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Preclinical Studies for Cartilage Repair: Recommendations from the International Cartilage Repair Society

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Abstract

Investigational devices for articular cartilage repair or replacement are considered to be significant risk devices by regulatory bodies. Therefore animal models are needed to provide proof of efficacy and safety prior to clinical testing. The financial commitment and regulatory steps needed to bring a new technology to clinical use can be major obstacles, so the implementation of highly predictive animal models is a pressing issue. Until recently, a reductionist approach using acute chondral defects in immature laboratory species, particularly the rabbit, was considered adequate; however, if successful and timely translation from animal models to regulatory approval and clinical use is the goal, a step-wise development using laboratory animals for screening and early development work followed by larger species such as the goat, sheep and horse for late development and pivotal studies is recommended. Such animals must have fully organized and mature cartilage. Both acute and chronic chondral defects can be used but the later are more like the lesions found in patients and may be more predictive. Quantitative and qualitative outcome measures such as macroscopic appearance, histology, biochemistry, functional imaging, and biomechanical testing of cartilage, provide reliable data to support investment decisions and subsequent applications to regulatory bodies for clinical trials. No one model or species can be considered ideal for pivotal studies, but the larger animal species are recommended for pivotal studies. Larger species such as the horse, goat and pig also allow arthroscopic delivery, and press-fit or sutured implant fixation in thick cartilage as well as second look arthroscopies and biopsy procedures.

Keywords

animal models, clinical trial, outcome measures, preclinical research, cartilage repair

Translation of new products from discovery to the clinic is a challenging endeavor. Only a handful of new repair methods ever attain regulatory approval for clinical use. In 2007, the Food and Drug Administration (FDA) issued a draft guidance to industry on steps that could be taken toward obtaining an Investigational Device Exemption (IDE) or Investigational New Drug (IND) application for new products intended to repair or replace damaged knee cartilage.[1,2] Recommendations included that the investigational device be adequately described, including all individual components and how they might interact with other materials or instruments, as well as basic manufacturing and sterilization information. Also, animal models would be used to evaluate the biological response to the product, the durability of the response, toxicology, and dose response. Animal cartilage repair models were suggested to control for cartilage defect type, preparation, and location. Assessments such as macroscopic,

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histological, biomechanical testing, and biocompatibility would be used as endpoints in proof of efficacy and safety. It was proposed that animal studies be based on rationale derived from the literature. The purpose of this review is to appraise published animal cartilage repair models and suggest guidelines for preclinical cartilage repair studies. Cartilage repair method development activity is now a global initiative throughout Europe, Asia, and the Americas, so in formulating this recommendation paper, it was necessary to consider the different regulatory and experimental environments in these regions and find some commonalities that would drive congruent and possibly shorter development pathways leading to highly efficacious cartilage repair methods.

A primary endeavor of experimental biology is to create model systems that are predictive of outcomes in patients that in themselves are highly variable. This is especially true in cartilage repair, where lesion size, location, depth, number and age, intercurrent disease, activity level, previous treatments, and other factors affect outcomes. For this reason, animal model use requires some assumptions and simplification to reduce the inherent variability seen in patients. The ideal animal model would develop spontaneous chondral lesions in the medial or lateral compartment of the femorotibial joint, and these lesions would enlarge over a few months, during which time they could be identified with noninvasive methods such as imaging or biomarkers. The ideal animal model would be large enough for gait analysis studies and manipulative tests, as well as high-resolution quantitative imaging and arthroscopic interventions, but not require expensive or special care facilities. Assessment of pain and lameness would be quantifiable and correspond to lesion severity. Relative cartilage thickness and geometry of the femoral condyles in the animal knee (stifle) joint would be similar to the human knee such that fixation and retention of repair constructs would be possible. Rehabilitation regimes, including reduced weight bearing, continuous passive motion, and physiotherapy, would be used, and progression of repair and healing would be evident because the aforementioned outcome measures would agree. Most important, the biologic repair response would be predictive of active adults, who comprise much of the patient population needing cartilage repair.

Clearly, the ideal model is not attainable, so stakeholders with specific interests have developed model systems that are practical for their goals. Since research and development groups in academic and industrial settings face daunting financial and time limitations, model conditions are optimized for specific applications. A simplified model system and stepwise approach may be necessary to elucidate the strengths and weaknesses of any new cartilage repair procedure. This approach initially leads research and development groups away from complex and potentially more highly predictive models to simpler models in rodents, guinea pigs, or rabbits to solve specific problems such as retention or fixation, survival of implanted cells, dose response, or modulation of the biologic response. To complete a series of such experiments, such laboratory animals are used in short- or medium-term experiments with several evaluation points usually lasting fewer than 90 days. The outcomes from such screening studies are primarily morphological with reliance on histology but may include other outcomes such as mechanical, biochemical, and molecular analysis of repair tissue depending on the interests or goals of the researchers. Once proof of principle for the cartilage repair procedure or product is established, pilot studies with large animal species, including the dog, pig, sheep, goat, and horse, are conducted, and finally, pivotal studies from 6 months up to a year or longer are required. Ideally, progression of cartilage repair is followed at intervals by procedures that mimic human clinical trials, including arthroscopic biopsies, *in vivo* imaging, and kinematic analysis. Human and veterinary specialists in musculoskeletal imaging, surgery, physiology, and pathology who are intimately familiar with the natural history of chondral lesions should be consulted for input into the design and analytical outcomes to ensure fidelity between models and clinical reality.

Other groups with a vested interest in the development of cartilage repair include investors, regulatory agencies, clinicians, and patients. Each can benefit from a better understanding of the predictive value and limitations of animal models. In return for their support, the research community needs to deliver timely, accurate, and predictive data that facilitate the arrival of new cartilage repair methods in the clinical setting.

In Vivo Preclinical Procedures

Animal Age and Cartilage Maturity

Any novel therapy aimed at treating adult patients should ideally be tested in experimental animals that have achieved skeletal and articular cartilage maturity. The underlying rationale for cartilage maturity is that chondral defects do not typically repair spontaneously, as described in detail below, as they do in the neonate and juvenile. The age of adult equivalency has been proposed for articular cartilage repair models on the basis of growth plate closure in the evaluation of biomaterials,[3] but these might not be adequate to predict the biological response of cartilage. It is important to note that skeletal maturity as defined by growth plate closure may be difficult to characterize because individual growth plates and secondary centers of ossification may remain radiographically evident for some time after exponential growth of long bones has ceased. For instance, the tibial crest apophysis may remain open up to 4 years of age in horses[4] and cattle, although this would have little impact

on cartilage repair in the femorotibal joint since the distal femoral and proximal tibial growth plates are closed by 2 years of age, and the femorotibal joint at this time meets more stringent criteria for cartilage maturity discussed here. Although sexual maturity in people and some animals occurs at nearly the same age as growth plate closure,[5] growth of long bones continues to occur in some species and varies according to anatomic location. Articular cartilage maturity, as indicated by the structural features of adult tissues such as zonal organization,[6] also occurs at different times compared to the closure of the epiphyseal growth plate. In humans, articular cartilage maturity as defined by these morphological properties is achieved near puberty, whereas the epiphyseal plate closure is reached significantly later.[7]

If growth plate closure is not a reliable indicator of the biologic response of articular cartilage to injury, a more orthodox approach would be to consider criteria that directly define adult cartilage maturity and its repair capacity. In this context, articular cartilage maturity is reached when chondrocyte growth and proliferation are completely arrested, a continuous layer of calcified cartilage separates the articular cartilage from subchondral bone, and the subchondral bone plate is minimally vascularized.[8,9] At this point, mature articular cartilage also demonstrates a degree of regional biochemical and biomechanical properties associated with a history of functional demands that are not found in juvenile tissue.[10,11] Irrespective of the criteria chosen, the primary age-related concern for cartilage repair models is an absence of the capacity for intrinsic repair, which is the case in the adult human. Intrinsic repair is the ability for chondrocytes themselves to proliferate and produce new functional matrix without the contribution of vascular elements.[12] Such scarless healing is well documented in embryonic and neonatal cartilage[13] and is not present in adult patients.

A common limitation of many rabbit cartilage repair studies is the use of immature rabbits with a robust intrinsic repair response that is not representative of the adult human. Skeletal and cartilage maturity of the rabbit is generally reached at 7 to 8 months of age. Wei and Messner[14] demonstrated that full-thickness articular cartilage repair in adult (8-month-old) rabbits was slower to reach the same level of fill and demonstrated inferior quality repair tissue than adolescent (5-month-old) and immature (3-month-old) rabbits at up to 12 weeks.[15] In a subsequent study by the same authors, fill was similar for all aged rabbits at 24 and 48 weeks postoperatively, and the superior lateral integration and tissue quality demonstrated in adolescent and immature rabbits at 24 weeks persisted to the 48-week time point. These data are compelling and in many ways set the gold standard by which the cartilage maturity of other species should be assessed. For this reason, rabbits aged to a minimum of 8 months are preferred since they more closely represent young adult patients. When rabbits less than 8 months old are used, such studies will overestimate the success of repair possible in other species, including humans. The main utility of the rabbit model is for proof of concept, formulation screening, mechanisms of action, and safety (see **Table 1**). A new product development program based solely on one species such as the rabbit is not recommended. The requirement for purpose-bred mature rabbits creates logistical barriers in most countries because the availability of retired breeders is limited, and purchasing younger rabbits to hold them in research facilities for months has practical limitations.

Goats and sheep are readily available from commercial and agricultural suppliers as 2-year-old or older animals. Animals of this age are considered mature based on the aforementioned criteria of zonal architecture, lack of spontaneous intrinsic repair response, and continuous calcified cartilage layer. Purpose-bred rather than random-source dogs are now required by most institutional review boards. Two-year-old dogs are considered mature based on the histology of their subchondral bone plate. Domestic and minipigs have closed distal femoral growth plates and most of the characteristics of mature cartilage by 18 months, although vascular penetration through the calcified cartilage is often present,[16,17] and slightly older animals would be preferable. Osteochondritis and cartilage growth deformities are common in domestic pigs unless their growth is restricted by diet and breeding.[18] The horse has been previously used as a clinical cartilage repair model in the distal femur and metacarpophalangeal joint. As in most large animal species, metaphyseal growth plate activity is variable from site to site. The horse is considered postpubescent at 18 months of age, with full maturation of the distal femoral cartilage, including formation of a tidemark, by 24 months of age.[19] Horses 2 years or older should be screened for naturally occurring disease, including osteochondritis dissecans and subchondral bone cysts in the femorotibial and femoropatellar joints before being admitted into a study.

It is important to consider the potential effect of age in repair models where the subchondral bone is fractured or debrided to elicit cartilage repair. The density and structure of subchondral bone and resident cell populations may be age dependent, but few published data are available. Morphometric criteria such as cell density and calcified cartilage thickness as well as markers of vasculature or the ability of subchondral-derived stem cells to proliferate and participate in cartilage repair might be useful in establishing comprehensive criteria for cartilage maturity. Until these parameters are established, researchers must carefully consider factors associated with cartilage maturity and the inherent variability in animals raised in different conditions when using animals for models of cartilage repair.

Table 1. Suggested Guidelines for Animal Models in Cartilage Repair

Species	Rodent: proof of principle, clearance, toxicology, safety Rabbit: proof of principle, developmental, formulation screening Horse, sheep, goat, pig: pivotal studies
Specific requirements of all animal models	Skeletally immature models may potentially be used for some initial or proof-of-concept studies. Only animals with fully mature cartilage structure should be used for pivotal studies. Control for lesion size, location, level of debridement, repair time Preplanned statistics on endpoints needed prior to initiating the study
Unilateral versus bilateral models	Ethics approval for bilateral models is typically institutional review board dependent. Bilateral models control for animal-to-animal variability in repair response. Unilateral models allow for less initial weight bearing on defect limb, eliminate para-effects, and are more amenable to partial- or full-joint immobilization.
Acute versus chronic defects	Acute defects are treated immediately after creation but overestimate efficacy. Chronic defects and delayed repair are more predictive of efficacy.
Lesion location and size	Location: femoral condyle or trochlea Multiple lesions may be used in some models. Size: critical-size defects for pivotal studies
Postoperative care	Consider analgesic regimens, pain monitoring, controlled weight bearing, and exercise
Duration of study and time points	Acute defect: 0 to 3 days (document level of debridement) Implant retention: 1 to 30 days (need a tracer that does not become diluted by cell division or hydrolyzed from the test article) Development of repair tissue: 1 to 6 months Pilot studies: 6 to 12 weeks (proof of concept, mechanisms of action, pharmacokinetics) Pivotal studies: 6 to 12 months (repair efficacy) Slowly degrading implants: may require studies exceeding 12 months
Durability	Estimate from long-term animal studies but more appropriately evaluated in human clinical trials
Dose response	Depends on mode of action, defect size Biologics: *in vitro* tests and then pharmacokinetic studies
Cartilage repair evaluation	Gross macroscopic scoring of the whole joint, repair tissue histology Other possible endpoints: biomechanics, magnetic resonance imaging, micro-computed tomography
Mechanical testing	Type of test depends on the repair strategy Indentation and dynamic compression tests are more sensitive. Consider intrasite variability of repair.
Safety	Pre- and postoperative body mass, food and water intake, lameness, urinalysis, synovial fluid, serum chemistry, histology of all synovial tissues, organ weights, major organs
Good laboratory practice (GLP) and statistical analyses	Requires traceable procedures, written documentation, certificates of characterization, or certificates of analysis for the test article(s) GLP-like with gap analysis suffices for most efficacy studies. Preplanned statistical analysis prior to study initiation with adequate animal numbers

Choice of Animal Species

The FDA draft guidance[1] and the ASTM guideline for devices intended for cartilage repair[3] contain many references to the utility of large animal species such as the goat, sheep, and horse in cartilage repair models. Nevertheless, smaller laboratory animal models are valuable tools in the early stages of development for cartilage repair therapies and products.

Rodents will continue to be widely used for screening of new biomaterials and cell-laden constructs as subcutaneous implants. The rat may be unique in that it is one of the only species where pain outcomes are well validated.[20] Although rats may be valuable for screening treatments or devices for osteochondral repair with drill-hole models, articular implants are less practical because of the very thin articular cartilage, making the defect volume miniscule compared to human chondral defects. Furthermore, growth plates remain open during adulthood, creating a highly vascularized epiphysis, which may contribute to intrinsic cartilage repair.[21,22] These factors necessitate the use of other animal models to corroborate rodent model results. Other laboratory animals such as the guinea pig are widely used in osteoarthritis models but are generally too small for cartilage repair experiments. Physical and behavioral limitations of laboratory animals make larger animals

such as the goat, sheep, and horse more valuable for the study of cartilage repair.

Possibly the most often studied species for cartilage repair models is the rabbit, which is widely selected because of its availability, relatively low cost, simple handling requirements, and abundant base of literature for comparison. The cartilage repair literature is replete with studies describing osteochondral healing in 3-mm diameter and 3-mm deep defects, but these are not representative of the chondral lesions found in patients. Careful creation of full-thickness chondral defects that do not penetrate the subchondral bone plate can be achieved in the rabbit[23,24] to facilitate study of cell-based and marrow stimulation repair methods. Complete filling with a fibrocartilage repair tissue is also reported in full-thickness chondral defects in adult rabbits treated with large-diameter subchondral drilling,[25–27] which provides the basis for comparison and testing of new repair techniques. Indeed, rabbit models of cartilage repair have provided a large part of the supportive documentation for devices and therapies in the past.[1,3,24,28,29] Recent meta-analysis of rabbit studies for cartilage repair[30] provides support for the use of this species as a standard in the early development of repair therapies or products. Notwithstanding, two concerns are the age of maturity mentioned previously and the thin cartilage in this species that often leads investigators to create osteochondral rather than chondral defects, making histological confirmation of defect depth mandatory.

The dog has been widely used for osteoarthritis,[31] joint injury,[32,33] and meniscal healing studies[34,35] and some cartilage repair procedures,[36–38] but its status as a companion animal makes it less attractive to researchers. As in the rabbit, pure chondral lesions are difficult to create in the thin cartilage of this species. Dog models of full-thickness defects have been created with and without damage to the subchondral plate to test the effect of cell delivery.[36,39] Dogs are easily trained, and thus controlled weight bearing and rehabilitation using swimming, underwater treadmills, and physiotherapy provides an advantage. Arthroscopic second-look procedures and biopsies are possible, but access to the femorotibial joints is limited. Perhaps the greatest strength of dog studies for cartilage repair is that kinematic and ground reaction forces can be used to assess gait, and weight bearing can be accurately characterized throughout the study.[40] Beagles and other chondrodystrophic breeds have short limbs, so the knee joints are more difficult to operate on and assess; for this reason, large breed dogs are recommended, although commercial availability is limited. The dog has a requirement for a high level of environmental enrichment, including contact with caregivers, that should be taken into consideration when working with this species.

Goats and sheep are frequently used in cartilage repair models because the knee joints are large enough to create lesions as large as those treated in patients (0.5–1 cm^2). These species are also popular models for osteochondral grafting[41–43] and meniscal repair.[44–46] Both sheep and goats share similar characteristics, although the goat has slightly thicker cartilage.[47] The larger defect size and thicker cartilage layer permit biochemical assays of cartilage repair tissue, including DNA, sulfated glycosaminoglycan, and collagen content, in addition to histology.[48,49] Like other larger farm animals, based on biomechanical testing, the subchondral bone plate of sheep and goats is well developed and very stiff compared to the human.[50] This feature of the subchondral bone dulls surgical tools and instruments quickly during bone cutting and drilling or debridement of calcified cartilage. Subchondral bone cysts are reported as a complication in these species around drill holes or when the subchondral plate is widely disrupted.[51–53] Access to the anterior one-third to one-half of the medial femoral condyle and the entire trochlear groove is possible via arthrotomy and by arthroscopy using a small (3.5-mm diameter) arthroscope, although partial resection of the fat pad may be necessary to improve access to the femorotibial joint. The thin cartilage (0.4–1.3 mm) in these species[47] makes retention of sutured flaps challenging,[54] although a high success rate has been reported.[55–57] Fixation of cell-laden constructs with fibrin glue or press-fitted into carefully prepared defects is possible but less successful than sutures.[57] Both the goat and sheep are amendable to magnetic resonance imaging (MRI)[58,59] because they fit into conventional superconducting MRI units; however, the knee joints themselves are much smaller. The flared geometry of the proximal limb makes use of human receiver coils challenging, which can be overcome with the use of customized coils. Anesthesia and analgesia protocols as well as housing conditions are well established and easily provided, making well-powered studies possible. These species are gregarious flock animals, and less environmental enrichment is needed if they are housed in small groups. Short-term (2–4 weeks) postoperative therapy and reduced weight bearing using a hind limb[60] or full-body sling[61] are possible in sheep and goats. Long-limb rigid casts can be used[62] to reduce the shear forces associated flexion–extension but do not abolish weight bearing and require daily monitoring for complications.

Domestic and mini-pigs have been used as models because of the thick cartilage in this species, particularly the domestic pig. Fast-growing domestic pigs commonly present with subchondral bone abnormalities and very soft, thin, and irregular subchondral bone plates due to osteochondritis dessicans and similar developmental lesions.[63] In many cases, domestic pigs are used in short-term studies before they are fully mature[64] because pigs older than 2 years of age are large and difficult to handle. The same cautions about using immature rabbits in developmental studies apply here.[64] Mini-pigs are more tractable, and although

critical-sized osteochondral defects are most often published,[65–67] chondral defects have also been created successfully and are more representative of human cartilage repair.[17] Osteochondral defect results are published for defect locations in both the femoral condyle and trochlea[68] for comparison. Subchondral cystic lesions similar to other species have been reported as a complication. Critical-sized cartilage-only lesions and age for cartilage maturity are not well characterized in the mini-pig. The long oropharynx, thick subcutaneous fat layer, and special handling requirements of this species create challenges during intubation, venipuncture, and surgery, and as with all species, investigators should obtain knowledge of the pharmacology, anesthesia, and housing requirements of this species.

The main advantages of the horse model are the large joint size and thick articular cartilage layer[47] with easy arthroscopic joint access.[69,70] As with all species, there are some challenges in the horse model. Unlike the human knee, the equine knee joint is compartmentalized into 3 synovial cavities. There is limited access to the femorotibial joints and menisci compared to the human, but the femoropatellar joint is accessible by a minimally invasive arthrotomy or arthroscopy. Standard size (4.5–5.0 mm diameter) arthroscopic equipment can be introduced through multiple portals, making complex manipulations such as fixation of cell-laden constructs[71,72] or osteochondral grafts[73] possible. Critically sized defect models have been developed in both the equine femoral trochlear ridges and medial femoral condyles.[47,70,71,74–77] Multiple critical-size defects can be made arthroscopically, and the calcified cartilage layer may be retained or resected as needed.[47] The calcified cartilage in horse is approximately twice as thick as human and other species,[47,78] and the subchondral bone is markedly harder and mineralized,[50,79] creating a substantial barrier to extrinsic repair from the bone marrow compartment. The large size and thickness of cartilage (1.5–3.0 mm) in the horse create an opportunity to harvest and analyze larger volumes of repair tissue so multiple outcomes are possible such as biochemistry, histology, and biomechanical studies. Microfracture,[69,75] osteochondral grafting,[73] and single-step[77,80] and multiple cell-based cartilage repair[72,81–83] have been successfully evaluated in this species.

As in the dog, horses can be monitored with respect to the clinical response to cartilage repair by assessment of lameness, but high-quality data from ground reaction forces recorded with force plates and motion analysis are expensive and time-consuming to acquire and analyze. Diagnostic imaging of the knee joint is limited because the bulk of the upper hind limb interferes with MRI. The carpus and tibiotarsal joints are more amenable to such imaging, but these joints have thinner cartilage (1 mm), and the joint geometry is less relevant to translational studies. A second-look arthroscopy and biopsy is usually the cornerstone of equine studies, allowing assessment of repair progression. Postoperative exercise but not the degree of weight bearing can be controlled. For this reason, most repair studies are done in the proximal-mid aspect of the medial or lateral trochlear ridge of the femur, which is thought to be partially protected from direct weight bearing. As mentioned previously, the articular cartilage in the femoropatellar and femorotibial articulations is closely comparable to human,[47] although the medial femoral condyle subchondral bone plate is very thick and has an ultimate strength 2 to 3 times that of human bone.[50,79] Use of horses with naturally occurring disease such as subchondral bone cysts can be used to evaluate repair strategies; however, the biology of these osteochondral defects in the femoral condyles is fundamentally different from chondral lesions in human patients.[83] In other joints such as the carpus and metacarpophalangeal joints, pathologic change in the subchondral bone drives joint disease in this species. Generally, the horse as research model is valuable in the context of cartilage repair, although in some jurisdictions, the horse, like the dog, is considered a companion animal. The specialized facilities and care that these animals require often make the horse a model that is used for late-stage development and pivotal studies.

Nonhuman primates have been used sparingly in cartilage repair research because of expense, availability, and societal concerns. Nonhuman primate use is indicated when dose response of biologics needs to be evaluated since the effective dose in the human is frequently different from laboratory and farm animals. Primates are also used when elucidation of immune compatibility is needed, for example, when animal origin biomaterials are developed.[84] Otherwise, at the present time, there is no consensus that primates are a necessary step in cartilage repair research.

Regional availability and familiarity with a species may influence the choice of species. Our present understanding of animal models makes them appropriate to explore cellular and molecular mechanisms, dose response, physical geometry, fixation, integration, and interactions between components. At the present time, it is clear that initial studies in rodents, rabbits, or pigs followed by developmental and then pivotal studies in the horse, sheep, or goat have been critical to the success of new cartilage repair methods now in clinical use. However, there is no specific animal model or group of models that predicts the completeness and durability of repair in human patients at the current time. Some of the reasons for this are discussed in the following sections.

Articular Cartilage Thickness in Animal Models

Interspecies differences are highlighted in several recent studies of articular cartilage thickness in the knee of animal species used for preclinical studies. Point-by-point mapping

of regional differences in the knee is needed to facilitate choice of species and location in animal models. Frisbie *et al*.[47] found the average articular cartilage thickness over 5 locations was 2.2 to 2.5 mm for adult human and 0.3 mm for rabbit, 0.4 to 0.5 mm for sheep, 0.6 to 1.3 mm for dog, 0.7 to 1.5 mm for goat, and 1.5 to 2.0 mm for horse. Average cartilage thickness reported by Archibald *et al*.[50] was 0.7 mm for dog, 1.2 mm for sheep, 2.9 mm for cows, 3.2 mm for pigs, and 3.2 mm for horses. The variability in thickness for the same species in these studies may be related to different ages or sites taken for analyses. On the basis of cartilage thickness alone, species such as the pig mimic the human joint and thus have been used to evaluate implantation of tissue-engineered constructs.[85] Depending on the particular treatment under investigation, other considerations may be as important for articular cartilage repair studies, such as relative joint size and arthroscopic access, which favor the horse and sheep or goat.

Calcified Cartilage and Subchondral Bone Plate Anatomy and Response to Injury

The primary reason for interest in the comparative anatomy of the subchondral bone plate is that the calcified cartilage layer is thought to be a barrier that limits the contribution of extrinsic repair to chondral defects (i.e., repair elicited from cells other than chondrocytes). This is relevant when there is uncertainty whether cell-based or other therapies are contributing directly to cartilage repair rather than directing or improving subchondral bone marrow–derived extrinsic repair. Cell tracking or other methods to confirm that the implanted cell population is represented in the repair tissue are valuable. Likewise, retaining an intact subchondral bone plate and calcified cartilage layer allows investigators to make stronger inferences about the origin of repair tissue in cell-based repair. In fact, such containment is probably not consistent with the application of cell-based therapies in humans where subchondral bleeding in the repair site is considered deleterious[86] but difficult to control in all situations. The influence of transplanted cells on reparative cells from multiple sources may be as or more important than the transplanted cells themselves.[84] A recent study has performed cell tracing using iron oxide particles in chondral defects with no intentional penetration of the subchondral bone plate and found that only 25% to 33% of the cells in chondral repair tissue at 12 weeks were from the transplanted population, whereas the majority of cells were from subchondral bone.[17] This result is consistent with previous studies tracing labeled cells using different techniques where only a small fraction of cells in repair tissue is found to be from the implanted cells.[87] One study is often cited as evidence for the persistence of implanted chondrocytes[88] but only reported labeled cells detected in 1 animal at 10 weeks or beyond after cell delivery, and this result should not be overinterpreted as general evidence of residency of implanted cells. The above studies and others also suggest that transplanted cells may migrate into the subchondral compartment, generating persistent cartilaginous tissue below the cartilage bone interface in a former bony site. If regulatory agencies require evidence about the provenance of repair tissue, then robust data tracing cell origin that is free of sampling bias need to be gathered.

In histology of human cartilage, the subchondral vasculature is often visible within the calcified cartilage layer.[89–91] As a result, once chondral lesions are full thickness, they may have access to marrow elements. Subchondral sclerosis in chronic lesions may reduce the availability of vasculature to contribute to the repair process, and the thicker, more mineralized subchondral bone plate of the larger animal species may mimic this condition.

In the healthy human knee, the calcified cartilage and subchondral bone plate are very thin. The subchondral bone plate is 0.2 to 0.5 mm thick[79,90] and composed of a condensation of a single horizontal trabecula supported by an open network of vertical trabeculae. The human subchondral bone plate has a very low bone volume fraction and low bone mineral density compared to farm animal species.[8,79,90] Although the dog, sheep, and goat may have a bone mineral density (BMD) comparable to humans, all animals have a 2 to 3 times thicker subchondral bone plate than the human.[79] Although not optimal, as mentioned earlier, this may be acceptable since some late-stage chondral lesions are accompanied by subchondral sclerosis and a thick subchondral bone plate (1.5–2.0 mm) that is similar to the horse and the other larger (farm) animals.

Since the large species of animals, including the horse, goat, sheep, and pig, are used in pivotal studies, the consequences of their relatively thick subchondral bone plate should be considered. Animal studies using marrow stimulation need to confirm that there is adequate penetration of the subchondral bone plate. Otherwise, there may still be a barrier between experimental chondral lesions and marrow elements. As mentioned previously, a common error in marrow stimulation surgical technique has been an incomplete or irreproducible removal of calcified cartilage layer, creating lower repair tissue volume and attachment in horses[70,75,92] and sheep.[53] These studies show that marrow-derived or transplanted repair tissue frequently fails to grow over and adhere to residual calcified cartilage.[70] Residual calcified cartilage may also change biomechanical signaling gradients in a manner that could impede chondrogenesis in the cartilage portion of the defect.[93]

The magnification available during arthroscopic surgery is beneficial in controlling calcified cartilage removal or retention, particularly in the horse,[69] where this aspect of defect creation has been well characterized. Removal of too much of the subchondral bone plate may result in subchondral bone collapse, subchondral cysts, and lack of support

for the new repair tissue in all species.[39,53,81,94–96] Since preparation of a standardized experimental defect is a critical step in cartilage repair experiments, a validation study evaluating depth, size, and characteristics of the chondral defect is warranted in nearly all cases. At surgery, the presence of punctate bleeding in the bed of the defect should be documented and digital photos taken to indicate whether the defect has reached vascularized subchondral bone during surgery. If using an arthrotomy, a surgical magnifying loupe to identify capillary bleeding should be used since such bleeding may be difficult to identify in the larger species with very dense subchondral bone plates found in species such as the sheep, goat, and horse. Partial removal of the calcified cartilage layer does not necessarily lead to punctate bleeding from small capillaries that occur within the bony fringes of the irregular calcified cartilage layer. Complete debridement of the calcified cartilage layer may not lead immediately to punctate bleeding that is evident by eye, depending on intraoperative blood pressure, anesthetics, and animal positioning. For these reasons, histological sections through whole, freshly prepared defects, at multiple levels in the defect, are needed during pilot work to quantify average thickness of residual noncalcified cartilage and calcified cartilage inside and outside the defects. A potential postsurgical complication is that load bearing during acute repair periods may induce cracks and fissures in the residual calcified cartilage layer[94] and hyaline cartilage, creating further injury and conduits for host repair cells[96] in addition to causing loss of some implants.[51] Thus, ideally, initial defects should be characterized not only at surgery but also afterward, permitting the animals to load-bear for up to 14 days with and without implant. Whether experimental lesions can be fully contained within the cartilage compartment postoperatively in the larger animal species may remain a contentious issue.

Some experimental conditions such as osteochondral drill holes[97] or the creation of a full-thickness defect with[94] or without any implant[17] result in distortion of the subchondral bone plate[97] and advancement of the tidemark during subchondral bone healing, which results in thin, poorly integrated repair tissue with altered mechanical properties. Subchondral bone overgrowth has been described in chronic chondral defects of patients, and once resected, this did not affect the outcome of cell-based cartilage repair,[98] but Minas *et al.*[99] described an advancing tidemark or "intra-articular osteophyte" as a potential mode of failure in patients after autologous chondrocyte implantation. Regardless of the origin, tidemark advancement and formation of new subchondral bone within the chondral repair zone should be considered undesirable in cartilage repair studies. Since this is a late complication, long-term animal studies ≥6 months in duration are required in late-stage development and pivotal studies to address this issue. Clearly, there is no animal that comes close to being a good anatomical correlate for the delicately thin human subchondral bone plate. Despite this, the response to injury and repair is predictive in several species as long as the tenets of cartilage maturity, critical-size defects, adequate statistical power, and long-term studies are respected. Although consideration of the subchondral bone should be respected in study design and analysis, the choice of species should be justified according to the specific experimental hypothesis or objective under investigation.

Recommended Operative Procedures

Unilateral versus Bilateral Models

In some jurisdictions, bilateral models are not permitted because of humane considerations, although this is becoming less of a concern with better analgesic and postoperative care protocols employing epidural administration[100] and slow-release formulations of analgesics such as fentanyl dermal patches. Unilateral models are considered less stressful for the animals and allow assessment of lameness progression, range of motion, and gait during the study. This simpler design ensures that no contralateral procedure, device, or implant affects the outcome. The main advantage is that postoperative weight bearing and joint motion can be controlled. The main disadvantage of unilateral models is that additional animals need to be used to create fully powered studies that include appropriate control groups. Sometimes this is less satisfactory than controlling animal-to-animal variation by having the control treatment in the contralateral limb. The final decision is a balance between a simpler and more robust statistical design with within-animal controls and the possibility of ipsilateral repair being affected by a contralateral control. Regional differences in animal care regulations and the need to control weight bearing may come into play in the experimental design.

Acute versus Chronic Defects

All patients are diagnosed with chondral defects after a variable period of symptom progression, so these lesions can range from subacute to chronic by the time they are operated. The cartilage surrounding these tissue deficits has a variable zone of injury extending centrifugally with associated matrix degradation and catabolic cartilage metabolism. Modeling of cartilage repair with acute defects is convenient, requires a single surgery for creation and treatment of the defect, and most important provides a reasonably reproducible initial lesion. However, use of acute chondral defects also creates ideal conditions for cartilage healing seldom encountered in the clinic and may be one factor responsible for the discrepancy between promising animal data obtained in an animal model and disappointing

performance in clinical trials. Creation of a chronic nonhealing critical-size defect followed by delayed repair[101,102] may improve external validity of the outcomes. The duration of delay between defect creation and treatment depends on the model, but for large animal pivotal studies, 2 to 3 months or more allows sufficient time for perilesional progression of degeneration.[103,104] This still would not mimic lesions that develop over longer periods in patients, but the inflammation-driven catabolic aspect in the synovial environment[105] and intraoperative bleeding[106] would constitute a more valid model. Cost and justification of multiple surgeries to animal care regulatory bodies create barriers to this model system, although creation of the index injury using arthroscopy rather than arthrotomy would be less invasive and potentially more acceptable.

Postoperative Care

Although in the past, intra- or postoperative intra-articular injection of local anesthetics was a valuable adjunct in the management of surgical pain, because of reports of chondrocyte toxicity, this practice should be discontinued in cartilage repair procedures. Epidural analgesics may be used in many species. The use of analgesics should be documented through the entire study, and preference should be given to products that do not bind to the osseous structure or interfere with bone repair. Pain can be monitored by implementing subjective assessment methods, activity monitoring, and assessment of animals' ability to socialize and compete with other animals in a small group (large animals) or to maintain body mass and caged activity (rodents, rabbits). Motion analysis and forceplate measurements are ideal but rarely used except in pivotal studies. The severity and duration of postoperative pain and hyperalgesia should be distinguished from chronic pain. Mechanical gait disorders, such as patellar luxation or periarticular fibrosis, may cause lameness that will not be resolved by better analgesia, so steps need to be taken intraoperatively to prevent these complications. Monitoring involves timely, periodic examination of the animal to assess its state of health and the maintenance of written records documenting the regular examination findings. If limb immobilization is recommended, then appropriate care will be required to monitor and change bandages or splints.

Drugs used for postoperative pain include narcotics, narcotic agonist–antagonists, and nonsteroidal anti-inflammatory drugs (NSAIDs). Intra-articular use of analgesics or any other drug should be avoided or at least carefully considered against possible deleterious effects on transplanted cells. NSAIDs may be COX1 or COX2 antagonists, but both are known to influence wound repair and bone healing and should be used sparingly in cartilage repair studies. Narcotics are very useful, and dermal patch delivery systems containing fentanyl are very convenient for rabbits, dogs, sheep, and goats, although they should be supplemented during the intraoperative and immediate postoperative phases with injectable narcotics. Strategies such as adhesive bandages need to be employed to prevent animals from ingesting these dermal patches. Maintenance of intravenous catheters for several days postoperatively is valuable as a conduit for drug delivery, although care must be taken, particularly in the horse, to prevent thrombophlebitis. Intraoperative intravenous antimicrobials are frequently warranted in experimental studies, but since these are classified as "clean" surgeries, postoperative antimicrobials are seldom warranted and may contribute to the development of resistant bacterial strains. The quinalone family of antimicrobials should be avoided because of their ability to interfere with cartilage metabolism. As mentioned previously, intra-articular use of analgesics or any other drug should be avoided or at least carefully considered because of possible deleterious effects on transplanted cells. Use of hyaluronate as a carrier for intra-articular injection of stem cells[107] has been used successfully, and postoperative intra-articular hyaluronate might be considered as a method of restoring joint homeostasis postoperatively.[42,105]

It is important to note that current postoperative rehabilitation regimes in patients usually avoid immediate full weight bearing but allow limited flexion–extension. This is difficult to employ in bilateral animal models. The residency of constructs in unilateral defects can be promoted by short-term (2–3 weeks) joint immobilization or restriction. However, complete immobilization (as in the classical Thomas splint that transmits much of the weight-bearing forces from the foot to the pelvis but prevents joint flexion) can be associated with articular cartilage degradation. Accordingly, partial immobilization is recommended, allowing for approximately 20 degrees of joint flexion and extension, permitting the animal to lie down and stand up spontaneously without aid and still allow weight bearing for balance and limited ambulation. A cast–bandage or hinged brace can be used to partially limit flexion–extension.[55] Whole-body hammock-like slings with cutouts for the limbs can be used for unloading of joints, but a period of preoperative conditioning to this device is recommended. Sling-like devices that partially immobilize or flex one limb may also be used to create a non-weight-bearing condition,[60] recognizing that controlled flexion–extension is harder to attain in animals compared to human patients wearing a knee brace.

Duration of Study

The recommended period for evaluation of preclinical models of cartilage repair is different for pilot or screening studies versus developmental and pivotal experiments. Early studies will necessarily be shorter to control costs and

hasten decision making. Biocompatibility testing experiments for rapidly absorbed biomaterials or scaffolds are usually timed according to the life span of the implant, often 6 to 12 weeks, with a positive result providing the impetus for longer safety and efficacy studies of 6 months or more. For biological or degradable scaffolds and cell-laden constructs, a positive result at the 3-month time point (i.e., good defect fill) leads to longer efficacy studies of 6 months or greater in duration. Early time points between 1 day and 3 months are often useful to study implant retention, mechanisms of failure, and interacting factors that mediate repair. The cell and molecular physiology of wound repair can be characterized to refine the repair method or dose response. At this time point in large animal models, most repair tissues are still immature, containing a mixture of fibrous connective tissue and fibrocartilage.[75] Maturation of this repair is a critical step that is not entirely predictable, but by 6 months, a mixture of well-attached hyaline-like and fibrocartilage should be present. There should be evidence of lateral integration between the repair tissue and host tissue at this time. Additional matrix organization and cell-type maturation should ensue between 6 and 12 months, but this may be highly dependent on local conditions within the joint, depending on activity, load history, and rehabilitation regime. Failure of animal cartilage repair tissue at the 12-month time point is a well-known phenomenon[106,107] as the initial organization, particularly the highly specialized superficial collagen network of the repair tissue, may not fully mature to withstand loading. Delamination, superficial fibrillation, and loss of lateral integration are frequently seen.[108] Specific models may have early time points where failure occurs, so in such cases, earlier, often arthroscopic evaluations or biopsies help make the decision of whether to continue the experiment. A longer 9- or 12-month time point should be pursued in a large animal model where controlled weight bearing and rehabilitation might be simulated, or if there are no safety concerns, phase 1 and 2 clinical studies in people may be more valuable.

The obvious exception with respect to study length is in the case of a slowly degrading biomaterial with long residency times where the implant itself remains, wholly or partly, as a scaffold or weight-bearing surface. ASTM guidelines (F2150, F1983, F981) for assessment of biomaterial degradation and biocompatibility should be followed, and these studies may require a year or even longer. However, products that are resident for shorter periods, such as fast-degrading biomaterials and possibly cell and biologically based products, could in principle be evaluated for efficacy using study durations that are 6 months in length, as stipulated in the ASTM guideline F2451.[3]

The issues of durability and long-term repair should be considered separate from the primary goal of initial repair and regeneration for the following reasons: assessment of durability of repair is complicated by the nonlinear and largely unknown relationship between human and animal aging and vast differences in load-bearing and behavioral activities. Laboratory animals with short (1–3 years) life spans cannot be scaled accurately to the human life span, although this does not limit their utility for short-term, developmental, or screening studies. Larger animals with life spans of 8 to 10 years or more such as the mini-pig, sheep, goat, and horse present an opportunity for more plausible scaling calculations, but the validity of any proposed scaling cannot be demonstrated at this time. A more widely accepted approach is to consider 6 months of implantation in any animal equivalent to 6 months of use and wear in patients in the case where load bearing and behavioral activities are equal. Thus, durability cannot be extrapolated from duration of implantation as a fraction of life span or from any other apparent experimental design criteria at the current time. Durability is not currently testable in animal models for prediction of durability in humans and can only be appropriately studied in human clinical studies at this time (**Table 1**).

Dose Response

Unlike pharmacological agents, which do require a full dose–response characterization *in vivo* to obtain a recommended clinical dosage, dose–response evaluation of cartilage repair products may not be relevant because of mechanisms of action that are not amenable to dosage variation or control. Some product compositions are not variable and are simply intended to fill the defect, and therefore the proper dosage may only be related to the defect volume. In this case, a dose–response animal study should seek to identify the dose that leads to restoration of the defect volume. Shrinkage of the repair material over time would be inappropriate, and various formulations would be sought that retain their initial volume but allow infiltration by adjacent chondrocytes or repair cells to achieve integration. In such cases, dose response may be linked to long-term integration and residency without evidence of cartilage degeneration on the opposing articular surface (bipolar or "kissing lesions").

Other products release active factors such as growth factors, so *in vitro* release kinetics is often established *in vitro* before pharmacokinetic studies to estimate dosing. In such cases, animal model studies are needed to study degradation of the carrier or scaffold as well as the release of bioactive components into the synovial environment from specific implants. Other products deliver live cells or cover the lesion with a layer of scaffolding, where the precise amount delivered may be related to the surface area of the defect but not necessarily, depending on mechanisms of action that are often not known. For both types of studies, synovial fluid collection, biomarkers of cartilage metabolism,[109] and histological assessments at multiple time points should be considered.

Retention of the implanted cell number and phenotype is also a major concern in passaged cells; dose response in this context may be related to cell density. If stem cells or genetically modified cells are implanted, a detailed characterization of their phenotype is needed because of concerns about their plasticity and the potential for tumorgenicity and escape from the transplant site. In products that deliver cell-based therapies, the dose response may be related to cell seeding density or maturation of constructs *in vitro* before they are implanted. Characterization of the chondrocyte phenotype using gene or protein expression may be logical steps that can be used for quality assurance and, as the product development matures, in good manufacturing practice (GMP). Biomechanical assessments may reveal important information when host tissue is substituted for the implant.

Therefore, it appears more reasonable to propose that the investigators provide a rationale for the applied dose or dose range that is used to demonstrate a beneficial therapeutic effect in animal studies as opposed to testing a series of different doses.

Mechanical Testing Outcomes

Specific procedures for mechanical testing should take into account the repair strategy. Mechanical testing of material properties of the initial implant may not be relevant with approaches aiming at stimulating cell recruitment, growth, and gradual maturation of repair tissue. Thus, inferior material properties of the initial implant may be appropriate (e.g., cell-based repair without supportive matrix). However, demonstration of implant residence at the site of application for an appropriate time after implantation that is consistent with the principal mode of action is important. In general, the characterization of tissue mechanical properties during repair and maturation provides assurance of functional restoration and one index of durability. Mechanical testing of tissues may involve procedures, such as tissue isolation, that hinder or prevent use afterward for other endpoints such as histology or biochemistry. Thus, the study design may need to include extra animals with defects specifically for biomechanical testing, methods that test only a portion of the repair site, or methods that are nondestructive.

For nonclinical mechanical testing of cartilage repair properties, confined compression or biphasic indentation methods have been proposed. These are classical methods developed decades ago to describe mechanical properties of cartilage and assume tissue structure to be homogeneous and isotropic. However, many physiologically important inhomogeneous and nonlinear tissue characteristics, such as compression–tension nonlinearity, are not captured using these methods.[110] Also, confined compression may not reveal a physiologically relevant dynamic response,[111] and biphasic indentation data that are reduced assuming tissue isotropy do not describe dynamic (instantaneous) properties accurately.[112,113] Although the methodology for these techniques is well established, they may not address the nonuniformity and complex geometry that is typical of repairing cartilage defects.

Unconfined compression, indentation, and tensile testing protocols can provide additional physiologically relevant information, especially concerning the important integrity of the cartilage collagen network.[114–118] Several advances in biphasic or poroelastic modeling, enabling more accurate characterization of the cartilage dynamic response, have been introduced and also describe experimental creep or stress relaxation data and to extract material parameters.[119,120] Therefore, the use of dynamic testing protocols that can capture physiologically relevant dynamic cartilage behavior should be included where mechanical testing is indicated. Furthermore, it should be noted that compression tests presume the availability of multiple, uniform-size osteochondral samples that are difficult to procure from repaired cartilage lesions in animals because of uneven repair tissue surfaces, the hard subchondral bone plate in large animals, and the thinness of the articular cartilage in small animals.

In addition, friction, wear, and fatigue properties are important mechanical properties that should be considered. In particular, excessive friction may be detrimental to the opposing tissue. Excessive wear or fatigue properties may lead to premature deterioration and loss of the implant. Although it is notable that friction and wear analyses of cartilage and implants are rapidly progressing fields, it would be desirable to show, directly or indirectly, that these properties of the implant are appropriate for the mode of action of the implant. The results of such tests are markedly affected by the lubricant fluid used.[121,122]

Possible *in vivo* methods to characterize the functional integrity of cartilage include indentation techniques. Although many of these techniques and instruments are still in developmental stages, those based on mechanical,[123] acoustic,[124] and electromechanical[125] methods are suitable for *in vivo* diagnostics and have been shown to provide quantitative information related to tissue structure, composition, and properties.

Imaging and Histology Outcomes

MRI and histological methods that are appropriate for animal studies are presented in 2 companion articles.[49,126,127] The reader is referred to these publications for further detailed information.

Good Laboratory Practice and Statistical Analyses

Proof-of-principle and developmental studies require rigidly controlled, meticulously documented conditions that are not strictly required by the FDA to be at the good

laboratory practice (GLP) level. Pivotal studies, frequently done in the larger animal species such as the pig, goat, sheep, and horse, are challenging because GLP conditions for all aspects of the study are difficult to establish. For instance, the prior medication and medical history for horses can be difficult to ascertain—for example, hay fed as feed would need a detailed analysis to be considered quality controlled. Instead, many experiments can be conducted in the spirit of GLP or with specific aspects such as the outcome measures conducted according to GLP with a gap analysis of non-GLP aspects. This may be a practical approach since non-GLP and GLP work may be conducted simultaneously in centralized animal facilities, such as an MRI suite or surgical unit. Although outside the scope of this article, a preplanned statistical analysis accounting for multiple outcome measures is essential to ensure proper control of type I and type II errors and the effects of multiplicity and missing values. A good review of these issues can be found in the companion article[128] on patient-reported outcomes for cartilage repair.

To conduct a cartilage repair study in the spirit of GLP, a study protocol would be written and approved prior to initiating the study, and the study design would ensure that sufficient animal numbers are included to obtain significant differences for the analyses that are to be performed. Standard Operating Procedures should be written and validated for all of the key procedures used in the study (i.e., preparation of the test article, surgery, postoperative care, necropsy, macroscopic scoring, histology, lameness tests). Written documentation in laboratory notebooks should be used, and certificates of characterization of all test articles and sterility tests should be documented. All of these steps will ensure the traceability and fidelity of the data obtained.

Conclusions

At the present time, it is clear that initial and developmental studies in rodents or rabbits followed by developmental and pivotal studies in the horse, sheep, and goat have been critical to the success of new cartilage repair methods now in clinical use. All developmental and pivotal studies should use animals with mature cartilage as defined by zonal organization of the extracelluar matrix and a continuous calcified cartilage layer and tidemark. Larger animals that allow arthroscopic procedures are more amendable to delayed repair models that mimic the chronicity and catabolic environment present in many patients' joints. Initial short-term trials with promising results could lead to pivotal experiments that should analyze repair outcome after a minimum of 6 to 12 months of repair. Outcome measures should minimally include implant residency (biomaterial clearance, cell tracking), macroscopic scoring, and histological analysis, and cartilage biochemistry, biomechanical testing, and 3-dimensional imaging are also recommended. Protocols, procedures, and written documentation carried out in the spirit of GLP can help generate FDA-compliant proof-of-efficacy documents. Quality assurance and preplanned statistical analyses can also help generate preclinical data useful in obtaining regulatory approval leading to a clinical trial.

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References

1. US Food and Drug Administration. Guidance for industry: Preparation of IDEs and INDs for products intended to repair or replace knee cartilage. Rockville, MD: US Food and Drug Administration, Center for Biologics Evaluation and Research; 2007. www.fda.gov/downloads/BiologicsBloodVaccines/.../ucm078710.pdf
2. Levine DW, Mondano L, Halpin M. FDA regulatory pathways for knee cartilage repair products. Sports Med Arthrosc Rev. 2008;16:202–207.
3. ASTM International. Standard guide for in vivo assessment of implantable devices intended to repair or regenerate articular cartilage. ASTM International F2451-07(2010). www.astm.org/Standards/medical-device-and-implant-standards.html
4. Fretz PB, Cymbaluk NF, Pharr JW. Quantitative analysis of long-bone growth in the horse. Am J Vet Res. 1984;45:1602–9.
5. Hunziker EB, Kapfinger E, Geiss J. The structural architecture of adult mammalian articular cartilage evolves by a synchronized process of tissue resorption and neoformation during postnatal development. Osteoarthritis Cartilage. 2007;15:403–13.
6. Rieppo J, Hyttinen MM, Halmesmaki E, Ruotsalainen H, Vasara A, Kiviranta I*, et al*. Changes in spatial collagen content and collagen network architecture in porcine articular cartilage during growth and maturation. Osteoarthritis Cartilage. 2009;17:448–55.
7. Malina RM. Physical growth and biological maturation of young athletes. Exerc Sport Sci Rev. 1994;22:389–433.
8. Müller-Gerbl M. The subchondral bone plate. Adv Anat Embryol Cell Biol. 1998;141:III–XI, 1–134.
9. Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18:419–33.
10. Gilmore RS, Palfrey AJ. A histological study of human femoral condylar articular cartilage. J Anat. 1987;155:77–85.
11. Brommer H, Brama PA, Laasanen MS, Helminen HJ, van Weeren PR, Jurvelin JS. Functional adaptation of articular cartilage from birth to maturity under the influence of loading: a biomechanical analysis. Equine Vet J. 2005;37:148–54.

12. Dell'accio F, Vincent TL. Joint surface defects: clinical course and cellular response in spontaneous and experimental lesions. Eur Cell Mater. 2010;20:210–7.
13. Wagner W, Reichl J, Wehrmann M, Zenner HP. Neonatal rat cartilage has the capacity for tissue regeneration. Wound Repair Regen. 2001;9:531–6.
14. Wei X, Messner K. Maturation-dependent durability of spontaneous cartilage repair in rabbit knee joint. J Biomed Mater Res. 1999;46:539–48.
15. Wei XC, Gao JZ, Messner K. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. J Biomed Mater Res. 1997;34:63–72.
16. Jiang CC, Chiang H, Liao CJ, Lin YJ, Kuo TF, Shieh CS*, et al*. Repair of porcine articular cartilage defect with a biphasic osteochondral composite. J Orthop Res. 2007; 25:1277–90.
17. Blanke M, Carl HD, Klinger P, Swoboda B, Hennig F, Gelse K. Transplanted chondrocytes inhibit endochondral ossification within cartilage repair tissue. Calcif Tissue Int. 2009;85: 421–33.
18. Carlson CS, Hilley HD, Meuten DJ, Hagan JM, Moser RL. Effect of reduced growth rate on the prevalence and severity of osteochondrosis in gilts. Am J Vet Res. 1988;49:396–402.
19. Fortier LA, Kornatowski MA, Mohammed HO, Jordan MT, O'Cain LC, Stevens WB. Age-related changes in serum insulin-like growth factor-I, insulin-like growth factor-I binding protein-3 and articular cartilage structure in Thoroughbred horses. Equine Vet J. 2005;37:37–42.
20. Henry JL. Molecular events of chronic pain: from neuron to whole animal in an animal model of osteoarthritis. Novartis Found Symp. 2004;260:139–45; discussion 145–53, 277–9.
21. Ghadially FN, Thomas I, Oryschak AF, Lalonde JM. Long-term results of superficial defects in articular cartilage: a scanning electron-microscope study. J Pathol. 1977;121:213–7.
22. Firth EC, Klarenbeek A. Transphyseal vessel involvement in repair of metaphyseal retained cartilage. Aust Vet J. 1995;72:452–5.
23. Hoemann CD, Sun J, McKee MD, Chevrier A, Rossomacha E, Rivard GE*, et al*. Chitosan-glycerol phosphate/blood implants elicit hyaline cartilage repair integrated with porous subchondral bone in microdrilled rabbit defects. Osteoarthritis Cartilage. 2007;15:78–89.
24. Brittberg M, Nilsson A, Lindahl A, Ohlsson C, Peterson L. Rabbit articular cartilage defects treated with autologous cultured chondrocytes. Clin Orthop Relat Res. 1996:270–83.
25. Menche DS, Frenkel SR, Blair B, Watnik NF, Toolan BC, Yaghoubian RS*, et al*. A comparison of abrasion burr arthroplasty and subchondral drilling in the treatment of full-thickness cartilage lesions in the rabbit. Arthroscopy. 1996;12:280–6.
26. Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg Am. 1976;58:230–3.
27. Im GI, Lee JH. Repair of osteochondral defects with adipose stem cells and a dual growth factor-releasing scaffold in rabbits. J Biomed Mater Res B Appl Biomater. 2010;92:552–60.
28. O'Driscoll SW, Keeley FW, Salter RB. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion: an experimental investigation in the rabbit. J Bone Joint Surg Am. 1986;68:1017–35.
29. O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion: a follow-up report at one year. J Bone Joint Surg Am. 1988;70:595–606.
30. Ahern BJ, Parvizi J, Boston R, Schaer TP. Preclinical animal models in single site cartilage defect testing: a systematic review. Osteoarthritis Cartilage. 2009;17:705–13.
31. Brandt KD, Schauwecker DS, Dansereau S, Meyer J, O'Connor B, Myers SL. Bone scintigraphy in the canine cruciate deficiency model of osteoarthritis: comparison of the unstable and contralateral knee. J Rheumatol. 1997;24:140–5.
32. Mrosek EH, Lahm A, Erggelet C, Uhl M, Kurz H, Eissner B*, et al*. Subchondral bone trauma causes cartilage matrix degeneration: an immunohistochemical analysis in a canine model. Osteoarthritis Cartilage. 2006;14:171–8.
33. Lahm A, Uhl M, Edlich M, Erggelet C, Haberstroh J, Kreuz PC. An experimental canine model for subchondral lesions of the knee joint. Knee. 2005;12:51–5.
34. Cook JL, Fox DB, Malaviya P, Tomlinson JL, Farr J, Kuroki K*, et al*. Evaluation of small intestinal submucosa grafts for meniscal regeneration in a clinically relevant posterior meniscectomy model in dogs. J Knee Surg. 2006;19:159–67.
35. Nishida M, Higuchi H, Kobayashi Y, Takagishi K. Histological and biochemical changes of experimental meniscus tear in the dog knee. J Orthop Sci. 2005;10:406–13.
36. Breinan HA, Minas T, Hsu HP, Nehrer S, Sledge CB, Spector M. Effect of cultured autologous chondrocytes on repair of chondral defects in a canine model. J Bone Joint Surg Am. 1997;79:1439–51.
37. Cook SD, Patron LP, Salkeld SL, Rueger DC. Repair of articular cartilage defects with osteogenic protein-1 (BMP-7) in dogs. J Bone Joint Surg Am. 2003;85A(suppl 3):116–23.
38. Cook SD, Barrack RL, Patron LP, Salkeld SL. Osteogenic protein-1 in knee arthritis and arthroplasty. Clin Orthop Relat Res. 2004:140–5.
39. Nehrer S, Breinan HA, Ramappa A, Hsu HP, Minas T, Shortkroff S*, et al*. Chondrocyte-seeded collagen matrices implanted in a chondral defect in a canine model. Biomaterials. 1998;19:2313–28.
40. Budsberg SC, Chambers JN, Lue SL, Foutz TL, Reece L. Prospective evaluation of ground reaction forces in dogs undergoing unilateral total hip replacement. Am J Vet Res. 1996;57:1781–5.
41. Hurtig MB, Novak K, McPherson R, McFadden S, McGann LE, Mul drew K*, et al*. Osteochondral dowel transplantation for repair of focal defects in the knee: an outcome study using an ovine model. Vet Surg. 1998;27:5–16.
42. Tytherleigh-Strong G, Hurtig M, Miniaci A. Intra-articular hyaluronan following autogenous osteochondral grafting of the knee. Arthroscopy. 2005;21:999–1005.

43. Lane JG, Massie JB, Ball ST, Amiel ME, Chen AC, Bae WC*, et al*. Follow-up of osteochondral plug transfers in a goat model: a 6-month study. Am J Sports Med. 2004;32:1440–50.
44. Mora G, Alvarez E, Ripalda P, Forriol F. Articular cartilage degeneration after frozen meniscus and Achilles tendon allograft transplantation: experimental study in sheep. Arthroscopy. 2003;19:833–41.
45. Chevrier A, Nelea M, Hurtig MB, Hoemann CD, Buschmann MD. Meniscus structure in human, sheep, and rabbit for animal models of meniscus repair. J Orthop Res. 2009;27:1197–203.
46. Guisasola I, Vaquero J, Forriol F. Knee immobilization on meniscal healing after suture: an experimental study in sheep. Clin Orthop Relat Res. 2002;(395):227–33.
47. Frisbie DD, Cross MW, McIlwraith CW. A comparative study of articular cartilage thickness in the stifle of animal species used in human pre-clinical studies compared to articular cartilage thickness in the human knee. Vet Comp Orthop Traum. 2006;19:142–6.
48. Hoemann CD, Sun J, Chrzanowski V, Buschmann MD. A multivalent assay to detect glycosaminoglycan, protein, collagen, RNA, and DNA content in milligram samples of cartilage or hydrogel-based repair cartilage. Anal Biochem. 2002;300:1–10.
49. Melrose J, Smith S, Ghosh P, (2004). Histological and immunohistological studies on Cartilage. In *Cartilage and Osteoarthritis*, Volume 2, Edited by De Ceuninck F, Sabatini M, and Patoureau P (eds). Humana Press, New Jersey pp 39-63.
50. Archibald M, Runciman J, Dickey J, Hurtig M. Do animal models approximate the subchondral bone and cartilage characteristics of humans? Presented at: 4th International Cartilage Repair Society, June 2002, Toronto, Canada.
51. Jackson DW, Lalor PA, Aberman HM, Simon TM. Spontaneous repair of full-thickness defects of articular cartilage in a goat model: a preliminary study. J Bone Joint Surg Am. 2001;83A:53–64.
52. Simon TM, Jackson DW. Articular cartilage: injury pathways and treatment options. Sports Med Arthrosc Rev. 2006;14:146–54.
53. Hoemann CD, Hurtig M, Rossomacha E, Sun J, Chevrier A, Shive MS*, et al*. Chitosan-glycerol phosphate/blood implants improve hyaline cartilage repair in ovine microfracture defects. J Bone Joint Surg Am. 2005;87A:2671–86.
54. Driesang IM, Hunziker EB. Delamination rates of tissue flaps used in articular cartilage repair. J Orthop Res. 2000;18:909–11.
55. Dorotka R, Windberger U, Macfelda K, Bindreiter U, Toma C, Nehrer S. Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. Biomaterials. 2005;26:3617–29.
56. Dorotka R, Bindreiter U, Macfelda K, Windberger U, Nehrer S. Marrow stimulation and chondrocyte transplantation using a collagen matrix for cartilage repair. Osteoarthritis Cartilage. 2005;13:655–64.
57. Brehm W, Aklin B, Yamashita T, Rieser F, Trüb T, Jakob RP*, et al*. Repair of superficial osteochondral defects with an autologous scaffold-free cartilage construct in a caprine model: implantation method and short-term results. Osteoarthritis Cartilage. 2006;14:1214–26.
58. Jones CW, Willers C, Keogh A, Smolinski D, Fick D, Yates PJ*, et al*. Matrix-induced autologous chondrocyte implantation in sheep: objective assessments including confocal arthroscopy. J Orthop Res. 2008;26:292–303.
59. Kangarlu A, Gahunia HK. Magnetic resonance imaging characterization of osteochondral defect repair in a goat model at 8 T. Osteoarthritis Cartilage. 2006;14:52–62.
60. Lu Y, Markel MD, Swain C, Kaplan LD. Development of partial thickness articular cartilage injury in an ovine model. J Orthop Res. 2006;24:1974–82.
61. van Susante JL, Buma P, Homminga GN, van den Berg WB, Veth RP. Chondrocyte-seeded hydroxyapatite for repair of large articular cartilage defects: a pilot study in the goat. Biomaterials. 1998;19:2367–74.
62. Wong MW, Qin L, Lee KM, Tai KO, Chong WS, Leung KS*, et al*. Healing of bone-tendon junction in a bone trough: a goat partial patellectomy model. Clin Orthop Relat Res. 2003;(413):291–302.
63. Hill MA. Causes of degenerative joint disease (osteoarthrosis) and dyschondroplasia (osteochondrosis) in pigs. J Am Vet Med Assoc. 1990;197:107–13.
64. Vasara AI, Hyttinen MM, Pulliainen O, Lammi MJ, Jurvelin JS, Peterson L*, et al*. Immature porcine knee cartilage lesions show good healing with or without autologous chondrocyte transplantation. Osteoarthritis Cartilage. 2006;14:1066–74.
65. Jung M, Kaszap B, Redöhl A, Steck E, Breusch S, Richter W*, et al*. Enhanced early tissue regeneration after matrix-assisted autologous mesenchymal stem cell transplantation in full thickness chondral defects in a minipig model. Cell Transplant. 2009;18:923–32.
66. Gotterbarm T, Reitzel T, Schneider U, Voss HJ, Stofft E, Breusch SJ. Integration of periosteum covered autogenous bone grafts with and without autologous chondrocytes: an animal experiment using the Gottinger minipig. Orthopade. 2003;32:65–73.
67. Petersen JP, Ueblacker P, Goepfert C, Adamietz P, Baumbach K, Stork A*, et al*. Long term results after implantation of tissue engineered cartilage for the treatment of osteochondral lesions in a minipig model. J Mater Sci Mater Med. 2008;19:2029–38.
68. Jung M, Breusch S, Daecke W, Gotterbarm T. The effect of defect localization on spontaneous repair of osteochondral defects in a Gottingen minipig model: a retrospective analysis of the medial patellar groove versus the medial femoral condyle. Lab Anim. 2009;43:191–7.
69. Frisbie DD, Trotter GW, Powers BE, Rodkey WG, Steadman JR, Howard RD*, et al*. Arthroscopic subchondral bone plate microfracture technique augments healing of large chondral defects in the radial carpal bone and medial femoral condyle of horses. Vet Surg. 1999;28:242–55.
70. Hurtig MB, Fretz PB, Doige CE, Schnurr DL. Effects of lesion size and location on equine articular cartilage repair. Can J Vet Res. 1988;52:137–46.
71. Nixon AJ, Fortier LA, Williams J, Mohammed H. Enhanced repair of extensive articular defects by insulin-like growth factor-I-laden fibrin composites. J Orthop Res. 1999;17:475–87.

72. Masri M, Lombardero G, Velasquillo C, Martínez V, Neri R, Villegas H*, et al*. Matrix-encapsulation cell-seeding technique to prevent cell detachment during arthroscopic implantation of matrix-induced autologous chondrocytes. Arthroscopy. 2007;23:877–83.
73. Bodo G, Hangody L, Modis L, Hurtig M. Autologous osteochondral grafting (mosaic arthroplasty) for treatment of subchondral cystic lesions in the equine stifle and fetlock joints. Vet Surg. 2004;33:588–96.
74. Hendrickson DA, Nixon AJ, Grande DA, Todhunter RJ, Minor RM, Erb H*, et al*. Chondrocyte-fibrin matrix transplants for resurfacing extensive articular cartilage defects. J Orthop Res. 1994;12:485–97.
75. Frisbie DD, Oxford JT, Southwood L, Trotter GW, Rodkey WG, Steadman JR*, et al*. Early events in cartilage repair after subchondral bone microfracture. Clin Orthop Relat Res. 2003;(407):215–227.
76. Fortier LA, Nixon AJ, Lust G. Phenotypic expression of equine articular chondrocytes grown in three-dimensional cultures supplemented with supraphysiologic concentrations of insulin-like growth factor-1. Am J Vet Res. 2002;63:301–5.
77. Fortier LA, Potter HG, Rickey EJ, Schnabel LV, Foo LF, Chong LR*, et al*. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. J Bone Joint Surg Am. 2010;92:1927–37.
78. Martinelli MJ, Eurell J, Les CM, Fyhrie D, Bennett D. Age-related morphometry of equine calcified cartilage. Equine Vet J. 2002;34:274–8.
79. Lowerison M, Allendorf S, Bell A, White L, Whiteside R, Marks P*, et al*. Subchondral bone plate characteristics in human and animal bone: implications for cartilage repair. Presented at the 9th World Congress of the International Cartilage Repair Society, September 26–29, 2010, Sitges, Barcelona, Spain.
80. Frisbie DD, Lu Y, Kawcak CE, DiCarlo EF, Binette F, McIlwraith CW. In vivo evaluation of autologous cartilage fragment-loaded scaffolds implanted into equine articular defects and compared with autologous chondrocyte implantation. Am J Sports Med. 2009;37(suppl 1):71S–80S.
81. Frisbie DD, Bowman SM, Colhoun HA, DiCarlo EF, Kawcak CE, McIlwraith CW. Evaluation of autologous chondrocyte transplantation via a collagen membrane in equine articular defects: results at 12 and 18 months. Osteoarthritis Cartilage. 2008;16:667–79.
82. Barnewitz D, Endres M, Krüger I, Becker A, Zimmermann J, Wilke I*, et al*. Treatment of articular cartilage defects in horses with polymer-based cartilage tissue engineering grafts. Biomaterials. 2006;27:2882–9.
83. von Rechenberg B, Guenther H, McIlwraith CW, Leutenegger C, Frisbie DD, Akens MK*, et al*. Fibrous tissue of subchondral cystic lesions in horses produce local mediators and neutral metalloproteinases and cause bone resorption in vitro. Vet Surg. 2000;29:420–9.
84. Muschler GF, Raut VP, Patterson TE, Wenke JC, Hollinger JO. The design and use of animal models for translational research in bone tissue engineering and regenerative medicine. Tissue Eng Part B Rev. 2010;16:123–45.
85. Shimomura K, Ando W, Tateishi K, Nansai R, Fujie H, Hart DA*, et al*. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell–based repair of cartilage in a large animal model. Biomaterials. 2010;31:8004–11.
86. Gomoll AH, Farr J, Gillogly SD, Kercher J, Minas T. Surgical management of articular cartilage defects of the knee. J Bone Joint Surg Am. 2010;92:2470–90.
87. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res. 1989;7:208–18.
88. Dell'Accio F, Vanlauwe J, Bellemans J, Neys J, De Bari C, Luyten FP. Expanded phenotypically stable chondrocytes persist in the repair tissue and contribute to cartilage matrix formation and structural integration in a goat model of autologous chondrocyte implantation. J Orthop Res. 2003;21:123–31.
89. Hunziker EB. Articular cartilage structure in human and experimental animal models. In: Kuettner KE, Schleyerbach R, Peyron JG, Hascall VC, editors. Articular cartilage and osteoarthritis. New York: Raven; 1992. p. 183–99.
90. Wang F, Ying Z, Duan X, Tan H, Yang B, Guo L*, et al*. Histomorphometric analysis of adult articular calcified cartilage zone. J Struct Biol. 2009;168:359–65.
91. Allendorf S, Lowerison M, Bell A, Hoemann C, Bursac P. Interaction of microfracture awl design with subchondral bone in early osteoarthritis. Presented at the 9th World Congress of the International Cartilage Repair Society, September 26–29, 2010, Sitges, Barcelona, Spain.
92. Frisbie DD, Morisset S, Ho CP, Rodkey WG, Steadman JR, McIlwraith CW. Effects of calcified cartilage on healing of chondral defects treated with microfracture in horses. Am J Sports Med. 2006;34:1824–31.
93. Richter W. Mesenchymal stem cells and cartilage in situ regeneration. J Intern Med. 2009;266:390–405.
94. Vasara AI, Hyttinen MM, Lammi MJ, Lammi PE, Långsjö TK, Lindahl A*, et al*. Subchondral bone reaction associated with chondral defect and attempted cartilage repair in goats. Calcif Tissue Int. 2004;74:107–14.
95. Jackson DW, Lalor PA, Aberman HM, Simon TM. Spontaneous repair of full-thickness defects of articular cartilage in a goat model: a preliminary study. J Bone Joint Surg Am. 2001;83A:53–64.
96. von Rechenberg B, Akens MK, Nadler D, Bittmann P, Zlinszky K, Kutter A*, et al*. Changes in subchondral bone in cartilage resurfacing: an experimental study in sheep using different types of osteochondral grafts. Osteoarthritis Cartilage. 2003;11:265–77.
97. Qi YY, Chen X, Jiang YZ, Cai HX, Wang LL, Song XH*, et al*. Local delivery of autologous platelet in collagen matrix simulated in situ articular cartilage repair. Cell Transplantation. 2009;18:1161–9.
98. Henderson IJP, La Valette DP. Subchondral bone overgrowth in the presence of full-thickness cartilage defects in the knee. Knee. 2005;12:435–40.
99. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation

after previous treatment with marrow stimulation techniques. Am J Sports Med. 2009;37:902–8.
100. Fischer BL, Ludders JW, Asakawa M, Fortier LA, Fubini SL, Nixon AJ, *et al*. A comparison of epidural buprenorphine plus detomidine with morphine plus detomidine in horses undergoing bilateral stifle arthroscopy. Vet Anaesth Analg. 2009;36:67–76.
101. Custers RJ, Saris DB, Dhert WJ, Verbout AJ, van Rijen MH, Mastbergen SC, *et al*. Articular cartilage degeneration following the treatment of focal cartilage defects with ceramic metal implants and compared with microfracture. J Bone Joint Surg Am. 2009;91:900–10.
102. Hurtig M, Chubinskaya S, Dickey J, Rueger D. BMP-7 protects against progression of cartilage degeneration after impact injury. J Orthop Res. 2009;27:602–11.
103. Hepp P, Osterhoff G, Niederhagen M, Marquass B, Aigner T, Bader A, *et al*. Perilesional changes of focal osteochondral defects in an ovine model and their relevance to human osteochondral injuries. J Bone Joint Surg Br. 2009;91B:1110–9.
104. Lee KB, Hui JH, Song IC, Ardany L, Lee EH. Injectable mesenchymal stem cell therapy for large cartilage defects: a porcine model. Stem Cells. 2007;25:2964–71.
105. Waddell DD, Bert JM. The use of hyaluronan after arthroscopic surgery of the knee. Arthroscopy. 2010;26:105–11.
106. Ghadially JA, Ghadially R, Ghadially FN. Long-term results of deep defects in articular cartilage: a scanning electron microscope study. Virchows Arch B Cell Pathol. 1977;25:125–36.
107. Howard RD, McIlwraith CW, Trotter GW, Powers BE, McFadden PR, Harwood FL, *et al*. Long-term fate and effects of exercise on sternal cartilage autografts used for repair of large osteochondral defects in horses. Am J Vet Res. 1994;55:1158–67.
108. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg Am. 1993;75A:532–53.
109. Hunter DJ, Losina E, Guermazi A, Burstein D, Lassere MN, Kraus V. A pathway and approach to biomarker validation and qualification for osteoarthritis clinical trials. Curr Drug Targets. 2010;11:536–45.
110. Park S, Krishnan R, Nicoll SB, Ateshian GA. Cartilage interstitial fluid load support in unconfined compression. J Biomech. 2003;36:1785–96.
111. Buschmann MD, Soulhat J, Shirazi-Adl A, Jurvelin JS, Hunziker EB. Confined compression of articular cartilage: linearity in ramp and sinusoidal tests and the importance of interdigitation and incomplete confinement. J Biomech. 1998;31:171–8.
112. Mow VC, Gibbs MC, Lai WM, Zhu WB, Athanasiou KA. Biphasic indentation of articular cartilage: II. A numerical algorithm and an experimental study. J Biomech. 1989;22:853–61.
113. Roemhildt ML, Coughlin KM, Peura GD, Fleming BC, Beynnon BD. Material properties of articular cartilage in the rabbit tibial plateau. J Biomech. 2006;39:2331–7.
114. Fortin M, Soulhat J, Shirazi-Adl A, Hunziker EB, Buschmann MD. Unconfined compression of articular cartilage: nonlinear behavior and comparison with a fibril-reinforced biphasic model. J Biomech Eng. 2000;122:189–95.
115. Langelier E, Buschmann MD. Increasing strain and strain rate strengthen transient stiffness but weaken the response to subsequent compression for articular cartilage in unconfined compression. J Biomech. 2003;36:853–9.
116. Laasanen MS, Töyräs J, Korhonen RK, Rieppo J, Saarakkala S, Nieminen MT, *et al*. Biomechanical properties of knee articular cartilage. Biorheology. 2003;40:133–40.
117. Bae WC, Temple MM, Amiel D, Coutts RD, Niederauer GG, Sah RL. Indentation testing of human cartilage: sensitivity to articular surface degeneration. Arthritis Rheum. 2003;48:3382–94.
118. Bae WC, Law AW, Amiel D, Sah RL. Sensitivity of indentation testing to step-off edges and interface integrity in cartilage repair. Ann Biomed Eng. 2004;32:360–9.
119. Huang CY, Mow VC, Ateshian GA. The role of flow-independent viscoelasticity in the biphasic tensile and compressive responses of articular cartilage. J Biomech Eng. 2001;123:410–7.
120. Soulhat J, Buschmann MD, Shirazi-Adl A. A fibril-network-reinforced biphasic model of cartilage in unconfined compression. J Biomech Eng. 1999;121:340–7.
121. Schmidt TA, Gastelum NS, Nguyen QT, Schumacher BL, Sah RL. Boundary lubrication of articular cartilage: role of synovial fluid constituents. Arthritis Rheum. 2007;56:882–91.
122. Schmidt TA, Sah RL. Effect of synovial fluid on boundary lubrication of articular cartilage. Osteoarthritis Cartilage. 2007;15:35–47.
123. Vasara AI, Nieminen MT, Jurvelin JS, Peterson L, Lindahl A, Kiviranta I. Indentation stiffness of repair tissue after autologous chondrocyte transplantation. Clin Orthop Relat Res. 2005;(433):233–42.
124. Hattori K, Takakura Y, Ohgushi H, Habata T, Uematsu K, Yamauchi J, *et al*. Quantitative ultrasound can assess the regeneration process of tissue-engineered cartilage using a complex between adherent bone marrow cells and a three-dimensional scaffold. Arthritis Res Ther. 2005;7:R552–9.
125. Quenneville Q, Garon G, Légaré L, Buschmann MD. Load and streaming potential responses of articular cartilage as a function of compression speed during indentation. Trans Orthop Res Soc. 2003;28:659.
126. Bae WC, Schumacher BL, Sah RL. Indentation probing of human articular cartilage: effects on chondrocyte viability. Osteoarthritis Cartilage. 2007;15:9–18.
127. Trattnig SWinalski CS, Marlovits S, Jurvelin JS. Welsch GH, Potter HG. Magnetic Resonance Imaging of Cartilage Repair-A Review. Cartilage. 2011;2:5-26.
128. Roos EM Engelhart L, Ranstam J, Anderson AF, Irrgang JJ, Marx RG, Tegner Y, Davis AM. ICRS Recommendation Document: Patient-Reported Outcome Instruments for Use in Patients with Articular Cartilage Defects. Cartilage.