A proposed model of naturally occurring osteoarthritis in the domestic rabbit

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Osteoarthritis affects one in eight American adults over the age of 25 y and is a leading cause of chronic disability in the US. Translational research to investigate treatments for this naturally occurring joint disease requires an appropriate animal model. The authors conducted a retrospective study to assess the potential of naturally occurring osteoarthritis in the domestic rabbit as a model of the human disease. Analysis of radiographic images showed that the presence and severity of osteoarthritis were significantly influenced by both age and body weight. The most commonly affected joints were the knee and the hip. The findings reported here suggest that the rabbit is an excellent model of spontaneously arising osteoarthritis that may be useful in translational research pertaining to the human disease.

Osteoarthritis is a slowly progressing degenerative joint disease that most commonly affects larger diarthrodial joints, such as the knee and shoulder[1,2]. Animal models of osteoarthritis are necessary to study its pathogenesis and pathology and to evaluate the efficacy of potential treatments[3,4]. Current animal models of osteoarthritis lack the spontaneous occurrence of the disease. Instead, the traumatic onset of osteoarthritis is induced by inflicting joint damage such as removal of the meniscus or transaction of the collateral or cruciate ligament[3,4]. Although traumatically induced osteoarthritis in animals may mimic certain aspects of the pathogenesis and pathology of naturally occurring osteoarthritis in humans, known differences between traumatically induced and naturally occurring osteoarthritis limit its applicability to the human disease. First, whereas human osteoarthritis usually takes years to develop, the disease progression in the animal model after surgical induction is much faster, making it a less realistic model[5,6]. Second, rodents will generally resume near-normal activity soon after experimental induction of osteoarthritis, whereas in humans the use of an affected limb is restricted after osteoarthritis-associated injury[3,4]. Finally, spontaneous human osteoarthritis results from a complex interplay of genetic, environmental and iatrogenic effects that are difficult, if not impossible, to replicate in an induced model[4]. For example, intra-articular injections of arthritis-inducing substances can induce osteoarthritis but may also result in other undesired changes, such as prolonged irritation resulting in granuloma formation and possible foreign body reaction[4,7,8]. A key to successfully identifying treatments for human osteoarthritis is the selection of an animal model that shares the natural onset of the human disease[9].

The practicality of using a particular animal model must also be considered. Studies investigating potential osteoarthritis treatments require subjects with joints that are large enough to allow surgical interventions and with sufficient tissue to allow in-depth tissue characterization. In cases where spontaneous osteoarthritis has been described in animals, the species used are small mammals such as mice, guinea pigs and Syrian hamsters[3,10–13]. In these small species, only the knee can be studied in most cases, and surgical approaches are technically demanding or impossible[4]. Therefore, although the pathology and pathogenesis of osteoarthritis in the guinea pig and Syrian hamster are likely

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to be similar to those of osteoarthritis in humans[3], their small size limits their utility in research. An intermediate-sized animal model of naturally occurring osteoarthritis is needed.

Complex surgical procedures and in-depth tissue characterization can be done on adult rabbit joints[14–17]. The rabbit has been used for the study of articular cartilage repair and other processes resulting from joint injury by defect creation or chemical insult[18]. These injuries induce an osteoarthritis-like condition but do not mimic the slow progression of disease that is seen in humans. To the best of our knowledge, naturally occurring osteoarthritis in the rabbit has not been previously described. Therefore, the aim of the present study is to report the occurrence and prevalence of naturally occurring osteoarthritis in the domestic rabbit and the possible effects of age and weight on its development.

Conventional radiography is the standard diagnostic imaging method for the evaluation of osteoarthritis progression, because radiographic features are reliable indicators of the presence of gross macroscopic lesions (cartilage and meniscal degeneration, osteophyte formation and tibial cartilage lesions)[19–21]. The clinical diagnosis of osteoarthritis usually is confirmed by radiography and graded using a semi-quantitative scoring system that has been validated in clinical and epidemiological studies[1,20,22]. In this study, radiographs were used to semi-quantitatively assess the presence and severity of naturally occurring osteoarthritis in a population of domestic rabbits. We hypothesize that rabbits develop osteoarthritis increasingly with age and weight and that the knee and the hip joints are most commonly affected due to the anatomic conformation of the species.

METHODS

Animals

In this study, we used skeletally mature (>1 y old) domestic rabbits of 13 different breeds that had been admitted to the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis, between January 2004 and November 2010. To be included in the study, each animal must have been admitted for reasons other than orthopedic disease and have had radiographic examination of at least one pair of appendicular joints (hip, knee, shoulder and elbow). We excluded animals with presence of lameness, history or evidence of orthopedic trauma, systemic disease affecting the skeleton (such as neoplasia), or metabolic disease. Of 330 rabbits in the medical record system, 187 met the criteria for inclusion in the study. We divided the rabbits into four groups according to their ages at the time radiographs were obtained: group 1 included rabbits that were 1–3 y old (n = 32); group 2, 3–6 y old (n = 54); group 3, 6–9 y old (n = 73); and group 4, greater than 9 y old (n = 28). Age and weight information was obtained from the medical records of each animal. The weight of each animal had been recorded on the first day of examination, using an electronic scale.

Image evaluation

We examined radiographs that had been acquired using a commercially available digital detector (Eklin Medical Systems, Inc., Santa Clara, CA). All digital images were evaluated on a medical-grade flat-screen monitor (NEC-Mitsubishi Electric Visual Systems Corp., Tokyo, Japan) using commercially available software (eFilm Workstation 2.1.0, eFilm Medical Inc., Toronto, Canada). No image enhancement or contrast agent was applied. At least two radiographs were examined per rabbit; in some instances, up to eight radiographs from one rabbit were examined. We used all of the radiographic images that were available in order to yield the most accurate diagnosis. An experienced, board-certified radiologist (E.R.W.) who was blind to the original diagnosis scored each radiograph for the

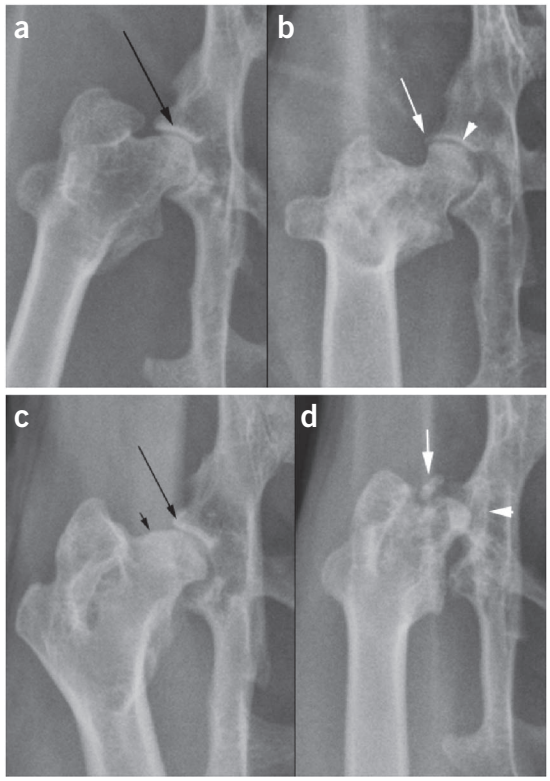

FIGURE 1 | Radiographic images of hip joints with increasing degrees of osteoarthritis severity. (**a**) Healthy hip joint (score = 0). Normal joint space (arrow). (**b**) Mild osteoarthritis (score = 1). Minimal periarticular new bone formation (arrow) and regional joint space narrowing (arrowhead) are visible. (**c**) Moderate osteoarthritis (score = 2). Mild subchondral bone sclerosis (long arrow) and remodeling of the femoral head and neck associated with new bone formation (short arrow) are visible. (**d**) Marked osteoarthritis (score = 3). Pronounced coxafemoral subluxation, a large osseous body in the joint space (arrow) and remodeling of femoral head and neck and acetabulum (arrowhead) are visible.

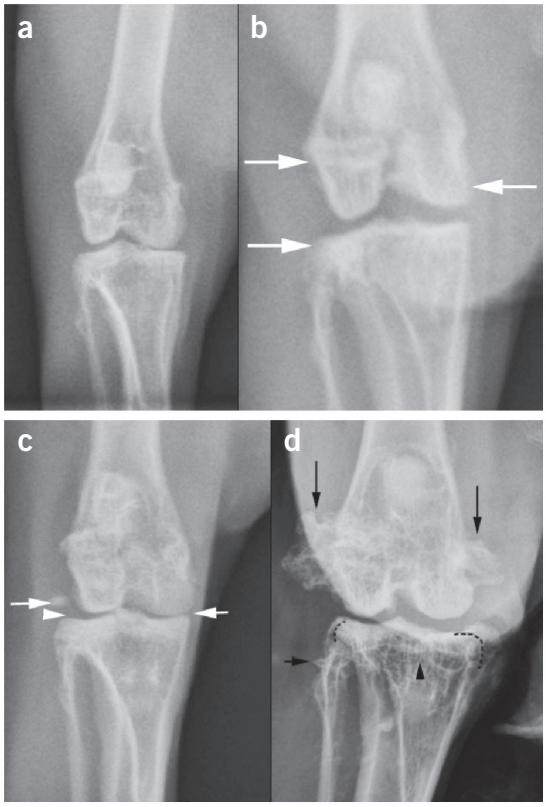

FIGURE 2 | Radiographic images of knee joints with increasing degrees of osteoarthritis severity. (**a**) Normal knee joint (score = 0). (**b**) Mild osteoarthritis (score = 1). Minimal periarticular new bone formation arising from the distal femur and proximal tibia (arrows) is visible. (**c**) Moderate osteoarthritis (score = 2). Minimal periarticular new bone formation involving the medial aspect of the proximal tibia (right arrow), meniscal mineralization (left arrowhead) and a separate osseous body in the joint space (left arrow) are visible. (**d**) Marked osteoarthritis (score = 3). Pronounced periarticular new bone formation involving the distal femur (long arrows) and proximal tibia (dashed lines), architectural remodeling of subchondral bone in the proximal tibia (short arrow) and subchondral sclerosis of the lateral and medial femoral condyles (arrowhead) are visible.

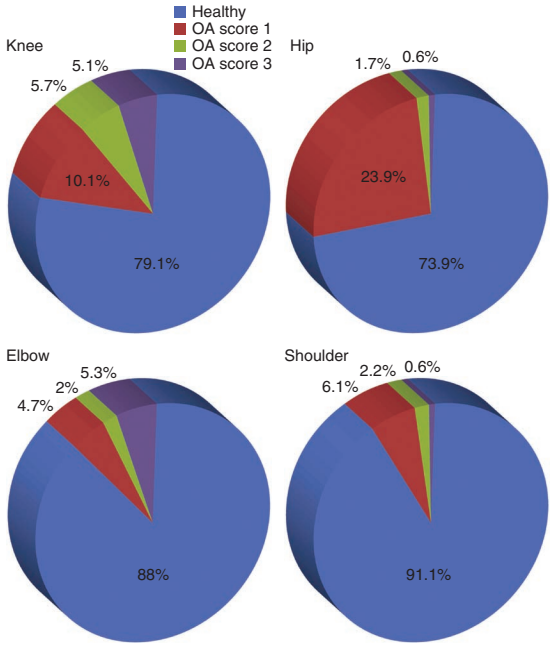

FIGURE 3 | The presence and severity of osteoarthritis detected in radiographic images of each of the examined joints in the total population. The most commonly affected joints were the hip (26.2%) and the knee (20.9%). OA, osteoarthritis.

presence and severity of osteoarthritis using a scale of 0 to 3 (**Figs. 1** and **2**). A score of 0 was given if no osteoarthritis was present. A score of 1 was given to indicate mild osteoarthritis if there was any evidence of meniscal mineralization; mild remodeling of subchondral bone; early signs of periarticular new bone formation; or small, separate osseous bodies within the joint space. A score of 2 was given to indicate moderate osteoarthritis if the previously described features were more pronounced. A score of 3 was given to indicate marked osteoarthritis if the previously described signs were even more pronounced, if large separate osseous bodies were evident within the joint space or if moderate subluxation was present. The radiologist's findings were later compared with the original diagnosis documented in the animal's records, which had originally been reviewed by a board-certified radiologist before being finalized. In this retrospective comparison, the current radiographic interpretations and those in the medical records were highly congruent. Therefore, we are confident that the presence and severity of osteoarthritic changes reported in this study are reproducible and reliable.

Statistical analysis

We applied the Cochran–Armitage trend test to determine whether there was an association between the occurrence of osteoarthritis and older age. We measured the increase in osteoarthritis score of the hip and knee joints between age groups with the Jonckheere–Terpstra test. We compared the distribution of osteoarthritis between the forelimb and hindlimb joints using McNemar's test. To determine the relationship between body weight and presence of osteoarthritis, we used binary logistic regression with the strength of association presented as odds ratios (ORs) with a 95% confidence interval (c.i.). We used binary logistic regression to estimate the influence of four categories of gender (intact female, intact male, spayed female, castrated male) on the probability of osteoarthritis occurrence. Finally, we used a χ^2 test for homogeneity to evaluate the association of rabbit breed with prevalence of osteoarthritis. For all tests, P values less than 0.05 were considered statistically significant.

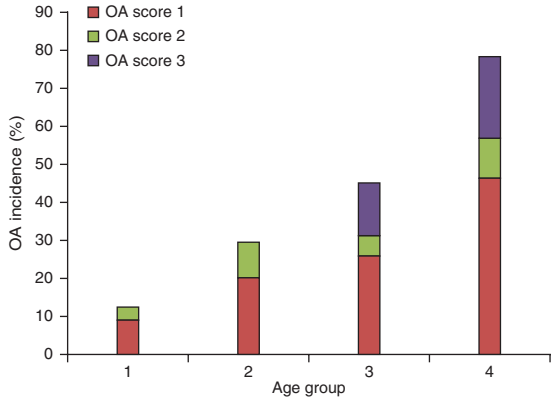

FIGURE 4 | The influence of age on the development and severity of osteoarthritis. The percentage of individuals affected by osteoarthritis was higher for older age groups ($P < 0.0001$). The severity of osteoarthritis was also greater for individuals in older age groups ($P < 0.001$). OA, osteoarthritis.

RESULTS

Prevalence of osteoarthritis

Overall, osteoarthritis was detected in radiographs from 40.2% of the studied population. The most commonly affected joints were the hip and the knee (**Fig. 3**). Of the affected rabbits, 37% had two or more pairs of joints affected, and 13% had three or more pairs of joints affected. Animals were significantly more likely to have a hindlimb (hip or knee joint) affected with osteoarthritis and a healthy forelimb (elbow or shoulder joint) than to have a forelimb affected with osteoarthritis and healthy hindlimb ($P < 0.0001$; data not shown).

Association between age and osteoarthritis

There was a significant association between age group and the presence of osteoarthritis in at least one joint ($P < 0.0001$; **Fig. 4**). Radiographic features indicative of osteoarthritis were observed in group 1 (12.5%) and increased in prevalence to >70% in group 4. In addition, there was strong positive correlation between multiple joint involvement and older age ($P < 0.001$; data not shown). At an age of ~6 y, about the midpoint of a domestic rabbit's life, approximately 50% of the population had radiographic signs of osteoarthritis in at least one joint. As age increased, the highest osteoarthritis score given for the hip or knee for any animal in the age group also increased ($P < 0.001$).

Effects of breed, gender and weight on osteoarthritis

There was a significant association between rabbit breed and the presence of osteoarthritis ($P = 0.0075$; data not shown), with dwarf rabbit breeds having a lower incidence (one case of osteoarthritis among 16 dwarf rabbits) than the other 12 non-dwarf breeds. No significant differences in osteoarthritis prevalence were observed between the non-dwarf breeds. There was no significant influence of gender on the presence of osteoarthritis. There was also no significant influence of weight on the presence of osteoarthritis, but rabbits weighing more than 5 kg ($n = 5$) tended to have radiographic signs of osteoarthritis more frequently (OR = 10.5, $P = 0.019$) than those weighing 5 kg or less ($n = 182$).

DISCUSSION

Nearly 27 million people 25 years of age or older in the US have osteoarthritis, and as the population ages, this estimate is likely to increase[2]. The estimated cost of this disorder, including medical costs and lost wages, exceeds $65 billion per year in the US[2,23]. Therefore, it is imperative to investigate treatments for osteoarthritis using an appropriate animal model that simulates the slow progression of the disease and accurately reflects its pathogenesis and pathology. Other spontaneous animal models of osteoarthritis, including the guinea pig, mouse and hamster, suffer from small joint sizes that hinder their utility in surgical interventions[4]. To our knowledge, the present study is the first to report the occurrence and distribution of naturally occurring osteoarthritis in the rabbit and to propose the rabbit as a possible animal model for human osteoarthritis.

The similarities between naturally occurring osteoarthritis in humans and in rabbits support the use of the rabbit as a model for osteoarthritis research. The prevalence of osteoarthritis in humans increases with age, and the joints most commonly affected are the knees and hips. The present study documents a similar progression pattern in the domestic rabbit; over 10% of rabbits between 1 and 3 y of age had radiographic signs of osteoarthritis and about 50% had radiographic signs of osteoarthritis by the age of 6 y. Hindlimb joints, including the knee and hip, were most commonly affected in this study population. In addition to age, body weight is an important predisposing factor for the development of spontaneous osteoarthritis in humans, which has been intimately linked to obesity[12,24]. In this study, we observed that osteoarthritis in rabbits was also associated with weight. For rabbits weighing up to 5 kg, weight did not have a significant influence on the prevalence of osteoarthritis, whereas for rabbits weighing more than 5 kg (greater than the normal range), weight did have a significant influence on the prevalence of osteoarthritis. These findings suggest that a population of older, heavier rabbits should have a high incidence of naturally occurring osteoarthritis.

The radiographic quantification method used in this study is the current standard for diagnostic imaging of osteoarthritis and is commonly used as an initial assessment and monitoring tool for ongoing clinical studies of treatments for osteoarthritis[25–28]. One major drawback of using radiographs to diagnose osteoarthritis is that radiographic features are evident only in the presence

of moderate to advanced lesions[25]. In the early stages of osteoarthritis, imaging techniques including radiography, ultrasound and magnetic resonance imaging are often considered insufficient[25,29]. For example, one study showed that the changes to the cartilage that occurred up to 40 weeks after meniscectomy in rabbits were not extensive enough to be detected by radiography[30]. The incidence of osteoarthritis reported in this study is therefore probably an underestimation of the true prevalence of naturally occurring osteoarthritis in the rabbit. Future studies evaluating naturally occurring osteoarthritis in the rabbit could consider incorporating magnetic resonance or computed tomography imaging methods for the earlier detection of the disease.

Previous studies have claimed that rabbit articular cartilage, unlike human cartilage, can be repaired[31,32], causing the rabbit model to lose favor[33,34]. Contrary to these claims, our study shows that, if allowed to live longer, rabbits begin to show cartilage deterioration and signs of osteoarthritis similar to those observed in humans. Any healing responses that may have occurred in our study population were likely to be temporary. Our findings are consistent with those of other studies reporting that the healing response after damage to the articular cartilage of adult rabbits generates only fibrocartilaginous repair tissue[35–37]. This tissue is prone to degeneration over the long term; the repaired tissue undergoes loss of matrix cellularity and proteoglycans, as well as loss of physical integrity of the surface[37,38]. In addition, the pattern of healing depends largely on the size of the defect and the time from cartilage insult to sacrifice[31,32,39]. At present, research efforts are directed toward enhancing natural healing potential of cartilage or replacing the damaged cartilage with engineered tissues[9]. These approaches, though promising, are far from reliable and are not yet sufficiently refined to be used in the clinical setting. Better understanding of cartilage repair processes relies on the selection of an animal model that mimics the natural osteoarthritis progression of humans[9].

When choosing an animal model for the study of osteoarthritis, the age of the animal is important. There is wide variability in the time at which bone maturity is achieved in rabbits[40]. When reviewing the radiographs in this study, we detected a few cases in which 9- to 10-month-old rabbits still had open physes, or growth plates, in the distal femur and proximal tibia (data not shown). We highly recommend that researchers confirm skeletal maturity in rabbits used to model osteoarthritis. As veterinary medicine advances and owner awareness increases, more pet animals are reaching an advanced age, allowing for the development of chronic disorders such as osteoarthritis to be observed.

Evaluation of cartilage healing has identified important and clinically relevant differences in young versus adult rabbits[36]. In the young rabbit (4 weeks old), trauma to cartilage perpendicular to its surface resulted in regression and necrosis of the tissue within 3 d; at 4–6 weeks after the trauma, there were demarcating fibers covering and protecting the cartilage fragment. In comparison, when the cartilage of adult rabbits (24 months old) was subjected to trauma, tissue splitting identified the absence of wound healing potential, and the repair process did not occur. In addition, in young rabbits, reaction of chondrocytes and mitotic activity were observed, whereas in adult rabbits, these responses were not observed[36]. The maturity of the cartilage is an important factor determining whether, and to what extent, cartilage repair occurs[41].

An appropriate animal model of any disease must feature a high level of 'translatability' to humans. In the case of osteoarthritis, researchers should attempt to match features of human osteoarthritis including spontaneous occurrence, lack of regenerative response and association of incidence and severity with age and weight. The results of this retrospective study suggest that osteoarthritis in rabbit mimics many characteristics of the human disease. We therefore recommend that future efforts should be directed toward the validation of the rabbit as an animal model for use in translational research.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Kinds, M.B. *et al.* A systematic review of the association between radiographic and clinical osteoarthritis of hip and knee. *Osteoarthritis Cartilage* **19,** 768–778 (2011).
2. Lawrence, R.C. *et al.* Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum.* **58,** 26–35 (2008).
3. Bendele, A.M. Animal models of osteoarthritis in an era of molecular biology. *J. Musculoskelet. Neuronal Interact.* **2,** 501–503 (2002).
4. Dinser, R. Animal models for arthritis. *Best Pract. Res. Clin. Rheumatol.* **22,** 253–267 (2008).
5. Bendele, A.M. Progressive chronic osteoarthritis in femorotibial joints of partial medial meniscectomized guinea pigs. *Vet. Pathol.* **24,** 444–448 (1987).
6. Bendele, A.M. & White, S.L. Early histopathologic and ultrastructural alterations in femorotibial joints of partial medial meniscectomized guinea pigs. *Vet. Pathol.* **24,** 436–443 (1987).
7. van der Kraan, P.M., Vitters, E.L., van de Putte, L.B. & van den Berg, W.B. Development of osteoarthritic lesions in mice by "metabolic" and "mechanical" alterations in the knee joints. *Am. J. Pathol.* **135,** 1001–1014 (1989).
8. van der Kraan, P.M., Vitters, E.L., van Beuningen, H.M., van de Putte, L.B. & van den Berg, W.B. Degenerative knee joint lesions in mice after a single intra-articular collagenase injection. A new model of osteoarthritis. *J. Exp. Pathol.* **71,** 19–31 (1990).

9. O'Driscoll, S.W. The healing and regeneration of articular cartilage. *J. Bone Joint Surg. Am.* **80,** 1795–1812 (1998).
10. Bendele, A.M. & Hulman, J.F. Spontaneous cartilage degeneration in guinea pigs. *Arthritis Rheum.* **31,** 561–565 (1988).
11. Bendele, A.M., White, S.L. & Hulman, J.F. Osteoarthrosis in guinea pigs: histopathologic and scanning electron microscopic features. *Lab. Anim. Sci.* **39,** 115–121 (1989).
12. Bendele, A.M. & Hulman, J.F. Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea pigs. *Arthritis Rheum.* **34,** 1180–1184 (1991).
13. Silberberg, R., Saxton, J., Sperling, G. & McCay, C. Degenerative joint disease in Syrian hamsters. *Federation Proceedings* **11,** 427–432 (1952).
14. Chen, H. *et al.* MRI and histologic analysis of collagen type II sponge on repairing the cartilage defects of rabbit knee joints. *J. Biomed. Mater. Res. B. Appl. Biomater.* **96,** 267–275 (2011).
15. Isaac, D.I., Meyer, E.G., Kopke, K.S. & Haut, R.C. Chronic changes in the rabbit tibial plateau following blunt trauma to the tibiofemoral joint. *J. Biomech.* **43,** 1682–1688 (2010).
16. Shirai, T. *et al.* Chondroprotective effect of alendronate in a rabbit model of osteoarthritis. *J. Orthop. Res.* **29,** 1572–1577 (2011).
17. Vaseenon, T. *et al.* Organ-level histological and biomechanical responses from localized osteoarticular injury in the rabbit knee. *J. Orthop. Res.* **29,** 340–346 (2011).
18. Arøen, A., Heir, S., Løken, S., Reinholt, F.P. & Engebretsen, L. Articular cartilage defects in a rabbit model, retention rate of periosteal flap cover. *Acta. Orthop.* **76,** 220–224 (2005).
19. Altman, R. *et al.* Design and conduct of clinical trials in patients with osteoarthritis: recommendations from a task force of the Osteoarthritis Research Society. Results from a workshop. *Osteoarthritis Cartilage* **4,** 217–243 (1996).
20. Boulocher, C.B. *et al.* Radiographic assessment of the femorotibial joint of the CCLT rabbit experimental model of osteoarthritis. *BMC Med. Imaging,* published online, doi:10.1186/1471-2342-10-3 (20 January 2010).
21. Rovati, L.C. Radiographic assessment. Introduction: existing methodology. *Osteoarthritis Cartilage* **7,** 427–429 (1999).
22. Hunter, D.J. & Felson, D.T. Osteoarthritis. *BMJ* **332,** 639–642 (2006).
23. Jackson, D.W., Simon, T.M. & Aberman, H.M. Symptomatic articular cartilage degeneration: the impact in the new millennium. *Clin. Orthop. Relat. Res.* **391** Suppl, 14–25 (2001).
24. Davis, M.A., Ettinger, W.H., Neuhaus, J.M. & Hauck, W.W. Sex differences in osteoarthritis of the knee. The role of obesity. *Am. J. Epidemiol.* **127,** 1019–1030 (1988).
25. Coan, P. *et al. In vivo* x-ray phase contrast analyzer-based imaging for longitudinal osteoarthritis studies in guinea pigs. *Phys. Med. Biol.* **55,** 7649–7662 (2010).
26. Dieppe, P.A. Recommended methodology for assessing the progression of osteoarthritis of the hip and knee joints. *Osteoarthritis Cartilage* **3,** 73–77 (1995).
27. Muraki, S. *et al.* Association of radiographic and symptomatic knee osteoarthritis with health-related quality of life in a population-based cohort study in Japan: the ROAD study. *Osteoarthritis Cartilage* **18,** 1227–1234 (2010).
28. Szebenyi, B. *et al.* Associations between pain, function, and radiographic features in osteoarthritis of the knee. *Arthritis Rheum.* **54,** 230–235 (2006).
29. Mollenhauer, J. *et al.* Diffraction-enhanced X-ray imaging of articular cartilage. *Osteoarthritis Cartilage* **10,** 163–171 (2002).
30. Messner, K., Fahlgren, A., Persliden, J. & Andersson, B.M. Radiographic joint space narrowing and histologic changes in a rabbit meniscectomy model of early knee osteoarthrosis. *Am. J. Sports Med.* **29,** 151–160 (2001).
31. Lietman, S.A., Miyamoto, S., Brown, P.R., Inoue, N. & Reddi, A.H. The temporal sequence of spontaneous repair of osteochondral defects in the knees of rabbits is dependent on the geometry of the defect. *J. Bone Joint Surg. Br.* **84,** 600–606 (2002).
32. Otsuka, Y. *et al.* Requirement of fibroblast growth factor signaling for regeneration of epiphyseal morphology in rabbit full-thickness defects of articular cartilage. *Dev. Growth Differ.* **39,** 143–156 (1997).
33. Little, C.B. & Smith, M.M. Animal models of osteoarthritis. *Current Rheumatology Reviews* **4,** 175–182 (2008).
34. Chu, C.R., Szczodry, M. & Bruno, S. Animal models for cartilage regeneration and repair. *Tissue Eng. Part B Rev.* **6,** 105–115 (2010).
35. Mitchell, N. & Shepard, N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J. Bone Joint Surg. Am.* **58,** 230–233 (1976).
36. Verwoerd-Verhoef, H.L., ten Koppel, P.G., van Osch, G.J., Meeuwis, C.A. & Verwoerd, C.D. Wound healing of cartilage structures in the head and neck region. *Int. J. Pediatr. Otorhinolaryngol.* **43,** 241–251 (1998).
37. Hunziker, E.B. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage* **10,** 432–463 (2002).
38. Wei, X., Gao, J. & Messner, K. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. *J. Biomed. Mater. Res.* **34,** 63–72 (1997).
39. Shapiro, F., Koide, S. & Glimcher, M.J. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. *J. Bone Joint Surg. Am.* **75,** 532–553 (1993).
40. Kaweblum, M. *et al.* Histological and radiographic determination of the age of physeal closure of the distal femur, proximal tibia, and proximal fibula of the New Zealand white rabbit. *J. Orthop. Res.* **12,** 747–749 (1994).
41. Silver, F.H. & Glasgold, A.I. Cartilage wound healing. An overview. *Otolaryngol. Clin. North Am.* **28,** 847–864 (1995).