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Animal Models of Osteoarthritis: Updated Models and Outcome Measures 2016–2023

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Abstract

Purpose—Osteoarthritis (OA) is a global musculoskeletal disorder that affects primarily the knee and hip joints without any FDA-approved disease-modifying therapies. Animal models are essential research tools in developing therapies for OA; many animal studies have provided data for the initiation of human clinical trials. Despite this, there is still a need for strategies to recapitulate the human experience using animal models to better develop treatments and understand pathogenesis. Since our last review on animal models of osteoarthritis in 2016, there have been exciting updates in OA research and models. The main purpose of this review is to update the latest animal models and key features of studies in OA research.

Method—We used our existing classification method and screened articles in PubMed and bibliographic search for animal OA models between 2016 and 2023. Relevant and high-cited articles were chosen for inclusion in this narrative review.

Results—Recent studies were analyzed and classified. We also identified ex vivo models as an area of ongoing research. Each animal model offers its own benefit in the study of OA and there are a full range of outcome measures that can be assessed. Despite the vast number of models, each has its drawbacks that have limited translating approved therapies for human use.

Conclusion—Depending on the outcome measures and objective of the study, researchers should pick the best model for their work. There have been several exciting studies since 2016 that have

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taken advantage of regenerative engineering techniques to develop therapies and better understand OA.

Lay Summary—Osteoarthritis (OA) is a chronic debilitating disease without any cure that affects mostly the knee and hip joints and often results in surgical joint replacement. Cartilage protects the joint from mechanical forces and degrades with age or in response to injury. The many contributing causes of OA are still being investigated, and animals are used for preclinical research and to test potential new treatments. A single consensus OA animal model for preclinical studies is non-existent. In this article, we review the many animal models for OA and provide a much-needed update on studies and model development since 2016.

Keywords

Osteoarthritis; Animal Model; Translational; Outcome Measures

Introduction

Definition of OA

Osteoarthritis (OA), the most common form of arthritis, is a degenerative joint disease resulting in chronic pain and is considered a global public health concern [1]. In the USA alone, there are over 50 million adults with arthritis with over 20 million having activity limitations imparting a huge socio-economic burden [2]. The risk of OA increases with age; while the etiologic association of aging with OA is not clear, increased oxidative damage, muscle weakening, diminishing proprioception, and thinning of cartilage may contribute to the disease progression [3, 4]. Other reported risk factors are female sex [5, 6], obesity [4, 7], and genetic variants [4, 8]. Changes to the joint can also be a risk factor for patients. Prior trauma such as anterior cruciate ligament (ACL) tear and/or meniscal tear can predispose a patient to developing knee OA due to changes in joint kinematics [4, 9]. Abnormal joint loading during activity can also predispose to OA. For example, activities of squatting and kneeling are associated with knee OA, while prolonged standing and lifting are associated with hip OA [4, 10]. Military personnel also suffer from OA through increased tactical tasks carrying heavy loads, bending, and kneeling [11] or lower limb amputation [12].

Current Treatments

While no cure for OA exists, several approaches are used to manage and minimize OA-associated pain including physical therapy, over the counter pain medications, minimally invasive injections, and surgical treatments. Surgical treatment such as joint replacement is the last resort after other failed treatments and is indicated when the patient has severe, debilitating disease. However, even joint replacement has ~ 20% of patients unsatisfied with their replacement and they may have activity limitations [13]. Physical therapy may help some, though exercises can be challenging for people with significant pain, restricted motion, and activity limitations [14]. Pain medications such as non-steroidal anti-inflammatory drugs, acetaminophen, and even opioids have been used to ameliorate symptoms but do not prevent progression of disease. Minimally invasive injections with cortisone and hyaluronic acid may provide some relief, but for many, the disease and

pain eventually become too severe and the patient will require joint replacement [15]. The treatments listed above only address symptoms but there are no cures. Currently, there are no United States Food and Drug Administration (FDA)-approved disease-modifying drugs or therapies for osteoarthritis despite many clinical trials [16].

Pathophysiology

Part of the challenge with drug development is that there is not a clear understanding of the pathophysiology as OA is a chronic, degenerative disease that initiates and worsens over many years. Osteoarthritis is thought to be a multifactorial disease consisting of systemic and intra-articular changes in inflammation, metabolism, biomechanics, cellular aging, tissue integrity, nociception, and endocrine signaling [17–19]. Figure 1 demonstrates classic radiographic features of joint space narrowing, osteophytes (bone spurs), subchondral sclerosis, and subchondral cysts [20]. Abnormal mechanical loading upon degraded cartilage can damage the underlying subchondral bone by recruiting immune and inflammation reactions to cause bone remodeling (sclerosis and osteophytes) as seen in Fig. 1 [21]. Loss of cartilage contributes to the narrow joint space with decreased collagen and glycosaminoglycan (GAG) content seen on magnetic resonance imaging (MRI). In addition to the established post-traumatic phenotype of OA, there are several risk factors that contribute to OA as discussed above and seen in the left of Fig. 1. The association with sex, obesity, and age suggests there is a complex interplay of endocrine and metabolic factors that contribute to progression of disease. The pathophysiologic process involves a chronic inflammatory state seen in obesity with adipokines that can upregulate degradative joint enzymes like metalloproteinases [22]. Given the complex and enigmatic nature of OA, there is an active pursuit of characterizing biomarkers of the disease.

Animal Models

To better understand the disease progression and molecular pathophysiology of OA, preclinical animal models are used to facilitate clinical care. Animal models are imperative in exploring investigational drugs, finding reliable biomarkers of disease, and identifying mechanisms. There is currently no animal model that perfectly recapitulates the complexities of human OA. Several challenges and limitations exist in selecting a consensus OA animal model: small animals are anatomically different from human, for example, mice have cartilage that is 70 times thinner than human cartilage [23]. Larger animals may be more anatomically similar to human, but there are high costs and technical challenges with animal maintenance for studies that allow the natural progression of OA over many years; surgically/chemically induced OA models do not mimic human pathogenesis of primary OA. The absence of an ideal animal model has created obstacles for investigational therapeutics and drug development.

Rationale and Strategy for This Review

We previously characterized animal models of osteoarthritis in 2016 [24] and denoted a new classification scheme in 2022 [25]. Since 2016, there have been promising preclinical studies and additional development of investigational therapies for human treatment. These advances have inspired an updated review on the literature. In this review, we aim to provide an update on the latest uses of animal models of OA. PubMed and bibliographic searches

were performed for the included studies of this review. Only studies published from 2016 onward were analyzed. Search terms included animal model and osteoarthritis, combined with the various sections and subsections of this review: natural, genetic, surgical, synthetic, dynamic, ex vivo, imaging, histopathology, biomarkers, etc.

Model Classification Scheme

Esdaille et al. recently updated our prior classification scheme from Kuyinu et al. [24, 25]. The updated scheme now includes a new tertiary in vivo model, in addition to the primary and secondary classification. Primary models consist of idiopathic development of OA without any post-birth intervention from the researcher. Secondary models consist of an induction method like surgery to destabilize the joint or chemical treatment of the intra-articular space to cause an inflammatory reaction that mimics osteoarthritis development or other synthetic means. The tertiary model combines two secondary techniques for example combining surgical destabilization with intense exercise to wear down the cartilage. Through the search, it became apparent that other models, including ex vivo models, are also in use, which is reflected in Fig. 2. We review the latest studies according to this classification and add additional ones from our previous 2016 review [24] as shown in Fig. 2.

In Vivo Animal Models of OA

In vivo models of osteoarthritis consist of small or large animal models. Large animals have cartilage size more similar to human [26], though the ambulatory mechanics of quadrupeds and anatomic differences in the fetlock and stifle joints (e.g., horse) make these models different than human. Specifically, the horse fetlock joint is a metacarpophalangeal joint affected by OA and is more analogous to the human knuckle [27]; the fetlock joint exhibits exquisite range of motion of 120° of both flexion and extension during running [28]. In human, the knee and hip joints affected by OA have less range of motion during running (arcs of ~ 120 and 60° respectively) and there are only two limbs in contact with the ground compared to horse [29]. While horse cartilage is described as most similar to human in terms of architecture, biochemical quantification, and limited self-healing capacity [26, 30–32], the model is still limited in mimicking human OA. For example, in testing autologous protein solution, a horse study reports improved lameness at 14 days [33], which correlates with reports of improved symptoms in human with autologous conditioned serum as a palliative therapy [34]. However, meaningful patient outcomes at long-term follow-up like time to joint replacement [34] are not feasibly studied in horse. One could surmise joint loading is different in horses with upright weight bearing and living in stables post-treatment [35] while humans are able to modify weight bearing and undergo targeted physical therapy. Moreover, most horse studies investigate the fetlock joint instead of the stifle joint [36]. These differences do not fully recapitulate the human biomechanics that could account for changes in efficacy of therapies [34].

Small animals like rodents are cheaper to maintain and still have cartilage that responds to surgical joint destabilization and chemical induction in a way that allows histologic scoring of cartilage lesions and treatment with regenerative therapies. However, the therapies are not always translatable because what works in rats may not always work in human. For example,

cartilage is thinner and chondrocyte numbers are higher in small animals compared to horses and humans, which suggests there may be differences in regenerative capacity among these species [26, 30, 36]. In rabbits, untreated cartilage defects may spontaneously repair with hypothesized contribution from bone marrow stem cells rather than local cartilage cell populations [35, 37]. In testing therapies, rats with adipose-derived mesenchymal stem cells have alleviated cartilage degradation [38], though such rigorous disease modification has not yet been established in human [39]. In addition, pain is an essential feature of human OA that the clinical trial drug must address. Even if cartilage is regenerated in the small animal and in the human, the drug must improve patient pain, function, or time to end-stage disease to seek approval. Pain study in animals is done though is not without limitations including lack of psychologic component, provoked pain, and ethical considerations [40, 41]. These are important considerations in using small animal models of OA, though their value is essential in research.

Primary Models

A primary OA model is one that the animal will develop spontaneously during its lifespan without any external intervention. For example, a naturally occurring model of OA will develop as the animal ages, similar to human. Another primary OA model is when the animal is genetically engineered or bred; an external intervention is performed to modify genes before the birth of the animal—otherwise, there is no intervention to induce OA.

Naturally Occurring—As the name suggests, these animal models manifest OA like humans with a time-dependent process. Naturally occurring models of OA are slowly progressing and may be costly in large animals; for example, the baboons may take 8 years to reach skeletal maturity [24]. Despite these constraints, the commercial pig/porcine model is one example of large animal that has been shown to spontaneously develop OA and exhibit varying clinical signs of lameness and microscopic destruction of cartilage within 3–4 years [42]. In smaller animals, the Hartley guinea pig is favorable because it reaches skeletal maturity in 6 months [24]. OA lesions develop in the guinea pig that can be detected and scored histologically with validated tools like the Osteoarthritis Research Society International (OARSI) [43]. OARSI histopathology scoring is also available for other species [43–49].

Ringe et al. used a naturally occurring guinea pig model to evaluate cartilage changes to assess potential disease-modifying therapy of hyaluronic acid (HA) + chemokine C–C motif ligand 25 (CCL25) injection [50]. The aging guinea pig serves as one appropriate OA model because guinea pigs develop localized, spontaneous lesions in the knee where there is no overlying meniscus like humans [43]. Accordingly, guinea pigs treated with HA + CCL25 had lower OARSI histologic scores at 16 months (Fig. 3) [50]. Although the studies have longer follow-up, preclinical guinea pig experiments provide valuable histologic scoring and cartilage changes to test a range of therapies, from injectable [50], to conservative measures like exercise [51].

Naturally occurring OA models may more closely mimic the insidious onset of primary human OA, though time, cost, and resources may impede their widespread use.

Genetic Engineering—Genetic engineering can be used to modify gene expression to study proteins that can contribute to, prevent, or explain osteoarthritis through gene knockout and mutation [24]. The genetically engineered animal models are commonly used to play a role in understanding mechanisms and pathogenesis of OA [52]. Recently, Burt et al. identified different isoforms of fibroblast growth factor 2 (FGF2) can contribute to the protection or induction of osteoarthritis [53]. Namely, mice lacking a gene encoding low molecular weight FGF2 demonstrate OA and catabolism of cartilage, while mice with high molecular weight FGF2 knockout have protection from OA and increased markers of cartilage anabolism [53]. Such studies are important to understand pathophysiology of OA and offer unique pathways for drug development or targeted gene therapy. Indeed, gene therapy is an active area of study [54], and viral-vector delivery of short-hairpin RNA (shRNA) in a mouse OA model has downregulated inflammatory cytokines and decreased cartilage damage [54]. It is noted, however, that the model in this study was a secondary model of OA and not a genetic engineering model.

Genetic engineering models of OA are reproducible and allow study of mechanisms but are not optimally suited to study therapeutics—changing just one gene may not reflect the complex interplay of several pathways contributing to the disease [36, 55].

Secondary Models

Secondary models can be separated into three categories, namely dynamic, surgical, and synthetic models [25]. An external stimulus provided by the researcher causes OA to develop in the animal. These can range from invasive surgical procedures to injections to completely non-invasive techniques like high-fat diet.

Dynamic Model—A dynamic model of OA involves non-invasive machine-guided joint loading and forces that injure and create an osteoarthritic defect in the animal. One example is an intra-articular tibial plateau fracture produced by a steel indenter driven by a tunable force generator machine [56]. More recently, Chang et al. combined a genetically engineered mouse model with a dynamic compressive load on the tibia to rupture the anterior cruciate ligament [57, 58]. In this study, a negative bone regulator in the Wnt pathway, sclerostin (Sost), was shown to play a significant modulatory role in osteophyte formation [57]—mice with knockout of Sost demonstrate significant osteophyte formation as seen in Fig. 4.

Ko et al. investigated the impact of a single tibial loading session on the mouse knee joint without rupturing the ACL [59]. The limb is fixed in a flexed position with a cyclic force transducer that provides a repetitive compressive force. The model successfully develops OA noninvasively without adversely affecting chondrocyte viability or causing morphological and compositional cartilage alterations. The method suggests a cell-mediated process leads to the OA development [59].

Dynamic models of OA consistently reproduce trauma and are non-invasive, but they are less commonly used because they require expensive machinery.

Synthetic Model—Synthetic models of OA consist of an iatrogenic chemical reaction in the joint to cause an insult and result in OA. They are less invasive than surgical techniques

and offer other advantages such as reproducibility, measurable changes in pain, and quick onset. A drawback of the synthetic model is that it does not precisely reflect the natural onset of OA, which is a chronic, idiopathic onset. Nevertheless, synthetic models are critical research tools.

MIA Model (Sodium Monoiodoacetate): One of the most used induction methods is intra-articular injection of sodium monoiodoacetate (MIA). MIA injections inhibit glycolysis within chondrocytes via inhibition of glyceraldehyde-3-phosphatase dehydrogenase leading to chondrocyte cell death and subsequent joint changes like fibrillation and decreased thickness of cartilage, reduced proteoglycans, decreased chondrocyte number, subchondral bone exposure, bone erosion/atrophy, oxidative stress, and inflammation [60–64]. With MIA injections, there are also measurable behavioral and pain-associated responses in animals, such as referred mechanical/thermal sensitivity, Von Frey hair algesiometry, electrophysiologic changes, weight bearing asymmetry/gait changes, and modulation of neuropeptides and inflammatory mediators [61, 64–69]. One such example of this outcome measure is via paw withdrawal latency—an infrared, hot stimulus is applied to the paw, and rats with MIA-induced joints will withdraw faster from this stimulus as in Fig. 5 [4] [70]. These changes in pain and function by the MIA OA model are of interest as they mimic the human experience.

For example, the MIA model has been used to test pain modulation of therapies. MIA OA induction in rats along with chronic constriction injury of the sciatic nerve (CCI) to induce mononeuropathy was used to study pain responses in rats. Photobiomodulation therapy was tested on the rats and provided analgesic effect evidenced by improved hind limb weight distribution and mechanical hyperalgesia [65]. The CCI model was also used by Khanal et al. to study the significant analgesic effects of a novel nanocomposite anesthetic delivery system [73]. Using such models in combination with the MIA model is important for developing therapeutics that target pain in OA research.

The MIA animal model was also used to test quercetin (a natural senolytic) [74], cannabis [66, 75], photobiomodulation [60, 65], hyaluronic acid [68, 76, 77], and exercise [78, 79]. Many of these studies focus on pain as an outcome measure because MIA can induce a chronic, pain at rest phenotype as well as other pain-related responses mentioned above [61–64, 80, 81]. Uniquely, the MIA rat model has also been used to study neuronal effects using electrophysiology and electron microscopy [66]. In these assays, cannabidiol (CBD) modulated neuropathic signals by decreasing nerve firing and preventing axonal demyelination (Fig. 5 [3]) [66]. Given these measurable changes in pain pathways, the MIA model is of interest to test the analgesic effects of investigational therapies.

Collagenase Model: Collagenase is another popular chemical means of inducing OA in animal by breaking down collagen in the extracellular matrix and subsequently allowing degradation of cartilage through native architectural disruption, inflammation, and joint laxity [82, 83]. This defect model is sufficient to allow for analysis of both structural and functional outcomes [84, 85]. For example, in rabbit, HA + growth factors improved both histologic scoring and lameness [84]. Collagenase model can also allow for biomechanical characterization of cartilage by detecting Young's modulus stiffness after nanoindentation

(Fig. 5 [1]) which were improved by the synthetic artificial stem cell (SASC) [71]. Collagenase-induced OA also causes gross erosive changes on rat knee that can be improved by an amnion hydrogel paired with adipose-derived stem cells (ADSC) (Fig. 5 [2]) [72]. These ranges of outcomes make collagenase an attractive model for OA. Aligning with the collagenase studies discussed, several clinical studies have been completed testing HA and amnion products, which demonstrated symptomatic improvement but no improved biomarkers at 1 year [86, 87].

Other Synthetic Models: Other synthetic models include papain injection, lipopolysaccharide (LPS) injection, quinolones, complete Freud's adjuvant, and high-fat diet. Some of these are less commonly used, though there have been recent studies using some of these models. In mice, feeding with a high-fat diet can mimic the effects of obesity and metabolic syndrome on the development of OA in animal model [88]. Hahn et al. and Griffin et al. used this dietary method to test exercise as a modality for treatment or characterization of osteoarthritis [89, 90]. LPS is a bacterial derived toxin that can induce OA [91–93]. In horses, LPS caused inflammation and lameness in the horses which was attenuated by hyaluronic acid (HA) treatment [94]. Papain is a protease that can degrade the extracellular matrix and contribute to OA [95]. A rat model with papain injection changed gait and pain responses and cartilage scoring, which responded to dexmedetomidine treatment [96].

Synthetic OA models provide quick and consistent induction of OA and are less invasive than surgery while still offering a full range of outcomes to study. However, there is still need for improved models because the synthetic model does not reflect primary OA and may not have a clear corresponding human phenotype.

Surgical Models—Surgical models involve invasive procedures that create a defect, instability, or modulate systemic endocrine signaling in the animal to induce OA. Several surgical models used are ovariectomy, orchiectomy, medial meniscus release, ACL transection, and subchondral/osteochondral bone defects.

ACL Transection: Anterior cruciate ligament transection (ACLT) is a commonly used model for OA development in animals. Exposure of the ACL for transection is seen in Fig. 6C. In some ways, this mimics the post-traumatic development of OA in humans—after a traumatic event, the joint is destabilized and abnormal joint biomechanics lead to degradative forces on the cartilage leading to OA. In rabbits, the ACLT model [97] exhibited decreased trabecular bone volumes vs. Sham surgery; treating with a cathepsin K inhibitor MIV-711 better preserved the bone volume and subchondral bone plate thickness as evidence by uCT and decreased markers of bone resorption (urinary HP-1) and cartilage degradation (urinary CTX-II) [97]. The post-traumatic ACLT OA model has also been used in both rodents and dogs demonstrating its versatility across species [98, 99]. In these studies, injectable hyaluronic acid (HA), HA hydrogels, and platelet rich plasma (PRP) were tested, which are clinically used therapies. Larger animal models like dog allow for the study of pain/functional outcomes that are more similar to human like range of motion, lameness, and gait symmetry [99]. Jeon et al. investigated the role of senescent cells (cells that no longer divide) in post-traumatic OA using the ACLT model [100]. With luciferase

fluorescent reporter, they tracked intra-articular senescent cells were prevalent after ACLT (Fig. 6A) and selective clearance of senescent cells through ganciclovir treatment yielded reduced progression of OA histologically and decreased OA symptoms (pain) in mice [100].

Medical Meniscectomy: The medial meniscectomy model is used to induce OA in various categories including partial, medial vs. Lateral, bilateral meniscectomies, and in combination with ACLT [101]. The meniscectomy can induce radiographic changes seen in human like joint space narrowing and osteophyte formation [101]. Zhou et al. 2019 demonstrated thicker cartilage, increased proteoglycans, and reduced inflammatory markers in meniscectomized rats treated with adipose-derived stem cells [38].

Osteochondral Defect: Osteochondral defects (OCD) are created by drilling and can degrade the cartilage and underlying subchondral bone. The surgical exposure with insertion of K-wire is shown in Fig. 6C [102]. Such technique is useful for evaluating scaffolds and hydrogels for cartilage regeneration [103]. A bio-engineered, piezoelectric poly-L-lactic acid (PLLA)-collagen scaffold was implanted in rabbits with OCD [104]. The rabbits were subsequently exposed to exercise to transmit a mechanical stimulus to guide cell migration and electrical charge-based cartilage regeneration. There was good histologic formation of hyaline cartilage and gross repair of defect compared to untreated controls as seen in Fig. 6B [104]. Although not all patients develop osteochondral lesions, such OCD model is still essential to develop therapies that target cartilage regeneration.

Systemic Surgeries for OA Induction: Systemic surgeries may involve an endocrine axis that contributes to OA development through molecular signaling pathways and inflammatory pathways. Park et al. used a bilateral orchidectomy in rats to induce testosterone deficiency and study metabolic contributions to OA [105]. Testosterone deficiency is thought to increase the inflammatory state and modulate insulin resistance and metabolic syndrome implicated in OA development [106, 107]. The ovariectomy model is used in female rodents to induce OA, which may mimic the post-menopausal phenotype [108, 109]. The endocrine and metabolic contributions to OA are active areas of study, and thus, exercise and metformin have been tested in the rodent ovariectomy model of OA [108]. Such work is important as the population of obese patients increases.

Surgical models are quick and consistent induction methods of OA and can mimic the post-traumatic and metabolic phenotypes. However, the OA defect may depend on the reproducibility of the surgeon/surgical technique and surgical models do not mimic primary OA. The surgical osteochondral defect model and ACLT model are shown in Fig. 6C from a study that looked at measurability of pain, which was better detected in the ACLT group [102].

Tertiary Model

The tertiary model of OA was recently described by Esdaille et al. [25]. These tertiary models pair a surgical procedure followed by intense exercise to induce OA changes in the joint. Several have used the tertiary model [110–113]. Tertiary models can be used to test therapeutics. In one study, a horse fetlock joint had an osteochondral chip fragment in the

metacarpophalangeal joint and the horse was exposed to intense exercise. In horse, bone marrow–derived mesenchymal stem cells (BMSC) (Fig. 7a–d) decreased osteophytes and synovial effusion compared to contralateral, untreated control joint (Fig. 7e–h) as evidenced by MRI (Fig. 7a, b, e, f), x-ray (Fig. 7c, g), and ultrasound (Fig. 7d, h) [112]. Umbilical cord–derived stem cells were also tested but did not produce the same therapeutic effect as BMSC [112].

Tertiary OA models may allow faster induction than surgery alone and mimic post-traumatic OA. Larger animal models are used and allow use of imaging modalities to determine the degree of OA as is done in human, though there are increasing reported uses of micro-CT scans in small animals like rat and rabbit [77, 114]. The tertiary model does require an additional methodologic step as there are 2 induction approaches which may be more time and cost intensive.

Miscellaneous Models (Ex Vivo Model)

A brief focus is now placed on other types of osteoarthritis models in addition to the animal/in vivo models discussed above. Such models can be useful in research to determine molecular pathways and how the cellular network interacts, though there is a challenge to recapitulate the intricacies of in vivo models.

Ex Vivo

Human—The ex vivo model consists of extracting living tissue from an animal or human and then sustaining the tissue in a laboratory environment through cell culture [23]. In human, such model has been used after extracting osteochondral plug tissue from human femoral heads or tibial plateaus during surgical hip or knee replacement, respectively [115]. Kleuskens et al. demonstrated human cell viability up to 4 weeks in culture conditions [116]. In a study by Li et al., human femoral samples underwent inflammatory induction with interleukin one beta (IL-1B) and tumor necrosis factor alpha (TNF-a) and subsequently measured upregulated gene expression of catabolic proteins like matrix metalloproteinase 3 (MMP-3) and inflammatory interleukins and down-regulation of anabolic genes like collage 2 and proteoglycan [115]. Such studies can aid in studying implicated pathways in OA development and screening for new drugs. It is noted that such explants come from donors with presumed pathology leading to surgical intervention without opportunity to compare to healthy human donors.

Animal—Ex vivo tissue has also been taken from animals like rats and pigs to study a post-traumatic model of OA. Similar to the dynamic model described above, an impact device was used to cause injury to the pig knee post-mortem [117]. Subsequently, the knee was treated with interleukin receptor antagonist protein, hyaluronan, dexamethasone, or mesenchymal stem cells to study therapeutic effects on gene expression of inflammatory and cartilage-damaging markers [117]. In the rat model, synovium from a surgical ACL and meniscotibial ligament transection was added to culture of harvested chondrocytes. The authors found that using early post-traumatic synovium yielded a protective effect from cartilage catabolism, but late post-traumatic synovium lost this protective effect [118]. Using animal models for extraction of tissue offers an advantage of in vivo manipulation followed

by ex vivo analysis and can overcome the challenge of finding healthy human donors. In addition, data collection is easier in an ex vivo model because the surrounding media can be continuously sampled, whereas repeated synovial or serum analysis is limited in vivo. Multiple osteochondral plugs can be harvested from the same animal and cultured for almost 2 months, which has potential to reduce the number of animals used experimentally [119]. Given the pig model's ex vivo use post-traumatically, one study also suggests that commercial pigs spontaneously developing OA could be used for tissue harvest and further study as an additional phenotype of OA [42].

Outcome Measures

There have been recent reviews that have looked at outcomes [41, 120]. Here, we discuss updates to outcome measures since 2016 [24]. Outcome measures are important to consider because human clinical trial initiation relies on preclinical data to justify their use in human. Outcomes in preclinical studies include both post-mortem and in vivo measurements. Depending on the study chosen, there may be more interest in a particular outcome measure. Of note, there is not a consensus in vivo biomarker studied in human clinical trials that predicts symptomatology, which makes the search for a disease-modifying therapy elusive. Some accepted measured methods in human trials are histomorphometric change based on radiographic changes in joint space width, presence of osteophytes and subchondral sclerosis/cysts, and more advanced imaging techniques like MRI to identify glycosaminoglycan content in the joint space [121–123]. Molecular markers like cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase (MMP) are used as surrogate markers in clinical trials though do not always correlate clinically [124]. There is also emerging study of biomarkers via bioinformatics approaches for which animal models play an important role [125–127]. Clinical trials must be symptom focused, which makes the preclinical trials left to also test pain/function in the animal, or use a surrogate marker that may have potential for disease modification, though there is no consensus on outcome measures in animals.

Histopathology

As discussed above, the Osteoarthritis Research Society International (OARSI) outlined a grading system for cartilage of several animal species [43–49]. Such scoring is demonstrated practically in Fig. 3, and Table 1 demonstrates the scoring criteria. We discussed these previously [24] and they are still relevant to current studies, though there are still efforts to improve these systems [128]. This group included peri-articular changes in their system as key features of OA and expand the tissue sections of interest to allow further analysis of the arthritic joint [128]. Other modalities beyond histologic scoring include immunohistochemistry, which has been used to identify protein expression of AMP-activated protein kinase and propose a mechanism for metformin modulation of post-traumatic OA in mice [129]. Post-mortem evaluation in animal can provide key insights into study of the disease and propose a role for pathways and therapeutics, though these measures are not feasible in human patients with active OA.

Imaging Modalities

As noted above in Fig. 4, CT scan can be used to measure bone volumes and degree of osteophyte formation, even in small animals like mice [57]—although these scans are not used routinely in all patients with OA, they provide a more precise means of measure compared to plain radiograph. Magnetic resonance imaging (MRI) and ultrasound are also used as seen with Fig. 7 and the tertiary model to identify soft tissue synovitis and osteophytes. Korchi et al. used advanced MRI and angiography to correlate with histologic changes in dog OA [130]. Such studies may be helpful to allow better parameters of imaging modality to disease progression. Developing a surrogate would be helpful in human studies where imaging is possible, yet histologic analysis in real time is not possible [130]. Another new imaging technique used recently was real-time detection of calcium signaling in the dorsal root ganglia for pain analysis in surgical mouse OA model, though this required a surgical procedure to capture camera images at specified wavelengths [40, 131].

Biomarkers

Biomarkers can be tested in vivo and may act as a surrogate for disease progression and modification. For example, serum or urine sample can reveal elevations in markers of cartilage and bone turnover/degradation. Cathepsin K inhibitors are an attractive drug class for disease modification of OA and osteoporosis because they act on osteoclasts to prevent cathepsin K proteolytic activity on collagen [132]. As such, animal studies in rabbit, dog, and monkey have noted decreased levels of serum and urine markers of bone turnover (collagen I cleavage product) and cartilage turnover (collagen II cleavage product): urine C-terminal telopeptide of type I collagen (CTX-I) and urine C-terminal telopeptide of type II collagen (CTX-II) in dog; serum CTX-I and urinary CTX-I, N-terminal telopeptide of type I collage (NTX-I) and CTX-II in monkey; urinary helical peptide (HP-1) and CTX-II in rabbit [97, 133].

An inflammatory state is implicated in osteoarthritis [17, 134]. Inflammatory biomarkers studied include C-reactive protein and prostaglandins, interleukins [135], and tumor necrosis factor alpha (TNF-a). Animals offer a benefit of synovial and post-mortem sampling that is not routinely feasible in human [135]. For example, an MIA rat model was used to implicate cell surface receptor P2X7R in the NF-kB inflammatory pathway [136]. Such animal studies are useful for better understanding molecular mechanism and identifying potential therapeutics.

Discussion

This review highlighted the latest animal models of osteoarthritis. Such information is pertinent as researchers continue to investigate disease-modifying osteoarthritis drugs and therapies. Despite years of research, however, such therapy remains elusive [137–139]. There is a continued need for use of animals in research as they can assist in bringing therapies to clinical trials [140]. As captured in this review, induction methods in vivo vary significantly from non-invasive primary models with natural or genetically engineered induction to minimally invasive secondary synthetic models via injections or feeding a high-fat diet. In addition, invasive techniques can mimic post-traumatic OA as seen in the

secondary surgical model and the tertiary model which adds intense exercise. More recent studies have tried to take advantage of ex vivo models using human tissue to study OA and reduce the number of animal studies [115, 116]; similarly, taking living tissue from animals may allow for decreased need of additional sacrifice time points if the tissue can be studied for 2 months [119]. We did not discuss in vitro models though researchers are also exploring this option to study pathogenesis and therapy targets of OA [23, 141–143].

As summarized in Table 2, there are several OA models in place for investigation identified in this review [23–25, 41]. Researchers can choose one that aligns with their investigation of interest during study design. For example, they may use a surgical model if the investigated treatment is for a post-traumatic phenotype [100] or may use a primary naturally occurring model if studying the natural progression of disease [50]. As the population ages with a higher percentage of obese patients, the high-fat diet model is of interest in evaluating systemic contributors to OA and better understanding the pathways involved [88].

Special consideration is also paid to the species selection when designing a study. Anatomically, larger animals like sheep and horse will have cartilage size similar to humans and display clinical deficits that play a role in therapy development [112, 144]. Rodent and rabbit models are more feasible in handling, cost, and scaling at larger quantity through housing in small cages [145]; indeed, they have proven useful in generating preclinical data [97]. There are many OA induction methods in these small animal models that researchers can tailor to a design that fits their needs [41]. Small animals also have qualities that allow study of outcome measures like pain, which is an important part of the human experience.

In 2016, we reviewed outcome measures of animal OA models and stressed the importance of using imaging modalities as in vivo means of assessing outcome measures. It was highlighted that such imaging techniques lacked standardization in animal [24]. Imaging modalities are still used in animal, though there is still no consensus technique for study in animal. In fact, there are human clinical trials to validate imaging outcomes for OA, yet the inconsistent correlation to altering the clinical course presents a significant challenge [146–148].

It should be mentioned that despite the numerous studies since 2016 reviewed here, a disease-modifying therapy for OA remains elusive. In our prior review, we provided a comprehensive analysis of animal models used in osteoarthritis and highlighted the primary and secondary models and subtypes [24]. In 2022, we expanded on the 2016 review to focus on regenerative engineering models and introduced the tertiary model, which consists of a traumatic insult followed by intense exercise to achieve a post-traumatic phenotype [25]. In this work, we provide significant updates to the models with updated literature review, and also explore the ex vivo model as a potential method of investigating therapeutics and mechanisms.

Several therapies have made their way to clinical trials with aid of preclinical studies [140]. For example, HA and PRP injections were studied in dog [99] and also have been actively studied in human clinical trial [149]. Systemic oral therapies (MIV-711 cathepsin

K inhibitor) have also been directly translated from animals rabbit and dog [97], to pharmacokinetic analysis in monkey [133] to a clinical trial [150].

There have been both safety and efficacy concerns of therapies that have left therapies out from gaining FDA approval [148, 151, 152]. Future work in preclinical and clinical investigation of OA is required to translate therapies to clinic. Animals serve an important role in research efforts, though it is noted that demonstrating efficacy of therapy in animal is not a guarantee of translatability. The preclinical and clinical research communities should work in synergy to develop therapies for OA in the coming years.

With the above discussed utility of OA models, there are several limitations of animal studies including an induction method that recapitulates the intricate pathogenesis of osteoarthritis; species selection; pain assessment; and standardization of outcome measures in animal studies. Reproducing the multifactorial pathogenesis of OA is a key drawback in preclinical studies. Using one method for animal OA induction like surgery may oversimplify the complex interactions of the human disease; changing multiple parameters still may not appropriately recreate the disease process and increase the number of animals needed. Species selection is also important in study design. Using large animals that better represent human cartilage are challenging because of scalability [41]. Many research labs studying OA may not have easy access to the facility, maintenance, and large number of animals needed for formal statistical study leading to use of smaller animals. Given the physiologic and cartilage differences in small animals, extrapolation of all results of a treatment should be interpreted with care [26, 35]. Beyond histologic evaluation, pain is studied in small animal models, but attempts at quantifying pain with nerve stimulation measurements and mechanical provocation of pain are difficult to interpret in the context of human symptoms [40, 41]. Furthermore, humans experience pain subjectively through higher order cognitive pain processing, which makes objective validity in these small animals an obstacle [153, 154]. A more representative pain model in animal study would be a seminal contribution to the field. Outcome measures must also be selected that fit the objective of the research study in animal. Standardization in measuring biomarkers in human OA remains elusive, but is an essential step in drug development [155]. Accordingly, without consensus in human disease, OA researchers must predict the most suitable outcome measure for their study, which presents a significant limitation in translating animal studies to clinical trials.

Conclusion and Future Directions

This review provides a much-needed update to the literature on animal models in OA. We also discussed the ex vivo model and regenerative engineering techniques that show promise in translating therapies. OA develops in humans in several ways including natural progression, post-traumatically, and with systemic metabolic contributions. A range of animal models exist to mimic these human phenotypes and allow for comprehensive study. Despite many animal models of OA, there are limitations that researchers must consider in developing treatments and understanding pathogenesis. In progressing treatments, small animals can initially provide evidence of cartilage regeneration, while larger animals may more suitably resemble human cartilage characteristics, though the patient experience

with the disease is not fully recapitulated in animals. To translate therapies to the clinic, researchers should continue to study pathogenesis and test disease-modifying therapies; when possible, focus on translatable outcomes should be considered. In light of the limitations discussed, animal models will play an essential role in the development of key therapies for treating osteoarthritis.

This review identified several promising experiments in regenerative engineering to study and treat OA [71, 72, 104]. Regenerative engineering converges the disciplines of advanced materials science, stem cell science, physics, developmental biology, and clinical translation to regenerate tissue like bone [156–161] and complex organ systems using several technologies including nanofibers [162]. This discipline serves as a path forward for developing a disease-modifying therapy for OA.

As mentioned, regenerative engineering techniques have been able to demonstrate cartilage regeneration [71, 72, 104]. In addition to performing additional studies to initiate translation of the therapy in the clinic, mechanistic studies can be pursued to further optimize the treatment. For example, time-lapsed micro-CT scans have been used to understand bone regeneration and certainly there are different stages of healing that occur in OA [163]. If the scientific community can characterize stages of healing of cartilage, then timed treatments can be pursued using the synthetic artificial stem cell, which recreates the stem cells' dynamic secretome [71]. Additionally, nanomaterials are used as an effective intra-articular therapy in OA models [164]. Injectable nanoparticles synthesized with hyaluronic acid could significantly inhibit cartilage destruction in knee joints [165] and chondroitin sulfate cross-linked nanoparticles within artificial anti-inflammatory macrophages decreased joint erosion and preserved glycosaminoglycans [166]. Anti-inflammatory properties of gold nanoparticles further demonstrate promising regenerative engineering techniques for translatable therapies for OA treatment [167]. Regenerative engineering has proven useful in developing translatable therapies for tissue regeneration of ligament and tendon [168–170], and such techniques are promising for use in cartilage regeneration to treat debilitating osteoarthritis.

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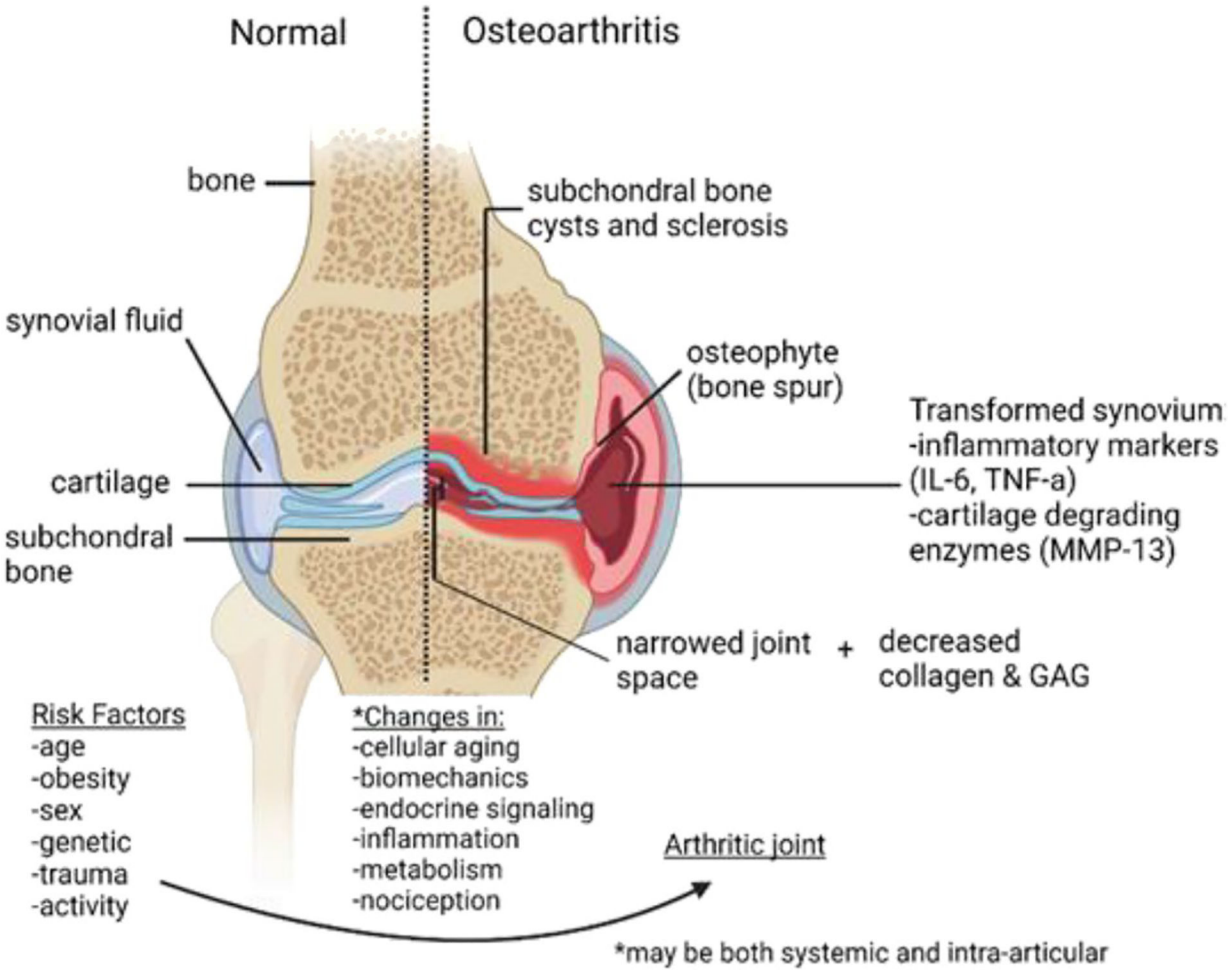


Fig. 1.

Characteristics of osteoarthritis. (Left) Highlights normal joint and risk factors of OA. (Center) Lists the pathophysiologic changes that contribute to (right) the arthritic joint. Classic radiograph findings of subchondral bone cysts/sclerosis, osteophytes, and narrowed joint space are shown. MRI findings of decreased collagen and GAG content are demonstrated. Synovial fluid changes are seen with inflamed synovium and cartilage-damaging enzymes. Created with BioRender.com

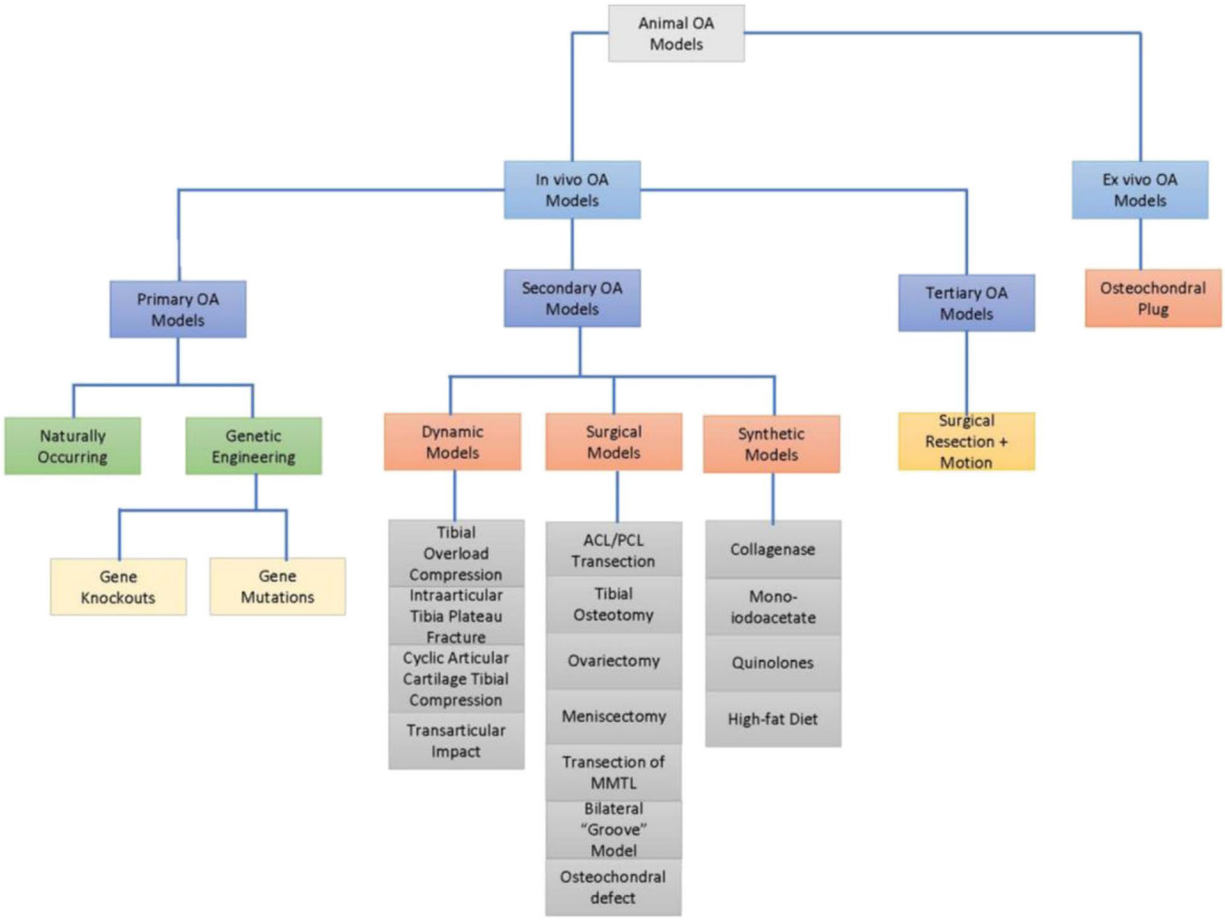
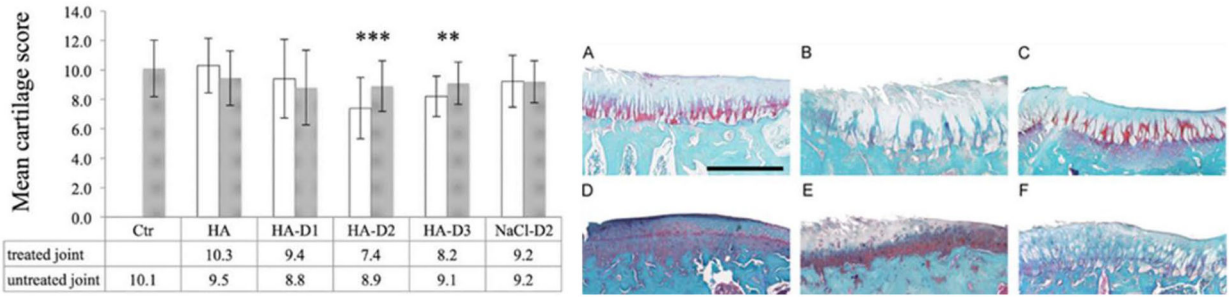


Fig. 2.

Classification scheme adapted from Esdaille et al. Additional models include the surgical osteochondral defect, synthetic high-fat diet, and new category of ex vivo osteochondral plug. The ex vivo model reflects manipulation of live tissue outside of the animal. Adapted from Esdaille et al. [25]



	Ctr	HA	HA-D1	HA-D2	HA-D3	NaCl-D2
treated joint		10.3	9.4	7.4	8.2	9.2
untreated joint	10.1	9.5	8.8	8.9	9.1	9.2

Fig. 3.

Naturally occurring model. In guinea pig, OARSI cartilage scores demonstrate naturally occurring OA at 16 months (left panel Ctr group score 10.1 and right panel, image A demonstrating loss of proteoglycans and fissuring). Guinea pigs were treated with weekly hyaluronic acid + chemokine C–C motif ligand 25 injection from age 11 months to 16 months at time of sacrifice. The medium and high dose groups (HA-D2 and HA-D3) had statistically significant lower OARSI cartilage scores compared to the contralateral control joint (left panel HA-D3 group 8.2 ** ($p < 0.01$) and HA-D2 group 7.4 *** ($p < 0.001$) groups). In right panel, HA-D2 and HA-D3 groups are shown in panel D and E and demonstrate more proteoglycan content and a smoother cartilage layer and less signs of destruction as compared to control group panel A. Scale bar = 500 μm. Adapted from Ringe et al. [50]

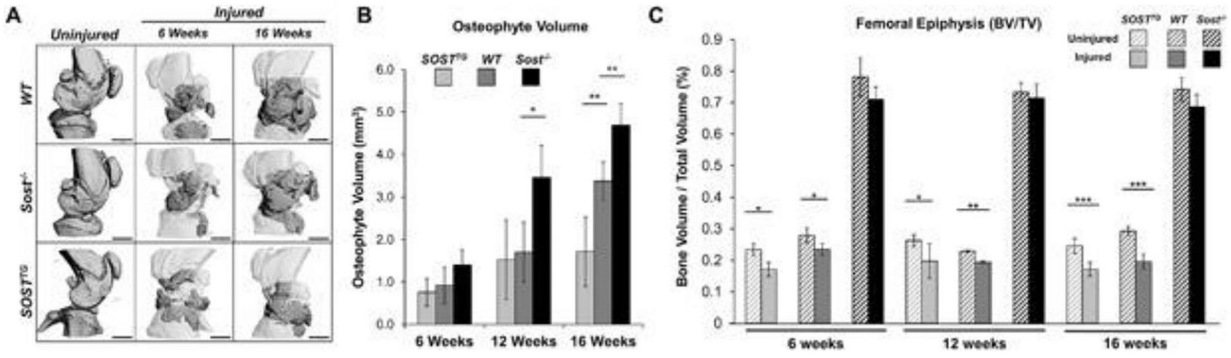


Fig. 4.

Dynamic/genetic model. Three different genotyped mice groups (wild type = WT, sclerostin knockout = Sost −/−, transgenic sclerostin = SOST TG) injured by dynamic tibial compression developed ACL rupture and subsequent OA. **A** uCT scans with gray highlight of osteophyte formation. Scale bar = 1 mm. **B** Osteophyte volume captured at 3 time points. **C** Femoral epiphysis bone volume to total volume ratio at 3 time points. SOST TG group had significantly less osteophyte formation relative to WT and Sost −/− groups (**A** and **B**) and relative subchondral bone volume compared to the WT and Sost −/− groups. From Chang et al. [57]

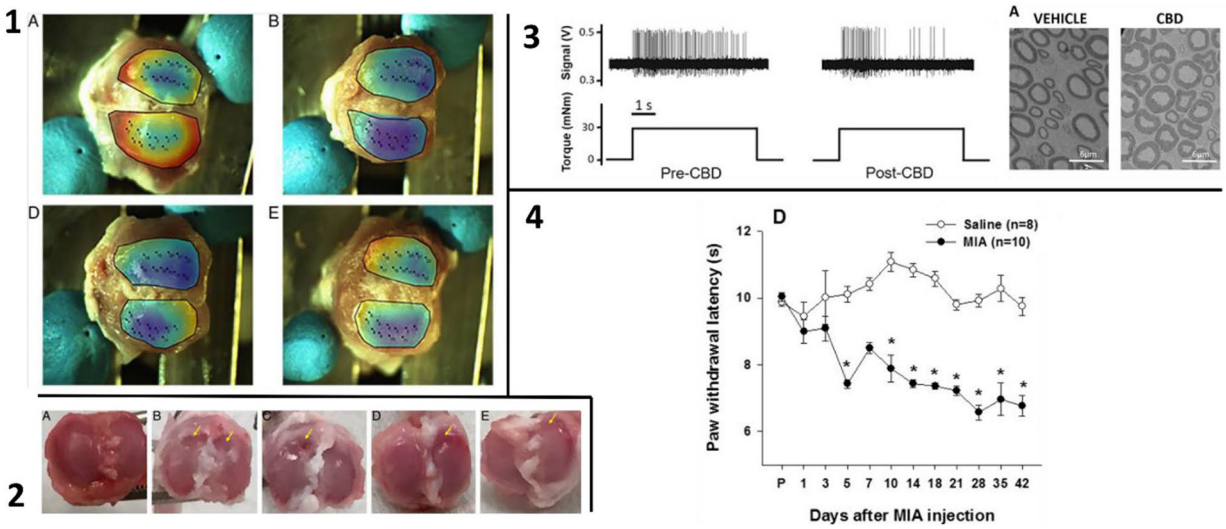


Fig. 5.

Secondary synthetic MIA and collagenase models. Left Figs. 5.1 and 5.2: collagenase model. Right Figs. 5.3 and 5.4: MIA model. Figure 5.1 at top left shows Tibial Young's modulus reflecting stiffness of the joint. Red regions reflect increased stiffness as seen in panel A, sham group (positive control); and blue regions reflect lower Young's modulus/ stiffness as seen in panel B, untreated OA group (negative control). There is recovery of stiffness (orange color) when treated with SASC as seen in panel E [71]. Figure 5.2 at bottom left shows gross articular surface with yellow arrows highlighting areas of erosion. Sham group with smooth surface shown at left panel A, negative control with erosion in panel B, and smoother appearing treatment group with amnion membrane + ADSC in panel E [72]. Figure 5.3 at top right shows both joint afferent firing electrophysiologic study (left figure) and electron micrograph of saphenous nerves axons (right figure). Left Fig. 5.3 demonstrates decreased nerve firing when a noxious torque is applied to the knee joint of rat post-CBD administration as evidenced by fewer signaled action potentials. Right Fig. 5.3 demonstrates electron micrograph with axonal demyelination (thinner membrane at left) shown in vehicle-treated group (negative control) compared to preserved axonal myelination (thicker membrane at right) shown in CBD-treated group. Adapted from Philpott et al. [66]. Figure 5.4 at bottom right shows time to paw withdrawal when infrared heat is applied to paw (painful). MIA-treated rats have short heat latency (remove paw quickly from heat) compared to saline-treated rats (leave paw on heat source longer) [70]

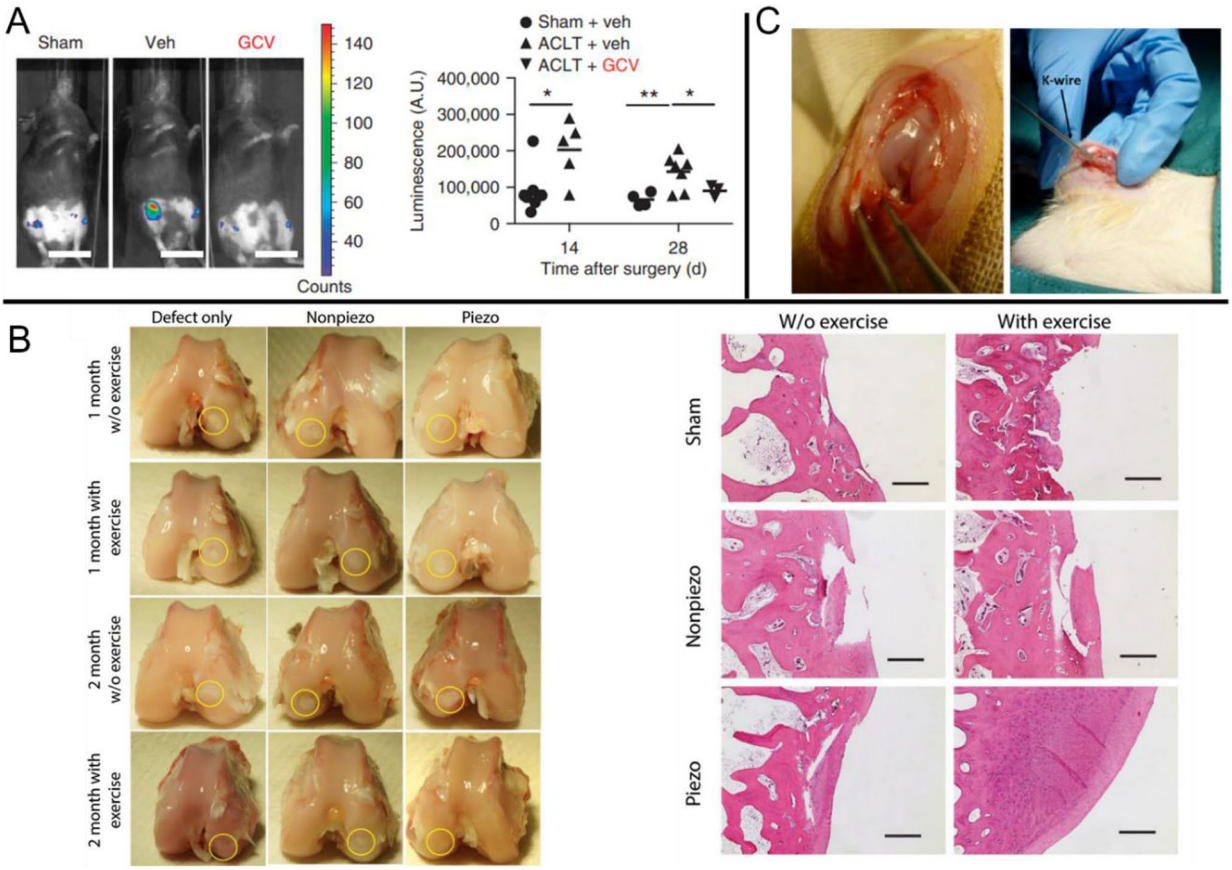


Fig. 6.

Secondary surgical models. **A** Top left shows mouse intra-articular, in vivo luminescence image that highlights increased senescent cells in vehicle-treated group compared to the ganciclovir-treated group at right without as much luminescence. Also evidenced by quantification at right of **A** by upside down triangle [100]. Scale bars = 2 cm. **B** Bottom left demonstrates gross image of osteochondral defects on rabbit femur showing fibrillation in defect only without exercise and near healing of the defect in the piezoelectric + 2 month exercise group. **B** Bottom right shows hematoxylin and eosin stain demonstrating hyaline cartilage in the piezoelectric + exercise group compared to sham treatment with degradation of cartilage. Scale bars at right = 500 μm. Adapted from Liu et al. [104] **C** Top right shows the surgical exposure technique of the ACL transection and osteochondral defect model similar to those noted in the other two studies in this Fig. [102]

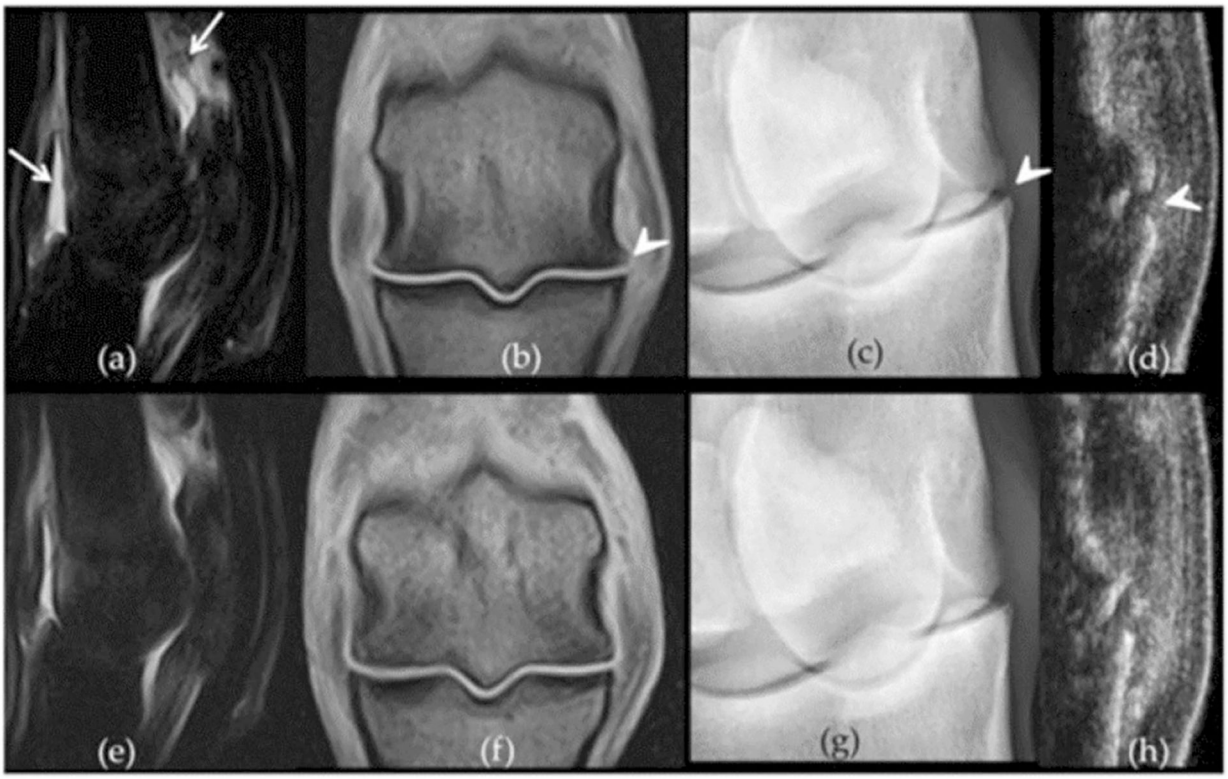


Fig. 7.

Tertiary models. Horse fore fetlock joints at 12 weeks post arthroscopic osteochondral chip fragment of the metacarpophalangeal joints. **a**–**d** (top) Left fore fetlock was treated at 3 weeks with placebo injection, and **e**–**h** (bottom) right fore fetlock was treated at 3 weeks with bone marrow–derived mesenchymal stem cells (BM-MSCs). Magnetic resonance image at left figure (**a**, **b**, **e**, **f**); plain radiograph (**c**, **g**); ultrasound at right figure (**d**, **h**). On the left placebo-treated joint, there is grade 3 synovial effusion (white arrow in **a**) compared to grade 2 synovial effusion in the right BM-MSC-treated joint (**e**, **f**); grade 3 osteophytes in placebo-treated left forelock (arrowheads in **b**–**d**) compared to grade 1 on the BM-MSC treated right on radiograph (**g**) and grade 2 on ultrasound (**h**). From Bertoni et al. [112]

Table 1

OARSI semi-quantitative histologic scoring for guinea pig cartilage. From Krauss et al. [43]

Parameter	Grade	Description
Articular Cartilage Structure	0	Normal, smooth uninterrupted surface
	1	Mild surface irregularities (undulations)
	2	Irregular surface, 1–3 superficial clefts (fissures)
	3	> 3 fissures and/or loss of cartilage in the superficial zone
	4	1–3 fissures extending into the middle zone
	5	> 3 fissures and/or loss extending into the middle zone
	6	1–3 fissures extending into the deep zone
	7	> 3 fissures extending into the deep zone and/or loss of cartilage to deep zone
	8	Fissures or loss of cartilage extending to the zone of calcified cartilage
Proteoglycan Content (staining by toluidine blue)	0	Uniform throughout articular cartilage
	1	Decreased in superficial zone only and for < half the length of the condyle or plateu
	2	Decreased in superficial zone for half the length or greater of the condyle or plateu
	3	Decreased in superficial and middle zones for < half the length of the condyle or plateu
	4	Decreased in superficial and middle zones for half the length or greater of the condyle or plateu
	5	Deceased in all 3 zones for < half the length of the condyle or plateau
	6	Deceased in all 3 zones for half the length or greater of the condyle or plateau
Cellularity	0	Normal (1/2 cells/lacuna)
	1	Diffuse/slightly hypercellularity
	2	Regions of hypercellularity and clustering
	3	Diffuse hypercellularity
Tidemark Integrity	0	Intact/single tidemark
	1	Crossed by vessels/duplication of tidemark
Additional Features		
Osteophyte		
	0	No osteophyte present
	1	Small osteophyte
	2	Meduim-sized osteophyte
	3	Large osteophyte

Osteophytes are tallied for three joint margins (medial and lateral tibial plateau, and lateral femoral condyle)

Total score 0–9

Table 2

Advantages and disadvantages of animal models

OA model	Advantage	Disadvantage
Primary		
Naturally occurring	• Mimics the spontaneous disease progression closely	• Takes a longer time for animal skeletal maturity
Genetic engineering	• Reproducible • Allows study of the mechanism of disease	• Not as suitable for therapeutic studies • Single gene change does not recapitulate the complex signaling pathway
Secondary		
Dynamic	• Reproducible trauma • Non-invasive	• Requires expensive machinery
Synthetic	• Quick onset of OA • Reproducible • Allows biomechanical characterization of cartilage (i.e., collagenase model) • Less invasive than surgery • Shows measurable changes in pain (i.e., MIA model)	• Does not reflect primary OA
Surgical	• Quick and consistent method of OA induction • Mimic post-traumatic OA development as in human (ACL transection) • Induce radiographic changes comparable to humans (medial meniscectomy) • Can be used to evaluate biomaterials in OA (osteochondral defect)	• Invasive to the animal • Costly • Relies on skill of surgeon • Surgical protocols require thorough planning
Tertiary	• May be used to treat therapeutics • May allow faster OA induction than surgery alone • Mimics post-traumatic OA	• Cost intensive • More invasive • Surgical and exercise protocols require thorough planning • Facilities/staff/housing of animals may not be available, especially in the case of large animal like horse
Miscellaneous (ex vivo)	• May look closely into molecular and cellular pathways ex vivo • Samples can be studied for months ex vivo allowing for several assays	• Challenge to recapitulate OA mechanisms as seen in in vivo models

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