TISSUE ENGINEERING: Part B
Volume 16, Number 1, 2010

DOI: 10.1089/ten.teb.2009.0452

Animal Models for Cartilage Regeneration and Repair

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Articular cartilage injury and degeneration are leading causes of disability. Animal studies are critically important to developing effective treatments for cartilage injuries. This review focuses on the use of animal models for the study of the repair and regeneration of focal cartilage defects. Animals commonly used in cartilage repair studies include murine, lapine, canine, caprine, porcine, and equine models. There are advantages and disadvantages to each model. Small animal rodent and lapine models are cost effective, easy to house, and useful for pilot and proof-of-concept studies. The availability of transgenic and knockout mice provide opportunities for mechanistic *in vivo* study. Athymic mice and rats are additionally useful for evaluating the cartilage repair potential of human cells and tissues. Their small joint size, thin cartilage, and greater potential for intrinsic healing than humans, however, limit the translational value of small animal models. Large animal models with thicker articular cartilage permit study of both partial thickness and full thickness chondral repair, as well as osteochondral repair. Joint size and cartilage thickness for canine, caprine, and mini-pig models remain significantly smaller than that of humans. The repair and regeneration of chondral and osteochondral defects of size and volume comparable to that of clinically significant human lesions can be reliably studied primarily in equine models. While larger animals may more closely approximate the human clinical situation, they carry greater logistical, financial, and ethical considerations. A multifactorial analysis of each animal model should be carried out when planning *in vivo* studies. Ultimately, the scientific goals of the study will be critical in determining the appropriate animal model.

Introduction

Articular cartilage has poor intrinsic healing potential. Consequently, traumatic and degenerative lesions of articular cartilage eventually progress to osteoarthritis, a leading source of disability worldwide. In the United States alone, osteoarthritis was estimated to cost over 60 billion dollars in 2001.[1] The tremendous clinical and financial burden of osteoarthritis motivates scientists and clinicians to investigate new strategies to improve repair and regeneration of articular cartilage.

In addition to cartilage injury and degeneration, advanced age, female sex, obesity, and joint injury are strongly associated with higher incidences of osteoarthritis.[2] Direct injury to articular cartilage, including creation of a focal defect,[3,4] joint destabilization,[5] or injection of chondrotoxic agents,[6] is a common method to induce cartilage loss and osteoarthritis in animal models. Trauma is reported to account for approximately 12% of osteoarthritis and is an under-appreciated cause of early disease.[7] Chondral defects are common in the symptomatic knee and progress to osteoarthritis with time.[8] As such, there has been renewed clinical interest in treating focal cartilage injuries. An exponential increase in studies on cartilage repair and regeneration, to include the new field of cartilage tissue engineering, has also arisen in the past 20 years focused on developing methods to heal focal chondral defects. Some of this research including autologous chondrocyte implantation in the United States and a wider range of novel scaffolds and cell–scaffold constructs internationally has been translated into clinical use.

When introducing such treatments into clinical practice, *in vivo* animal studies are essential to closing the gap between *in vitro* experiments and human clinical studies.[9] This review summarizes the benefits and limitations of animal models commonly used in cartilage repair studies. A discussion of murine, lapine, canine, caprine, porcine, and equine models follow in order of size. Animal size roughly corresponds to the size of the joint and cartilage thickness, two factors important to determining utility in modeling applications to human disease.[10] Although much can be learned from the repair of chondral and osteochondral lesions of varying sizes, the average volume of human cartilage defects is approximately 550 mm^3, and human cartilage lesions requiring treatment generally measure 10 mm or more in diameter.[10–12]

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Rodents

Rodent models are cost effective in providing proof-of-concept data to serve as a bridge between *in vitro* experiments and more costly large animal preclinical studies. Chondrogenesis has been extensively studied in murine models by subcutaneous,[13,14] intramuscular,[15] and intraarticular implantations[16–20] of various biomaterials and cells. Rodent joints, however, are small with thin cartilage (Fig. 1). In addition, the presence of open growth plates through advancing age likely increase intrinsic healing potential that now confound repair and regeneration studies in rodent models.

Mice

Mice offer strong advantages for mechanistic *in vivo* studies due to the availability of athymic, transgenic, and knockout strains. Mice are affordable and manageable to purchase, breed, and house. Availability of immunocompromised mice provides the opportunity to perform studies involving allogenic or xenogenic cells and tissues. Major disadvantages of the mouse model for cartilage repair studies include the small size of the joint and the extreme thinness of the articular cartilage,[10] which consists of only a few cell layers (Fig. 1). Repair processes that may be successful in restoring a small diameter defect extending a few cell layers deep may not work with larger defects. The small joint size and thin cartilage also mean that it is not practical, feasible, or meaningful to study the effects of surgical implants in this model.

On the other hand, athymic mice, which have a limited cellular immune response, permit initial *in vivo* study of allogenic and xenogenic cartilage regeneration strategies. Vacanti implanted human chondrocytes seeded onto biodegradable suture material subcutaneously on the dorsum of athymic mice to generate hyaline cartilage and launched the field of cartilage tissue engineering.[21]

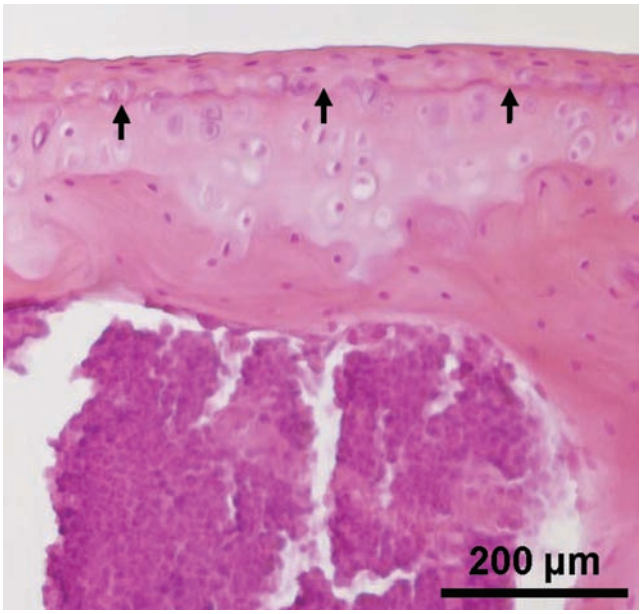

FIG. 1. Histology of mouse articular cartilage. Histological section of the murine distal femur shows the extreme thinness of the articular cartilage consisting of only a few cell layers above the tidemark (arrows). Color images available online at www.liebertonline.com/ten.

In addition to strains of mice in which osteoarthritis (OA) occurs spontaneously,[22] transgenic and knockout mice are potentially available. Transgenic animals are used to study the effects of a particular gene or protein on cartilage repair and regeneration.[23–28] For example, in the MRL/MpJ (JAX®, Jackson Laboratories, Bar Harbor, ME) mouse strain, the cartilage defects healed better than similar defects in C57Bl/6 mice. This was postulated to result from lower circulating levels of the proinflammatory cytokine interleukin 1α and higher levels of antiinflammatory cytokines in MRL/MpJ (JAX) mice compared to their wild-type counterparts.[29] While transgenic and knockout mice can be difficult and expensive to generate and maintain, use of these models can provide mechanistic information on factors important to cartilage repair. Improved understanding of the molecular basis for cartilage regeneration may generate new treatment options for further study in larger animal models.

Rats

The rat model has similar economic advantages as the mouse, while their larger size improves the feasibility and reproducibility of studies involving creation of cartilage defects (Fig. 2). Anraku *et al.*, using a custom-built device on rats, created trochlear defects highly consistent in size.[30] In addition, athymic rats are available. While the costs for procurement and husbandry of athymic rats exceed that of wild-type rats, the ability to create osteochondral defects within which xenogenic cells can be implanted provides a unique opportunity to study the repair potential of human cells within the diarthrodial environment. An *in vivo* study by Pagnotto *et al.* showing the persistence of adenoassociate virus transgene expression and the effects of sustained release of transforming growth factor β1 on human bone marrow cells (BMCs) implanted into osteochondral defects provides an example of the utility of athymic rats for cartilage repair studies.[20] Other xenogenic cells can be used in nude rats, as illustrated by the study of Matsumoto *et al.*, showing a better osteochondral repair potential of murine muscle-derived stem cells isolated from males as compared to females.[18] The successful use of murine cells in athymic rats raises the possibility of studying the effects of different genes and proteins on cartilage repair by using cells from transgenic and knockout mice.

The rat model also provides a cost-effective means for initial testing of the *in vivo* degradation characteristics and safety profile of new biodegradable scaffolds and polymers.[16] Ferretti *et al.* used the rat osteochondral defect model to show that degradation of PEG-genipin can be altered *in vivo* within osteochondral defects by changing the concentration of genipin and that PEG-genipin is biocompatible within osteochondral defects[16] (Fig. 2). Such scaffolds are core elements for many cartilage restoration strategies,[31] as they provide a three-dimensional environment for cell growth, deliver chondrogenic agents, or facilitate both.

While larger than mice, the rat model suffers from similar limitations due to joint size, thin cartilage, and potential for improved intrinsic repair because of life-long open growth plates.[32] The thin cartilage is likely easier to damage, and may also be easier to repair than human articular cartilage.

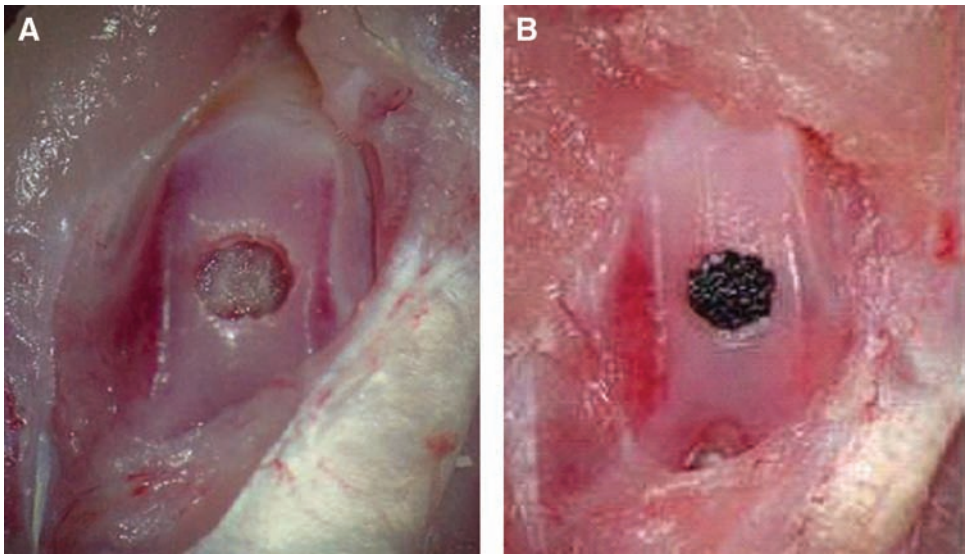

FIG. 2. Rat osteochondral defect model. (**A**) 1.5 mm osteochondral defect drilled into the rat trochlear groove. (**B**) Defect filled with PEG-genipin scaffold. Color images available online at www.liebertonline.com/ten.

Although longitudinal assessment technologies may be limited for joints of this size, Watrin-Pinzano *et al.* were able to evaluate spontaneous cartilage repair in rat patella with an 8.5 T magnetic resonance imaging (MRI) system.[33]

Overall, rodents are attractive models for cartilage research due to the availability of immune-masked and transgenic animals, as well as lower costs to purchase and house. Furthermore, access to hundreds of probes, antibodies, and reagents (http://invitrogen.com) offers additional advantages to investigators using rodent models. However, their small joint size and very thin cartilage limit translational potential. In the context of cartilage repair and regeneration, rodent models are most useful as cost-effective means for *in vivo* mechanistic studies, feasibility studies, and preliminary testing of new therapies.

Rabbits

The lapine model has been widely used for research on cartilage regeneration.[34–38] This is based on ease of handling, the use of compact caging, and the relatively low cost for animal purchase and care (http://dlar.pitt.edu). Similar to other small animal models, the rabbit also affords the opportunity to use a large quantity of genotypically similar subjects. In the early years of cartilage tissue engineering research, the rabbit was a popular model for osteochondral repair studies because the condyles of mature New Zealand White rabbits were large enough for creation of 3–4 mm defects. This was believed, at the time, to be a size permitting both the study of new implants and also a size where intrinsic repair processes predictably fail.

Subsequently, remarkable endogenous healing potential has been described in rabbits.[42,43] Shapiro *et al.* used 122 animals to investigate the cell origin of repair tissue in full-thickness cartilage defects. They concluded that repair was mediated entirely by proliferation and differentiation of mesenchymal cells from the bone marrow without participation from the residual, adjacent articular cartilage.[38] In contrast, cartilage defects in humans, if left untreated, show little to no spontaneous repair.[44] This makes it difficult to evaluate the translational potential of treatments using this model.

The rabbit model also suffers from relatively thin cartilage. An elaborate analysis revealed a mean cartilage thickness at the trochlear groove of 0.44 ± 0.08 mm and at the anteromedial femoral condyle of 0.3 ± 0.07 mm.[39] This limits the size and depth of the articular cartilage defects that can be made. In fact, the most common depth of experimental osteochondral defects reported in the literature is approximately 3 mm,[40–42] which means that more than 80% of the defect volume is located within subchondral bone. Further, rabbits have unique joint loading conditions. Due to the high degree of knee flexion, they use the trochlea groove as a partial weight-bearing surface, which, in the connection with low body weight (2–4.5 kg), creates much different loading conditions than in humans or large animals.[10]

In summary, the rabbit appears to be a practical model for early stages of therapy evaluation due to relative cost effectiveness, ease of handling, and reasonable joint size for surgical procedures. However, the rabbit model has lost favor in recent years due to high potential for spontaneous healing, sizable variation from human joint loading conditions, and thin cartilage.

Dogs

The dog has been used in studies of articular cartilage repair.[45–49] Similar to humans, dogs lack significant intrinsic ability to heal cartilage defects. Dogs also suffer from cartilage problems such as osteochondritis dissecans and osteoarthritis.[50] As such, studies of cartilage repair in these larger animal models may more closely model the human situation than rodent or lapine studies. For example, compared to implantation of autologus chondrocytes performed in rabbits, the canine model showed inferior results.[46] However, defect diameters and cartilage thickness in all but the largest dogs remain small, with 4 mm being the most common.[10] This is significantly smaller than clinical defects in humans.

In medium to large dogs, the thickness of the cartilage (range: 0.95–1.3 mm)[10] is greater than that of rodent and lapine models. This thickness renders it potentially feasible to create and study partial thickness cartilage injuries in canine and larger animals (Fig. 3). However, canine cartilage is still relatively thin compared to humans.[51] Consequently, repair

studies in canine models generally use small-diameter osteochondral defects.[12]

A potential advantage of the canine model is that the relatively exposed stifle joint facilitates arthroscopic examination of the tibio-femoral joint.[52] Canines are additionally well suited to studies requiring specific exercise and rehabilitation protocols. Dogs are able to accept bandages, braces, and slings, and they can be trained to use treadmills.

The strong bond between dogs and humans and their popular status as family pets have highlighted ethical concerns regarding animal research. These concerns have led to efforts to use canines bred for research as well as efforts to reduce, refine, and replace the use of animals when possible. In situations where postoperative management or rehabilitation protocols cannot be replicated in other animal models, the research question may require use of the canine model. However, the size and cartilage thickness of canine joints remain significantly smaller than human joints limiting assessment to defects averaging 4 mm, which are well below the sizes (>10 cm) of greatest clinical interest for humans.

Goats

The caprine model is commonly used in cartilage research.[5,53–56] This model offers advantages regarding joint size, cartilage and subchondral bone thickness and consistency, accessibility for arthroscopic procedures, and limited intrinsic healing capacity. The caprine joint is typically larger than the canine joint with the most frequently reported defect size being 6 mm in diameter, a size that has been shown to be unable to heal on its own.[10,54] The proportion of cartilage to subchondral bone and the subchondral bone consistency in goats is also reportedly closer to humans than small animal, canine, or sheep models.[10,54]

When compared to other large animal models, goats are relatively inexpensive and easy to handle if adequate facilities are available (http://dlar.pitt.edu). The larger size of the joint facilitates creation of chondral and osteochondral defects in goats compared to smaller animals. It also allows for examination of the knee arthroscopically, should longitudinal evaluation be required.[57] Protected weigh-bearing and exercise protocols are difficult to implement in goats, making them less well suited for studies where these factors are important.

Goats have been successfully used for evaluation of new implants for treatment of osteochondral defects.[56,57] Niederauer *et al.* treated osteochondral defects with various implantable constructs and achieved repair with hyaline-like cartilage and good underlying bone.[56] Researchers have also been able to fill cartilage defects with autologous BMCs previously aspirated from goat iliac crests.[53] On the other hand, fibrin, which is commonly used as a sealing component for cartilage defects, is not commercially available for goats. Preparation of autologous fibrin is not as commonly performed as in equines.[58]

For evaluation of chondral defects, the caprine model provides an opportunity to study the healing of partial and full-thickness defects, as the cartilage thickness on the medial femoral condyle is reported to range from 0.8 to 2.0 mm.[57] Furthermore, despite the fact that the size of the goat knee allows for the creation of defects that are, by volume, in the lower range of commonly observed cartilage defects in humans,[10] the tibiofemoral joint is still significantly smaller in goats compared to humans (Fig. 4).

If the limitations of a large animal model, including higher cost and the need for adequate facilities can be overcome, the goat model is a feasible large animal model for study of chondral and osteochondral defects. The size of the lesions studied, however, remain on the smaller end of what is considered clinically relevant for humans.

Pigs

The porcine joint size, weight-bearing requirements, and cartilage thickness more closely imitate the human condition than canine and smaller animal models.[59–61] Nevertheless, the porcine model has been relatively underutilized in cartilage research. This is, in part, because swine are large and can be relatively aggressive, making them more difficult to handle in research facilities.[62,63] Use of mini-pig strains, however, can overcome many of these limitations. Miniature swine bred for research are generally docile and bred to maintain an adult weight and size comparable to adult men. While the size of the mini-pig stifle joint remains significantly smaller than humans, chondral and osteochondral lesions measuring 6–8 mm in diameter and larger can be created in either the femoral condyles or the trochlear groove.[9] Similar to humans, adult pigs also have limited capability for endogenous repair of chondral and osteochondral defects.

To reduce potential for intrinsic cartilage repair found in immature swine,[61] it is important to use skeletally mature pigs. Mini-pigs such as the Gottingen mini-pig (GMP) reach skeletal maturity with closure of the growth plates occurring between 18 and 22 months of age (http://minipigs.dk). Further, studies have shown that the physiological and chemical parameters of the GMP, such as blood count, blood clotting, electrolytes, and liver enzymes are similar to values from humans.[9,64] Histomorphometric analysis of peripheral bone has also shown that the bone apposition rate and trabecular thickness in the GMP resembles human bone. Despite the lack of any detailed quantitative analysis on the structure and organization of adult minipig articular cartilage, Kaab *et al.*[65] were able to show by freeze-fracture scanning electron microscopy analysis that the collagen fiber arrangement in pig articular cartilage is very similar to the leaf-like arrangement found in humans.

The relative thickness of pig cartilage (about 1.5 mm)[66] is another advantage of the model because this thickness permits creation of full and partial thickness cartilage defects (Fig. 3). Further, arthroscopic evaluation of the knee joint (i.e., second look surgery) is feasible.[67,68] Although swines can tolerate slings, they are not well adapted to protected weight-bearing or exercise protocols.

Several studies of both chondral and osteochondral defects have been performed using mini-pigs. Hembry *et al.* used both immature and mature mini-pigs to localize matrix metalloproteinase activity in partial thickness cartilage defects.[69] Gotterbarm *et al.* showed that both osteochondral and chondral defects of 6.3 mm in diameter do not heal completely in the Gottingen mini-pig (GMP), thereby establishing the utility of this strain for articular cartilage repair

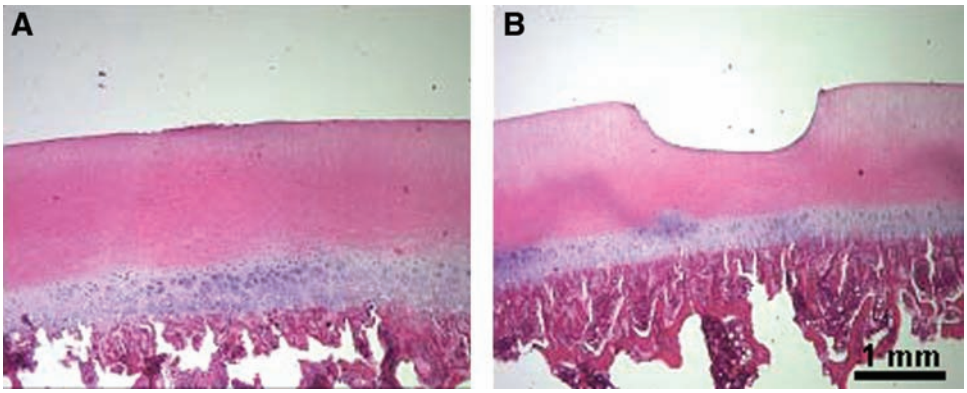

FIG. 3. Histology of porcine articular cartilage. (**A**) Histological section of porcine articular cartilage shows cartilage thickness greater than 1 mm. (**B**) Histological section of porcine articular cartilage after radiofrequency treatment shows that this tissue is thick enough for the creation of a partial thickness defect. Color images available online at www.liebertonline.com/ten.

studies.[9] Harman *et al.* evaluated the viability and healing of osteochondral autograft using a Sinclair mini-pig.[70] Using the Yucatan mini-pig model, Mainil-Varlet showed that control defects of 4 mm diameter healed with a more inferior fibrous cartilage than defects implanted with a tissue-engineered cartilage-like implant.[71] Chang *et al.* showed poor spontaneous healing of 8 mm osteochondral and chondral defects in the Lee-Sung miniature pig.[59,72] These studies support the feasibility and utility of the mini-pig model for use in studying the repair and regeneration of partial thickness, full-thickness, and osteochondral defects approaching the sizes of interest for human clinical study.

Mini-pigs have also been used in organ transplantation research and, therefore, have potential to become an important large animal model for studying the use of allograft and xenograft tissues for cartilage repair. Bourget *et al.* showed that prolonged tolerance to large musculoskeletal allografts can be induced in major histocompatibility-antigen (MHC)-matched pigs with a short course of cyclosporine.[73] Recent studies using α1,3-galactosyltransferase–(α-1, 3GalT)-deficient pigs indicate that long-term survival and function of porcine xenografts may be achievable in nonhuman primates.[74] Transgenic pigs have also been developed to express human regulators of complement activation,[24] indicating a possibility for transgenic work in large animals. These exciting findings have interesting implications for the potential use of xenograft tissue in joint reconstruction and osteochondral transplantation.[75]

The miniature swine offers several advantages for use as a cartilage repair model. While these specially bred pigs can be costly to procure and maintain, they are appropriately sized for cartilage research and are typically well characterized due to their use for a variety of biomedical research applications. The successful use of minipigs in transplantation research may increase interest in using this model for cartilage replacement and regeneration studies.

Horses

The equine model offers several advantages for cartilage repair studies. Similar to humans, horses suffer from cartilage problems ranging from osteochondritis dissecans to cartilage injury and osteoarthritis. Largely due to the racing industry, the clinical treatment of osteochondral and chondral injuries in horses is well developed.[76] Consequently, injury and repair of articular cartilage are better understood in horses than in most other animal models. Similar to humans, equine articular cartilage shows low intrinsic capability for repair.[79,80] Convery *et al.* showed that large defects (up to 21 mm) made to weight-bearing areas of femoral condyles in Shetland ponies failed to heal.[79]

Because it permits study of cartilage repair processes in defects approximating the size and depth of lesions seen in humans, the equine model is highly beneficial for preclinical evaluation of the efficacy of new cartilage repair techniques and technologies. Cluster analysis of studies involving single cartilage defects on the distal femur placed horses as the only animal model in the same group with humans in regard to defect dimension.[10] Cartilage thickness in the horse stifle joint is reported to be 1.75 mm, which is closest to human cartilage thickness (2.35 mm) among commonly used animal models[66] (Table 1). Therefore, full and partial thickness cartilage defects can be created with very close correlation to clinically relevant-sized defects in human cartilage.[10,58,77,78] Evaluation of chondral and osteochondral defects of 15 to 20 mm is possible in horses.[77–79] Additionally, the large joint dimension, thick articular cartilage, and fully extended, upright stifle joints during gait are closer to human knee anatomy than small animal models.[9]

The dimensions of the equine stifle joint are also suitable for arthroscopy. This means that both primary intervention and arthroscopic second-look assessments can be readily performed. Wilke *at al.* reported on arthroscopic implantation of mesenchymal stem cells, previously aspirated from horse sternums, into large cartilage defects in horses. Thirty-day arthroscopic assisted biopsy revealed significant improvement in cartilage repair as compared to defects filled only with fibrin.[81] This study highlights the well-developed clinical techniques for arthroscopic treatment and assessments of cartilage injury and repair in the equine model.

Due to the clinical need for effective cartilage repair in equines, cartilage repair techniques are well studied in this model. The availability of complementary scientific and clinical data offers additional advantages for translation of basic research into preclinical study in horses. Overall, equine bone marrow cells (BMCs) have been shown to have good chondrogenic potential.[82] Also, implantation of genetically modified chondrocytes infected with adenovirus vector encoding bone morphogenetic protein-7 has been shown to accelerate the appearance of hyaline-like repair tissue in

Table 1. Mean Cartilage Thickness

Species	*Murine*	*Lapine*	*Canine*	*Porcine*	*Caprine*	*Equine*	*Human*
MFC cartilage thickness (mm)	0.1	0.3	0.95	1.5	1.1	1.75	2.35

This table shows the mean cartilage thickness on the medial femoral condyle (MFC) from Frisble *et al.*[66] Values are reported in millimeters.

equine experimental cartilage defects.[78] Moreover, Litzke *et al.* showed that autologous implantation of chondrocytes in horses improves cartilage healing when compared to control, untreated defects.[83] Finally, multiple biochemical, molecular, gene therapeutic, and immunohistochemical assays have been described for various equine joint tissues and fluid. This can be attributed in part to the clinical need for cartilage regeneration in horses, to allow animals involved in racing to maintain their athletic performance.[58,77,78,82,84–88]

The horse is the largest of the animal models commonly available for cartilage research (400–500 kg). Due to their weight and physiology, equine jointloading conditions and the consequent hardness of equine subchondral bone are of some concern.[89] Consequently, any potential treatment applied over the cartilage defect will be subjected to greater loading than in humans. Additionally, the horse is unable to maintain protected weight-bearing protocols that are typical following human cartilage repair procedures. In horses, joint loading starts as early as during recovery from anesthesia and restrictions to loading or joint motion raises potential life-threatening health problems. Therefore, the location of the defect should be carefully considered to avoid early overloading. The horse stifle joint can be divided into the primary weight-bearing medial and lateral compartments, as well as the patellofemoral compartment, which is relatively unloaded during stall confinement and supervised walking. Therefore, the lateral trochlea of the femur has been frequently used for cartilage repair and regeneration studies in the equine model.[10]

While protected weight-bearing is difficult, horses can be readily led and trained for supervised exercise. Specific rehabilitation programs involving exercise at varying speeds and durations can be implemented in training areas or on treadmills. The equine model is therefore suitable for studies evaluating or requiring specific rehabilitation protocols.

Although the horse stifle joint is large, MRI of the joint for longitudinal evaluation of cartilage repair and osteochondral incorporation is not feasible. Use of the tibiotarsal joint in horses does permit *in vivo* MRI for longitudinal evaluations.[90,91] The equine tibiotarsal joint is smaller than the stifle joint with thinner cartilage. However, arthroscopic procedures to this joint are also performed clinically. The potential to perform both second-look arthroscopy and noninvasive MRI studies of this joint indicate that and the equine tibiotarsal joint may be useful for studies where longitudinal evaluations of cartilage regeneration and repair are needed.

The major disadvantages of equine models include high expense in procuring and caring for the animals and access to appropriate facilities and veterinary support for surgical procedures and perioperative care. Only large, highly specialized, and well-equipped facilities can conduct experiments on equines, which is in contrast to the previously discussed large animal models (http://dlar.pitt.edu). High joint loading conditions, high price, and the need for highly specialized facilities pose potential limitations for researchers interested in using the equine model. Nevertheless, factors such as joint size, morphology of the cartilage, and fully extended stifle during gait make the equine model the most attractive large animal available for preclinical studies. Therefore, horses should be considered a logical and translational model for studying the efficacy and safety of new cartilage treatments before human clinical trial.

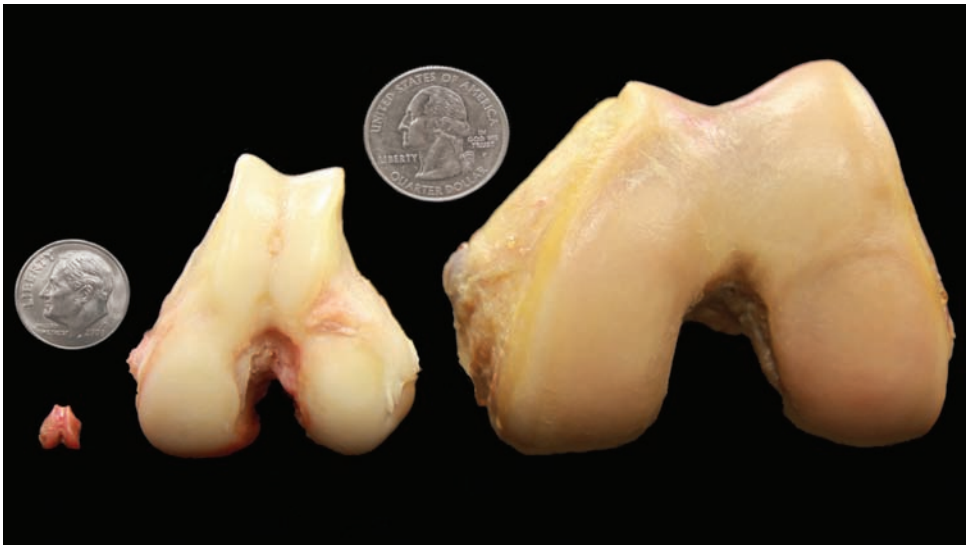

FIG. 4. Comparative size of distal femurs. Note the significant differences in size between the distal femurs from a rat (left), a goat (center), and a human (right). The quarter and dime provide a reference to show the relative size of the condyles.

Table 2. Age of Skeletal Maturity

Species	*Murine*	*Lapine*	*Canine*	*Porcine*	*Caprine*	*Equine*
Age of skeletal maturity	life-long	16–39 weeks	12–24 months	42–52 weeks	48–36 months	60–72 months

This table shows the average age growth plate remains open from Ahern *et al.*[10]

Discussion

This article provides a general review of animal species commonly used to study new techniques for cartilage repair and regeneration. The present clinical focus of cartilage tissue engineering is to develop methods with consistent regenerative potential to form a durable hyaline repair cartilage. A variety of scaffolds, in combination with chondrogenic cells, have been shown to have good ability to regenerate hyaline cartilage both experimentally and clinically.[4,92,93] Several studies have shown that new cartilage can be engineered *in vivo* by transplanting chondrocytes seeded into a three-dimensional scaffold.[4,94–96] Rodent models offer advantages for mechanistic study, evaluation of allogenic and xenogenic strategies, and initial feasibility studies. To demonstrate efficacy and safety before human clinical use, long-term large animal studies evaluating a new treatment in full-thickness cartilage lesions of the weight-bearing areas are needed.[4]

Multiple factors need to be considered in selecting an appropriate animal model. With limited research funding, costs of animal purchase and housing are important factors. In general, cost increases proportional to animal size (http://dlar.pitt.edu). For this reason, most researchers conduct initial experiments on smaller models, like rodents and rabbits, to show proof-of-concept. Use of small animal models also permits study of genetically similar individuals, which can reduce variability in the results. On the other hand, institutional review boards may not permit the study of new cartilage treatments in humans without supportive data from large animal studies. Such a requirement in cartilage research is based on the fact that joints of small animals do not adequately mimic those of humans (Fig. 4). Small animal joints are much smaller and have thinner cartilage, both of which do not allow the ability to create defects of comparable dimensions to human defects (Table 1).[66] Furthermore, significant involvement of subchondral bone in defect healing may not be avoidable when smaller species are used.

The stifle joint has been most commonly used for cartilage repair and regeneration studies, as it is the largest weight-bearing diarthrodial joint. Smaller joints can be considered when cartilage defect size and cartilage thickness are not the primary consideration. An example would be use of the tibiotarsal joint in equines when longitudinal MRI scans are desired. Finally, while designing an animal experiment, skeletal maturity of the subject needs to be carefully considered (Table 2).[10] If the affected joint is surrounded by open growth plates, these can interfere with the applied treatment. Germinal cells from the physis may supply regenerating cartilage and alter the study results.

Many of these limitations can be reduced by using skeletally mature larger animal models. However, the cost of purchase and housing for older and larger animals is generally greater than those of smaller and younger ones. Additionally, large animals more often require specialized husbandry and veterinary care, which is expensive. Appropriate space, facilities, and personnel may not be accessible or available for large animal studies. If, however, these limitations can be overcome by the investigator, large animal models will better replicate the human clinical scenario.

Canine, caprine, swine, and equine models are considered large animal models. Although the ability to achieve critical-size defects, defined as defects for which spontaneous repair does not occur, is present for each of these models, only the equine model permits ready examination of defects at dimensions comparable to those in humans for which clinical treatment is required.[10] Defect diameters averaging 4 mm in canine, 6 mm in caprine, and 8 mm in swine do not heal spontaneously, but are also much smaller than clinically challenging defects in humans, which are typically greater than 10 mm in diameter. Similar to what is seen clinically in humans, genetic diversity in canine, caprine, and equine models may account for differing healing potentials resulting in inconsistent results and the need for larger numbers of experimental animals to properly power a cartilage repair study. These factors may justify the additional expense of procuring more genetically similar individuals such as mini-pig strains developed for research.

In summary, no animal is ideal for every type of project in cartilage research. As every animal model has its advantage and disadvantage, a comprehensive analysis of each available species needs to be conducted when planning an animal study. Cost effectiveness, anatomy, maturity, and joint biomechanics as well as postsurgical protocol must be taken into account. The research question ultimately drives the choice of animal model.

Disclosure Statement

The authors have no conflicts to disclose.

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Received: July 8, 2009
Accepted: October 14, 2009
Online Publication Date: January 14, 2010