitations for their use in all studies of osteoarthritis.

2.2.2 **DOGS**

Unlike rodents, and in similarity with humans, dogs lack the ability to significantly repair damage to the cartilage. As mentioned above, dog models are commonly used when studying lesions to the articular cartilage (Section 2.1.2.) Dogs also suffer naturally from osteoarthritis and osteochondritis dissecans and therefore, they can more closely resemble the human model than rodent or lapine models. However, their popular status as pets has raised ethical concerns regarding their use as animal models. Furthermore, the defects present in dog cartilage (~4mm) are still significantly smaller than those of interest in humans (>10cm).
2.2.3 GOATS

The caprine model presents a model that offers several advantages in regard to the size of the joint, the cartilage thickness, and the subchondral bone thickness. Additionally, most cartilage defects in goats tend to be larger than 6mm, thus rendering unable to heal on their own. However, while goats are less expensive to house and handle in comparison with other large animals, the cost for adequate facilities remains high. And while caprine models would be a feasible way to study chondral and osteochondral defects, the size of the lesions remain small than those of interest in humans.

2.2.4 HORSES

Horses present a unique situation when studying them as models due to the racing industry. Because of that, clinical treatment of osteochondral and chondral injuries in horses is advanced, thus providing a better understanding for cartilage repair in them. However, horses have a significantly different loading pattern than humans, thus presenting some challenges when trying to study treatments from horse models in humans. Additionally, there are significant cost and ethical concerns due to their common place in human lives.

2.3 DISCUSSION

As explained, no animal model presents an all-in-one model for all the studies concerning osteoarthritis. Therefore, when selecting a model it its important to take into account factors such as cost effectiveness, anatomy, and joint biomechanics. Ultimately, the research question determines the choice of the appropriate animal model.
CHAPTER III

Mouse Models for Osteoarthritis
Mouse models are cost effective, present no logistical challenges in their housing, and are very useful to provide proof-of-concept studies. Furthermore, the availability of athymic, transgenic, and knockout strains of mice allows for in vivo mechanistic studies and are useful in evaluating the cartilage repair potential of human cells and tissues. In most mouse strains, osteoarthritis tends to develop in the medial aspect of the knee (similar to humans). This has led for research to focus in destabilizing the knee via damaging the ACL or the medial meniscus, thus forcing the mice to develop medial compartment cartilage degeneration.

Each strain of mice presents its advantages for osteoarthritis research. Athymic mice permit the initial in vivo study of allogenic and xenogenic cartilage regeneration strategies due to their limited immune response. Transgenic mice are generally used in the study of specific genes or proteins involved in cartilage regeneration. For example, Fitzgerald et al. showed that in the MRL/Mpj mouse strain, cartilage defects healed better than in the C57B1/6 strain of mice. They attributed this finding to lower levels of the proinflammatory cytokine interleukin 1α and conversely, higher levels of anti-inflammatory cytokines in the MRL/Mpj mice.

Studies of osteoarthritis in mouse models mostly focus on investigating the deterioration of articular cartilage in order to develop potential treatments. This allows for these strategies to have the proof-of-concept needed before moving on to more expensive trials with larger models. In order to induce the instability of the knee, three major techniques are used with mouse models: (1) genetic deletion (knockout), (2) deterioration through treadmill running, and (3) surgical mechanical injury.
3.1 KNOCKOUT – GENETIC DELETION

Genetic deletions, or knockout models, consist in deleting a gene encoding a specific protein or molecule of interest. These models tend to be more expensive than other mouse models, but they provide vital information on factors important to cartilage repair. Additionally, because it can be hard to generate significant defects in mice cartilage, genetic deletions tend to be the most common study in mouse models.

In recent years, knockout strains of mice have greatly accelerated the process of identifying genetic players in articular cartilage homeostasis, thus making significant contributions to the understanding of osteoarthritis pathology. In fact, since the mid 1990s, gene deletion experiments have yielded more than 40 different strains of knockout mice.

Most genetic ablations in knockout can be classified into three types: (1) Extracellular matrix (ECM) degrading enzymes, (2) ECM molecules and receptors, and (3) cytokines and growth factors. Appendix I provides a very useful table put together by Raducanu et al., which shows various knockout models relevant in osteoarthritis research.

3.1.1 ECM DEGRADING ENZYMES

The ECM is composed of two major structures: (1) type II collagen fibrils, and (2) aggrecan, a proteoglycan (PG) which is able to resist static and dynamic compression. When one of these two major structures is disturbed, it is generally accepted that osteoarthritis will develop by means of aggrecanolysis (aggrecan degradation) and collagenolysis (collagen degradation). In fact, proteoglycan depletion is one of the first signs of osteoarthritis, as shown by Figure 6. In addition, knockout models have been able
to identify several families of proteinases and proteinase inhibitors that play an important part in the progression of osteoarthritis, as they are present in both normal and osteoarthritic human joints. Three major families of such molecules are:

1) Matrix metalloproteinases (MMPs): zinc- and calcium-dependent proteinases that are involved in the developmental, repair, and pathological processes of the ECM.

2) Adisintegrin and metalloproteinases (ADAMs): MMP-related proteinases that together with ADAMTSs (ADAMs with thrombospondin motifs) are able to break down aggrecan.

3) Tissue inhibitors of metalloproteinases (TIMPs): tissue inhibitors that regulate ADAMTSs and MMPs.

Figure 6. Loss of aggrecan is common in early stages of OA. 

One recent study studied MMP3 because of its ability to degrade not only aggrecan but other cartilage ECM components as well. The study compared the incidence of spontaneous osteoarthritis between MMP3-null and wild type mice. The results showed
that MMP3 seems to initiate the breakdown of cartilage by activating procollagenases, thus implying that MMP3-regulated matrix turnover is important to maintain normal cartilage homeostasis.9

In order to prevent uncontrolled ECM degradation in connective tissues, a balance between MMPs and TIMPs is of utmost importance. One specific TIMP, TIMP3, is able to inhibit both MMPs and several ADAMs and ADAMTSs. Mice that were depleted of TIMP3 showed degradation of the articular cartilage, as evidence by elevated levels of both aggrecanases and collagenases.9

3.1.2 ECM MOLECULES AND RECEPTORS

Small leucine-rich proteoglycans (SLRPs) are extracellular molecules that bind to growth factors, TGFβs, collagens, and other ECM molecules. There are four widely expressed SLRPs: (1) biglycan, (2) decorin, (3) fibromodulin, and (4) lumican. These SLRPs, when absent, provoke the formation of abnormal collagen fibrils in the ECM. A study to investigate the role of SLRPs was conducted in which SLRP-deficient mice were created. The SLRP-deficient mice developed various diseases including osteoarthritis, osteoporosis, and muscular dystrophy. Therefore, it was postulated that mutations in SLRPs might play an important part in becoming genetic factors for the aforementioned diseases.14

In hyaline cartilage the collagen-aggrecan networks are interconnected through molecules containing SLRPs and non-collagenous glycoproteins such as cartilage oligomeric matrix protein (COMP) and matrilins. Matrilin-1 and -3 are expressed during cartilage development and mutations in them can lead to various skeletal disorders. The levels of COMP, matrilin-1 and matrilin-3 serve as markers for changes in cartilage metabolism. Studies have shown that COMP- and matrilin-1-deficient mice had no signs of
osteoarthritis. However, the role of matrilin-3 is more controversial due to diverse results in studies conducted with matrilin-3-deficient mice. In fact, it was found that matrilin-3 polymorphisms were only associated with hand osteoarthritis in humans, but not in knee OA. The differences in results could be explained by the distinct mice that were used, thus demonstrating the need for more consistent and detailed studies.

3.1.3 CYTOKINES AND GROWTH FACTORS

Soluble growth factors and cytokines mediate the communication of chondrocytes with their environment by interacting with specific cell receptors and controlling gene expression. Among cytokines, IL-1 cytokines are the most studied as the pro-inflammatory cytokines playing an important role in osteoarthritis. Studies with knockout mice lacking IL-1β, ICE (IL-1β-converting enzyme) or iNOS (inducible nitric oxide synthase) showed that these mice developed accelerated osteoarthritis after knee surgery; thus, suggesting that IL-1β maintains a balanced metabolism in articular cartilage.

Members of the transforming growth factor (TGF)-β family play a pivotal role in the biology of articular cartilage. Initially, in vitro studies of TGF-β showed that it could lead to the synthesis of chondrocyte proteoglycan and collagen II, while also suppressing the expression of IL-1-regulated genes. These studies led to the belief that TGF-β could be used to treat osteoarthritis. However, further studies in mice showed that excessive levels of TGF-β in the knee joints led to osteophyte formation and cartilage degradation.

3.1.4 DISCUSSION

One main advantage of genetically manipulated mouse models over surgically induced models of osteoarthritis is that the molecular etiology is known and the incidence and severity of osteoarthritis can be controlled.
The extensive use of various transgenic, knockout, and knock-in mice has yielded extensive knowledge in the role of cytokines, growth factors, and the ECM and their interactions. This knowledge has allowed for new genes to be identified as potential targets for the study of osteoarthritis, and has provided valuable insight into the pathogenesis of osteoarthritis and cartilage degeneration. With new advances in the field, genetic deletion models will be at the forefront of mouse models for osteoarthritis for years to come.

3.2 TREADMILL RUNNING

Treadmill running in mouse models is a good way to induce rapid deterioration of the knee joint in mice. However, simply using treadmills on mice would provide little new insight into the study of osteoarthritis. Therefore, it is very common for treadmill running models to be used in conjunction with other models, most frequently with genetic manipulation models. Nevertheless there have been studies (Lapveteläinen et al.) where a strain of C57BL mice was subjected to intense daily running. Such study found that extended running in C57BL mice led to a higher incidence of osteoarthritis as opposed to those mice that were no subjected to a running regime.\textsuperscript{16}

Li et al. conducted a study in which the role of ADAMTS5 was investigated by injecting mice with TGF-β1 and enforcing uphill treadmill running (TTR model). Both wild type C57B1/6 mice and ADAMTS5-deficient C57B1/6 mice were evaluated for cartilage changes, meniscal damage, and fibrotic ingrowths.\textsuperscript{11} Using surface imaging it was found that the wild type (WT) femoral condyles showed surface roughening and fibrosis at the joint margins unlike their ADAMTS5 -/- counterpart. The WT tibial plateaus also showed some surface erosion and even cracking and collapse of the cartilage layer as well as fibrosis and cartilage deposition as shown in Figure 7.
Li et al. concluded that ADAMTS5 is responsible for more than simply cartilage aggrecan degradation as the absence of it did not eliminate aggrecanase activity from the articular cartilage but blocked fibrosis and resulted in cartilage deposition.
Another study involving both a treadmill running regime and genetic deletion was conducted by Ameye et al. The purpose of the study was to investigate double-deficient mice lacking biglycan (bgn) and fibromodulin (fm). These SLRPs are highly present in tendons and bones, and excessive use of the joint in double-deficient mice increased osteoarthritis and ossification.17

After 3 months, osteoarthritis-like lesions were present in the bgn/fm-deficient mice, and in 6 months, complete erosion of the articular cartilage was present. Additionally, osteophytes developed in the tibial and femoral surfaces.17 In comparison, WT mice showed no OA-like lesions and in even the oldest mice, their AC was smooth and even.17

3.2.1 DISCUSSION

Treadmill running provides a useful model for the development of spontaneous osteoarthritis on knockout mice. This is of special importance because the running regime allows for an early onset of the disease and a rapid progression, thus allowing researchers to study different “long-term” strategies for the treatment of osteoarthritis.

3.3 SURGICAL MECHANICAL INJURY

In chapter 2 it was mentioned that mouse models present significant difficulties to creating articular cartilage defects. However, some studies have been able to surgically destabilize the knee in mice by transecting structures in the joints. The destabilization of the medial meniscus (DMM) has been used to show to that in ADAMTS5 -/--mice there was a diminished presence of fibrosis and erosion of the articular cartilage.11

Another study using ADAMTS5 -/- mice transected the medial miscotibial ligament to induce osteoarthritis. The mice had one knee surgically altered and another one was left unoperated. Additionally, the results were compared between WT and ADAMTS5 -/- mice;
and it was found that the joints in ADAMTS5 -/- mice had a significantly thinner subchondral plate and less epiphyseal trabecular bone. Furthermore, this study also confirmed what studies like Li et al. confirmed - ADAMTS5 -/- have significantly less cartilage damage.

Another technique of induced surgical injury is the anterior ligament transection (ACLT) in mouse models. A study using 129/SvEv mice used ACLT to induced osteoarthritis; in fact resulting in a severe form of OA, chondrogenesis of the joint capsule, and even sever subchondral erosion of the posterior tibial plateau in some cases. However, Glasson et al. concluded that ACLT is not recommended as a viable technique due to the surgical proficiency needed (Figure 8) and the development of severe OA which may limit the potential research of the disease.

In comparison, when the same ACLT technique was used in rats, the results vastly changed. Hayami et al. observed articular damage and subchondral bone loss 2 weeks post-surgery and osteophyte formation was observed 10 weeks after surgery. This rat model allowed to observed similar characteristics in the development of osteoarthritis between rats subjected to ACLT and humans. Furthermore, their results suggested that this model is suitable to evaluate bone resorption inhibitors as potential therapeutic therapies.
CHAPTER IV

Conclusion
The development of various mouse models has allowed for new advances in the study of osteoarthritis, not only in the search of treatment strategies but also in the understanding of the disease’s pathology. As mentioned before, not all animal models provide the same advantages and disadvantages, but mouse models remain at the front of osteoarthritis research because of their feasibility and cost-effectiveness. New strains of knockout mice and new techniques, such as treadmill running, have expanded the range of structures and molecules that can be studied in the joints. Meanwhile, surgical induction of osteoarthritis in mice remains a viable way to conduct proof-of-concept studies that could lead to new research in larger animal models and eventually in humans.

This thesis does not intend to provide a summary of all the literature that has been written in all available mouse models (such list would be very extensive and might even be counterproductive). Instead, this work has attempted to provide a review of the available animal models, specifically mouse models, and some of the main results that have been obtained using such models and techniques. It is our belief that mouse models will remain an integral part of osteoarthritis research because of their unique advantages such as genetic alteration, fast gestation period, and minimal ethical and logistical constraints. Therefore, we encourage new researchers to further the study of osteoarthritis and its complexity by innovating new uses for the existing models as well as new techniques that could be applied to them.
APPENDIX I

Table of genetically modified mouse models
<table>
<thead>
<tr>
<th>Protein/Gene</th>
<th>Model</th>
<th>Articular Cartilage Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteolytic Enzymes and Related Molecules</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADAM15</td>
<td>KO</td>
<td>Accelerated knee OA; modulation of integrin signaling?</td>
</tr>
<tr>
<td>ADAMTS-1</td>
<td>KO</td>
<td>Normal susceptibility to experimentally induced arthritis</td>
</tr>
<tr>
<td>ADAMTS-4</td>
<td>KO</td>
<td>Normal susceptibility to experimentally induced arthritis</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>KO</td>
<td>Protection from experimental arthritis; major aggrecanase in OA</td>
</tr>
<tr>
<td>ADAMTS-4/-5</td>
<td>KO</td>
<td>Similar to ADAMTS-5 KO, AC releases intact aggrecan</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>TR</td>
<td>Joint destruction associated with sever bone defects</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>KO</td>
<td>Osteopetrosis, OA was not investigated</td>
</tr>
<tr>
<td>MMP2</td>
<td>KO</td>
<td>Age-dependent AC destruction, bone defects</td>
</tr>
<tr>
<td>MMP3</td>
<td>KO</td>
<td>Decreased susceptibility to spontaneous OA and inflammatory arthritis</td>
</tr>
<tr>
<td>MMP9</td>
<td>KO</td>
<td>Increased susceptibility to surgically-induced OA</td>
</tr>
<tr>
<td>MMP13</td>
<td>TR</td>
<td>Spontaneous OA-like changes in young mice</td>
</tr>
<tr>
<td>MMP14</td>
<td>KO</td>
<td>Accelerated OA, ectopic collagen X expression</td>
</tr>
<tr>
<td>Runx2 (+/-)</td>
<td>KO</td>
<td>Amelioration of surgically induced OA, reduced MMP-13 expression</td>
</tr>
<tr>
<td>TIMP3</td>
<td>KO</td>
<td>Mild OA, increased cleavage of aggrecan and collagen II</td>
</tr>
<tr>
<td><strong>ECM Molecules and Receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a1 integrin</td>
<td>KO</td>
<td>Accelerated OA, increased MMP2 and MMP3 expression</td>
</tr>
<tr>
<td>Aggrecan (Cmd/+)</td>
<td>KO</td>
<td>No reported OA-like changes</td>
</tr>
<tr>
<td>Aggrecan “Jaffa”</td>
<td>KI</td>
<td>Partially protected from experimental arthritis</td>
</tr>
<tr>
<td>Aggrecan “Chloe”</td>
<td>KI</td>
<td>Normal susceptibility to experimentally induced arthritis</td>
</tr>
<tr>
<td>Biglycan</td>
<td>KO</td>
<td>OA-like changes, ectopic tendon ossification</td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>KO</td>
<td>Age-dependent OA-like changes, ectopic tendon ossification</td>
</tr>
<tr>
<td>Biglycan/Fibromodulin</td>
<td>dKO</td>
<td>Early onset OA, ectopic tendon ossification, accelerated TMJ OA</td>
</tr>
<tr>
<td>Lumican</td>
<td>KO</td>
<td>OA was not reported</td>
</tr>
<tr>
<td>Lumican/Fibromodulin</td>
<td>dKO</td>
<td>Early onset OA, ectopic tendon ossification</td>
</tr>
<tr>
<td>Collagen II, a1 (+/+ )</td>
<td>KO</td>
<td>Higher prevalence of OA in aging mice, softer AC</td>
</tr>
<tr>
<td>Collagen II, a1 (dmn/+ )</td>
<td>NO</td>
<td>Early-onset OA, AC thinning, increased cell density, reduced matrix</td>
</tr>
<tr>
<td>Collagen II, a1 (Del/+ )</td>
<td>TR</td>
<td>Early-onset OA, up-regulation of cathepsin K</td>
</tr>
<tr>
<td>Collagen IX, a1</td>
<td>KO</td>
<td>Early onset OA, up-regulation of DDR2 and MMP13 expression</td>
</tr>
<tr>
<td>Collagen IX, a1</td>
<td>TR</td>
<td>Early onset OA</td>
</tr>
<tr>
<td>Collagen XI, a1 (cho/+ )</td>
<td>NO</td>
<td>Early onset OA, up-regulation of DDR2 and MMP13 expression</td>
</tr>
<tr>
<td>Collagen XI, a2</td>
<td>KO</td>
<td>Mild abnormalities of the AC, no obvious OA</td>
</tr>
<tr>
<td>Proteoglycan 4</td>
<td>KO</td>
<td>Surface changes followed by AC deterioration</td>
</tr>
<tr>
<td>Matrilin-3</td>
<td>KO</td>
<td>No obvious OA in one study; high OA prevalence at 12 months in another study</td>
</tr>
<tr>
<td>Matrilin-1/Matrilin-3</td>
<td>dKO</td>
<td>Similar incidence of OA in controls and double mutants at 14 months</td>
</tr>
<tr>
<td>Perlecan</td>
<td>KO</td>
<td>OA-like changes on the femoral head</td>
</tr>
<tr>
<td><strong>Cytokines and Growth Factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>TR</td>
<td>OA-like changes of the AC, osteophyte formation</td>
</tr>
<tr>
<td>BMPR1A</td>
<td>cKO</td>
<td>Progressive AC degradation</td>
</tr>
<tr>
<td>Fgf13</td>
<td>KO</td>
<td>Premature OA, increased MMP13 expression</td>
</tr>
<tr>
<td>IL-1β</td>
<td>KO</td>
<td>Acceleration of surgically induced OA</td>
</tr>
<tr>
<td>IL-6</td>
<td>KO</td>
<td>Age-dependent OA and subchondral sclerosis in males</td>
</tr>
<tr>
<td>ICE</td>
<td>KO</td>
<td>Acceleration of surgically induced OA</td>
</tr>
<tr>
<td>Ltbp-3</td>
<td>KO</td>
<td>Progressive AC degradation, abnormal chondrocyte hypertrophy</td>
</tr>
<tr>
<td>Mig-6</td>
<td>KO</td>
<td>Early-onset OA, osteophyte formation</td>
</tr>
<tr>
<td>NOS</td>
<td>KO</td>
<td>Acceleration of surgically induced OA</td>
</tr>
<tr>
<td>Smad3</td>
<td>KO</td>
<td>Progressive OA, osteophyte formation, increased Col10 expression</td>
</tr>
<tr>
<td>TGFβRII</td>
<td>TR</td>
<td>Progressive OA, osteophyte formation, increased Col10 expression</td>
</tr>
</tbody>
</table>

Note: KO, knock-out; dKO, double knock-out; cKO, conditional knock-out; KI, knock-in; NO, naturally occurring; TG, transgenic. Borrowed from Raducanu et al. 13

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REFERENCES


