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The OARSI Histopathology Initiative - Recommendations for Histological Assessments of Osteoarthritis in the Guinea Pig

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Abstract

Objective—This review focuses on the criteria for assessing osteoarthritis (OA) in the guinea pig at the macroscopic and microscopic levels, and recommends particular assessment criteria to assist standardization in the conduct and reporting of preclinical trails in guinea pig models of OA.

Methods—A review was conducted of all OA studies from 1958 until the present that utilized the guinea pig. The PubMed database was originally searched August 1, 2006 using the following search terms: guinea pig and osteoarthritis. We continued to check the database periodically throughout the process of preparing this chapter and the final search was conducted January 7, 2009. Additional studies were found in a review of abstracts from the OsteoArthritis Research Society International (OARSI) conferences, Orthopaedic Research Society (ORS) conferences, and literature related to histology in other preclinical models of OA reviewed for relevant references. Studies that described or used systems for guinea pig joint scoring on a macroscopic, microscopic, or ultrastructural basis were included in the final comprehensive summary and review. General recommendations regarding methods of OA assessment in the guinea pig were derived on the basis of a comparison across studies and an inter-rater reliability assessment of the recommended scoring system.

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Results—A histochemical-histological scoring system (based on one first introduced by H. Mankin) is recommended for semi-quantitative histological assessment of OA in the guinea pig, due to its already widespread adoption, ease of use, similarity to scoring systems used for OA in humans, its achievable high inter-rater reliability, and its demonstrated correlation with synovial fluid biomarker concentrations. Specific recommendations are also provided for histological scoring of synovitis and scoring of macroscopic lesions of OA.

Conclusions—As summarized herein, a wealth of tools exist to aid both in the semi-quantitative and quantitative assessment of OA in the guinea pig and provide a means of comprehensively characterizing the whole joint organ. In an ongoing effort at standardization, we recommend specific criteria for assessing the guinea pig model of OA as part of an OARSI initiative, termed herein the OARSI-HISTOgp recommendations.

Introduction

Osteoarthritis (OA) in the guinea pig can be induced chemically and surgically, and can occur spontaneously (Table 1). Silverstein and Sokoloff were the first to report spontaneous OA-like changes in the guinea pig [1], although this report described only the bony manifestations of the disease and lacked information about articular cartilage alterations. Bendele *et. al.* next noted spontaneous OA-like changes in non-operated knees, contralateral to partial medial meniscectomized knees [2]. This observation was quickly followed by a characterization of spontaneous knee OA in the Hartley guinea pig [3], and an in-depth light and electron microscopic description, including an assessment of shoulder and elbow joints [4]. Coincident with these observations, the guinea pig continued to be utilized for surgical models of OA, induced by either medial meniscectomy [2,5], or section of the lateral collateral ligament, with or without section of the medial collateral ligament [5].

The majority of studies of OA in the guinea pig have utilized the outbred albino Dunkin-Hartley guinea pig, although a few other strains of guinea pig have been evaluated and display similar susceptibility to OA, such as the English Short Hair (Kraus and Huebner, unpublished observation), or lesser susceptibility to knee OA, including Strain 13 [6], Bristol Strain 2 [7], and the GOHI strain [8]. The evaluation of OA has been performed primarily in male animals, mainly because of a more rapid weight gain that results in more consistent pathological alterations [9]. However, spontaneous lesions have also been observed in female animals [4], and it has been reported that the progression of knee OA can be accelerated with ovariectomy [10,11]. The attraction of the guinea pig as an OA model system is its histopathological similarity to human disease. The appearance of joint pathology in guinea pig and humans is both age-related and subject to a variety of well-recognized OA risk factors shared in common with humans including body weight [12], mechanical load [13], and high bone turnover [6]. Spontaneous lesions in the knee are bilaterally symmetric, and appear first, and are more pronounced, in the medial knee compartment in the area not covered by the meniscus. This corresponds to the pattern of localization of idiopathic, non-traumatic knee OA in humans. Femoral lesions lag behind the appearance of tibial lesions.

In addition to the histological similarities of OA between the guinea pig and humans, a variety of OA-related biomarkers, used to evaluate human OA, are detectable in guinea pig cartilage or body fluids [14]. The guinea pig is also amenable to evaluation by a variety of methods for assessing disease status including magnetic resonance imaging [15–22], micro-computed tomography [23–31], radiography [7,32–39], and dual x-ray absorptiometry to quantify bone density [28,40–43]. Joints are also large enough to enable cartilage harvest for molecular analyses and to accommodate cartilage and bone biomechanical analyses that can provide valuable quantitative outcomes to complement histological assessments [30,44–47]. Due to the fact that guinea pigs are relatively sedentary, it may also be possible to simultaneously

evaluate the effects of a therapeutic agent on both spontaneous knee OA and surgically induced OA in the guinea pig because unilateral knee meniscectomy does not appear to alter the course of spontaneous knee OA in the contralateral limb (Bendele, unpublished observation). Finally, much is known about the general care and manipulation of this animal [48], which facilitates the ease with which OA studies can be conducted. This review focuses on the methods of assessment (macroscopic, microscopic, and ultrastructural) of OA in this animal model system and recommends particular methods of assessment to assist standardization in the conduct and reporting of clinical trails in the guinea pig model of OA.

I. Anatomy and Joint Pathology

Anatomy

Although classified in the order Rodentia, the guinea pig has a phylogenetic origin closer to lagomorphs than rodents [49]. The guinea pig knee, like the human knee, is a diarthrodial joint composed of cartilage, bone, synovium, and supporting structures such as ligaments. The age-related pathological process known as OA affects all of these tissue components. Bone growth in the guinea pig ceases by 4 months of age, evinced by stable tibial condyle widths after 3 months of age [16]. However, skeletal maturity of the limb, defined by growth plate fusion, does not occur until between 7 months (femur) and 12–18 months (tibia) of age [50]. The structures of the guinea pig knee (at age 12 months) are shown in Figure 1.

Pathology

The preferential occurrence of spontaneous OA in the medial compartment of the guinea pig knee is due to the fact that the medial aspect of the knee is loaded predominantly in the guinea pig as in humans [3]. Cartilage degeneration is characterized by the breakdown of type II collagen and the loss of proteoglycan. Changes in bone associated with the development of OA include subchondral bone thickening, osteophyte formation, and alterations in trabecular bone. The OA-related changes in cartilage and bone are evident microscopically after 2 months with a steady age-related worsening in joint pathology by histological appearance (Figure 2A), apparent upon quantification of histological changes using the histochemical-histological scoring system proposed herein (Figure 2B). Even earlier pathological changes are apparent at 2 months of age in the form of collagen fibril disruption detected by polarized light microscopy (imaging sections stained with picosirius red)[51]. The synovium has been characterized in only a few studies in the guinea pig [12,33,34,52–57] but should not be overlooked. Like other rodents, guinea pigs are prone to meniscal calcification [25,58]. The calcification of the menisci is due to basic calcium phosphate crystal deposition that becomes apparent at 2-months of age. This calcification is theorized to contribute to OA by altering joint loading and producing a pro-inflammatory stimulus upon shedding of crystals shed into the joint [58].

II. Scoring of Alterations in Joint Structures

II. a. Macroscopic scoring (Staging of the disease process)

Macroscopic images of guinea pig knees are shown in Figures 3A and 3B, demonstrating the overall joint dimensions (1 × 1 cm) and varying severities of lesions. Macroscopic scoring, both with [58–60] and without [61, 62] Indian ink staining has been utilized in the guinea pig. The use of Indian ink on the cartilage surface is a method of quantifying depth of cartilage loss in the macroscopic scoring process. The use of Indian ink staining has also been advocated prior to tissue fixation and sectioning as an aid to histological interpretation; Indian ink particles enter fissures that communicate with the synovial space and do not appear subsequently in artifactual irregularities created during the fixation and sectioning process that occur after the Indian ink staining [63]. The results of scoring of Indian ink stained sections have been

expressed semi-quantitatively as the percentage of surface with overt superficial fissuring or fibrillation and the percentage of surface with bone exposure.

Indian ink staining method—As published by Richardson et al [59] and used subsequently by Tessier et al [18], the cartilage surface is painted, for 15 seconds, with a 20% (v/v) dilution of blue Indian ink (Parker, Quink) in phosphate buffered saline containing protease inhibitors. Others have successfully used 10% (v/v) dilution of Indian ink without protease inhibitors (J DeGroot unpublished). Any excess is blotted off with a moist cotton swab. For frozen joints, the specimens are thawed by immersion in phosphate buffered saline, pH 7.4 for 20 minutes prior to the application of Indian ink.

Semi-quantitative scoring methods—The severity of histological OA can be assessed semi-quantitatively or quantitatively with the aid of digital images of the cartilage surfaces and image analysis software [59,60], expressing the damaged area (that took up stain) as a percentage of the surface area of cartilage. The Outerbridge semi-quantitative scoring scheme [64], or a variation thereof, has been a mainstay of macroscopic scoring for many years for human and animal studies. The intra-observer and inter-observer *kappa* coefficients were reported to be 0.80 and 0.52 when cartilage damage was graded arthroscopically in humans with a mean inter-observer *kappa* coefficient of 0.72 between two physicians in practice 5 years or more, thus demonstrating good reliability for trained graders [65].

- For guinea pig studies, we recommend a variation of the Outerbridge classification that has been used in rabbit experimental OA [66] and is amenable to guinea pig studies as summarized in Table 2.

II. b. Microscopic scoring of cartilage alterations (Grading of cartilage degeneration)

Methods of fixation, embedding, and decalcification—An extensive review of the literature has revealed that the most frequently used method of preparing guinea pig joints for histological evaluation of OA is fixation in 10% buffered formalin, decalcification in either a solution of 10% EDTA (in 0.1 M phosphate buffer, pH 7–8 for approximately 8–10 weeks, solution changed once a week) or 5% formic acid (diluted in water, for approximately 1 week), and embedding in paraffin. Approximately halfway through decalcification, the process can be hastened by transecting the joints in half coronally with a sharp razor blade (using the tibial collateral ligament as a guide), then returning the joint halves to the decalcification solution. While EDTA decalcification is optimal for proteoglycan preservation and highly recommended for sections destined for *in situ* hybridization [67], formic acid decalcification has the advantage of being rapid. Decalcification is adequate when an aliquot of the decalcification solution no longer produces a calcium precipitate upon addition of ammonium oxalate using the method of Rosen [68]. Briefly, remove a 0.5 ml aliquot of the EDTA decalcification solution and add 10 ml of a citrate-phosphate buffer (0.2 M citric acid and 0.16 M dibasic potassium phosphate, pH 3.2–3.6 achieved with NaOH), and 2.5 ml of saturated ammonium oxalate (5% solution) and vortex. The failure to form a cloudy white precipitate over the course of 20 minutes on two successive days of testing indicates decalcification is complete. When decalcification is complete, the whole joint is embedded in paraffin and sectioned (usually front to back), or the two halves of the joint are embedded (in paraffin) to facilitate production of central sections.

- For simple histological scoring we recommend the use of formic acid decalcification for its ease and time efficiency.

Methods of sectioning—Although some studies have used sagittal sections through the knee joint in order to separate the medial and lateral compartments, frontal (coronal) sections are feasible in a joint of this size and optimal for assessing all surfaces of the femorotibial joint

simultaneously and in their correct anatomic arrangement. When using a spontaneous model of OA, it is important to grade histological damage in the central section of the joint to assess the site of maximal pathology and to compare animals in a consistent manner. This can be accomplished by observing anatomical landmarks characteristic of a central section, such as the anterior cruciate ligament insertion site as well as the medial and lateral menisci (see Figures 4A and 4B). If OA is induced surgically, it may be necessary to verify the lesion location by macroscopic evaluation in order to direct microscopic scoring to the appropriate region, or to section and histologically examine the entire joint. Paraffin embedded sections are cut at 5–8 μm on a microtome along the coronal plane of limbs fixed in a slightly flexed position as described by Cashin [35] and shown in Figure 5. A clever alternate method utilized for studies involving bone histomorphometry calls for the preparation of thick (300 μm) methylmethacrylate sections for histomorphometry with subsequent use of these thick sections to generate thin (5 μm) sections for histology [69].

- For simple histological scoring we recommend 5–8 μm paraffin sections be used.

Methods of staining—Both Safranin O and Toluidine blue are cationic dyes that stain proteoglycans as well as glycosaminoglycans [Toluidine Blue (0.04%) in 0.1 M sodium acetate buffer, pH4; Safranin-O (0.04%) in 0.1 M sodium acetate buffer, pH4]. Toluidine blue has been reported to provide more intense staining, due to the fact that it has a higher affinity for the sulfur in cartilage compared to Safranin O [70]. Although Safranin O staining is proportional to proteoglycan content in normal cartilage, it has been reported that it is not a sensitive indicator of proteoglycan content in cartilage in which glycosaminoglycans have been depleted [70].

- For simple histological scoring of proteoglycan content, we recommend staining with Toluidine Blue

Standardization procedures for semi-quantitative scoring—For assessment of spontaneous OA in the guinea pig, it is sufficient to score the site of maximal OA at the joint center, encompassing the region not covered by the meniscus (Figures 1, 4A and 4B). However, as noted above, assessing the maximal extent of surgically induced OA may require a survey of the entire joint due to localization of the lesions in other than the central location. For spontaneous OA, several studies have relied on scoring a single central section. Brismar et al defined the central portion of the condyle, corresponding to an area of 3–4 mm^2 , as the middle of 5 sections (taken at intervals of 250 μm) without cruciate ligament remnants [71]. Hyttinen et al [72] adopted a sectioning and scoring system previously used in mice [73] that relied on the mean of the grades of three sections: the first was from the level of insertion of the anterior cruciate ligament, the second 200 μm distant, and the third 400 μm posterior to the first one, thereby grading an area that approximated the central third of the tibial condyles. For the evaluation of guinea pig OA, the relative merits are currently unknown of grading a single central section versus several sections encompassing the middle third of the joint.

Grading should be performed blinded to the age and intervention, and the number of graders reported. Grading of the central section can be performed by two blinded observers and averaged [14]; alternatively, 3–4 central sections can be graded by a single observer and the scores averaged [3,74]. Scores should be tabulated for the various quadrants or condyles separately (medial tibia, medial femur, lateral tibia, and lateral femur) to capture data for site-specific changes, since the disease manifests to varying degrees at these different sites. These scores can be used to calculate compartmental (medial and lateral) and total joint scores.

- For simple histological scoring of spontaneous OA in the guinea pig, we recommend two blinded observers, and scoring of one intact central section. For scoring of

surgically induced OA, step through sections encompassing the entire joint are required to insure accurate lesion localization.

Semi-quantitative scoring systems—Five distinct semi-quantitative scoring schemes have been employed in guinea pig OA studies (summarized in Tables 3 and 4 for ease of comparison). The majority of studies performed in the guinea pig to date use a scoring scheme based on one first introduced by Mankin in 1971 [75] or some adaptation of this (Table 4) made to better conform to the features of OA in the guinea pig [6,7,14,19,20,26,27,31,36,38,43,45,60,61,69,76–82]. The scoring scheme in Table 4 was an adaptation developed by Carlson [83] for studies in primates, but has been used successfully by numerous investigators to evaluate guinea pig knee OA [69,82,84]. This scoring scheme permits site-specific and separate scoring of cartilage structure and proteoglycan content. Accurate scoring requires the appropriate identification of the zones of articular cartilage as depicted in Figure 6. Variations in the feature of tidemark integrity are not very prominent in spontaneous OA. Recently, Mapp, *et al.* characterized osteochondral and synovial angiogenesis in the spontaneous model of OA and concluded that guinea pigs display low vascularity throughout the development of the disease [57] however, this feature may be of importance for surgically induced OA, as described in the rat meniscal transection model [57], and for drug intervention trials. Therefore, scoring of tidemark duplication and penetration by vasculature can be considered optional components of the scoring method, dependent on the range of variation in these parameters observed with the particular intervention. Similarly, the cellularity feature of the original Mankin scoring system can be assessed (Figure 7c), however, differentiation of scores 0 and 1 can be difficult in the guinea pig model due to the high density of chondrocytes present in the articular cartilage. In addition, osteophyte and synovitis grading, as well as bone histomorphometry, as described below, can complement the scoring scheme to provide a comprehensive assessment of the whole joint organ. This approach is an attractive one for more sensitively evaluating target-specific effects of drugs in a clinical trial setting. Figure 7a-c demonstrates the entire range of lesions of various grades for all aspects of the recommended scoring scheme as described in Table 4, with mean histological scores shown for each image based on an assessment by two independent experienced graders.

- For semiquantitative scoring of histological OA in the guinea pig, we recommend the histochemical-histological scoring system summarized in Table 4 with grading of cartilage structure (Figure 7a) and proteoglycan content (Figure 7b). Grading of tidemark for duplication or vascular penetration (Figure 7b), cellularity (Figure 7c), and osteophyte (Figure 7c), are optional features and are recommended for inclusion when the intervention warrants it on the basis of potential for change in these parameters with the intervention.

Quantitative scoring systems—Cartilage histomorphometry has been used to quantitatively assess OA in the guinea pig [69,74]. Measured parameters have included chondrocyte density [74], cartilage thickness, cartilage surface fibrillation index [74,85], cartilage proteoglycan content [34,86], and cartilage volume [85] (Table 5).

- Quantitative histological assessment is not required for routine assessment of OA severity, however these methods can provide complementary information tailored to the needs of the investigation.

II. c. Microscopic scoring of synovial alterations (Grading of synoviopathy)

Representative images of the guinea pig knee are shown in Figure 8 demonstrating varying severities of synovitis. Three methods could be found, used in six published studies, that graded synovitis semi-quantitatively as a separate entity from cartilage and bone alterations in the guinea pig joint [12,33,34,54,56,57] (summarized in Table 6). The method of Williams et al

[87], developed in dog and used for a guinea pig study by Han et al [56] is a simple 5 level (0–4) grading scheme based on synovial hyperplasia. In the study by Han et al [56], synovial hyperplasia was reported in only 3 of 8 animals with spontaneous OA, and in the study by Mapp, et al [57] in only 3 of 24 animals. Therefore, this histological parameter is likely to be of greater importance in surgical models of OA than in spontaneous OA. In addition, a more detailed method, published by Pelletier et al [88] for canine studies, although not yet applied to guinea pig, is preferred for clinical trial testing of a drug with an anti-inflammatory mechanism of action because the scale is broader and more detailed, potentially providing a means of discerning small changes in this parameter with treatment.

- For synovitis assessment we recommend the more detailed scoring system of Pelletier [66,88], summarized in Table 6, for its comprehensiveness, need for only haematoxylin and eosin staining, and cross-species utility demonstrated by its previous use in canine [88], rabbit [66], and human [89] studies.

II. d. Microscopic scoring of bone alterations

As summarized in Table 5, there has been a wealth of bone histomorphometric measurements made in the guinea pig model of OA. Bone histomorphometry has been performed with the aid of micro-computerized tomography (micro-CT) on guinea pig knees or on polymethylmethacrylate embedded thick (300 µm thick) sections. The American Society of Bone and Mineral Research devised a unified system of terminology for bone histomorphometry [90], intended to standardize reporting of bone histomorphometric data. This document serves as a source of standardized terms and abbreviations and a universally applicable method for reporting all data. Several bone histomorphometric studies in the guinea pig have published results in conformity with this nomenclature [26,27,30,31,69,91] (Table 5). Methods for cartilage and bone histomorphometry have been published by Pastoureau et al [74,92] including analyses utilizing an automated computerized system. They demonstrate the feasibility of this more quantitative method for histological characterization of OA in the guinea pig model. Currently, there is no agreed upon core set of elements that is most indicative of disease severity and progression.

- Quantitative histomorphological assessment is not required for routine assessment of OA severity, however these methods can provide complementary information tailored to the needs of the investigation.

II. e. Microscopic scoring of ultrastructural alterations

Changes in joint cartilage ultrastructure have also been characterized by electron microscopy by a total of eleven studies [4,10,56,72,93–99], and graded qualitatively [10]. In addition, there have been several studies in which the ultrastructural properties of type II collagen fibers have been assessed by birefringence under polarized light microscopy [51,56,72] demonstrating the utility of ultrastructural assessment to detect early alterations of cartilage.

II. f. Scoring of other alterations - osteophytes, menisci, ligaments, cysts

Osteophyte—These should be scored separately from cartilage structure and proteoglycan content as they reflect a separate reparative process characterized by distinct stages of chondrocyte differentiation within the osteophyte [100], and distinct from cartilage degeneration. One published sum score tabulated osteophytes for 3 joint margins: range 0–9 (medial tibial plateau 0–3, lateral tibial plateau 0–3, lateral femoral condyle 0–3) (see Table 4) [81]. Osteophyte of the medial femoral condyle was not scored because it could not be reliably distinguished from normal variation of the femoral margin. Patellar osteophytes have been indirectly quantified by measuring patellar perimeter on a lateral radiograph [7]. Another method of scoring osteophytes relied on a range of 0–3 based on the µm measurement from

the original tidemark using an ocular micrometer [9]. Finally, the growth of tibial osteophytes has been estimated by the increase in length of the medial tibial plateau in coronal sections using Bézier curve fitting [16]. This is done by measuring the distance along the length of the medial tibial plateau, from the tip of the intercondylar eminence to the osteochondral junction on the medial aspect of the joint. Alternatively, the volume of osteophytes (mm^3) has been quantified [101].

- For purposes of general histological scoring we recommend the scoring of osteophytes at the margins of the joint on a 0–3 scale based on the photomicrographs provided in Figure 7c.

Other anatomical features that do not currently form part of a standardized histological scoring system include scoring of meniscus, ligaments and subchondral cysts and are described below.

Meniscus—Significant ossification of the meniscus has been reported to increase in the guinea pig with age and is thought to contribute to the pathogenesis of OA by altering mechanical forces in the joint [25]. The qualitative assessment of meniscal calcification has been demonstrated with von Kossa’s specific stain for calcium [58]. In addition to meniscal ossification, it is possible to observe decreased proteoglycan content of the meniscus.

Ligaments—The central mid-portion of the posterior cruciate ligament in the Dunkin-Hartley has been shown to form a fibrocartilage matrix by the age of 3 months [97]. Proteoglycans were demonstrated by electron microscopy through fixation of the ligament in the presence of the cationic dye cuprolinic blue. It has been reported that the Dunkin Hartley guinea pig also has significant laxity of the anterior cruciate ligament and that this may predispose this strain to early onset of OA [102].

Subchondral cysts—These structures, termed pseudocysts, have been noted to be an early feature of the disease process in the guinea pig. They appear on the femoral and tibial surfaces of the knee joint in the regions of the cruciate ligament insertions [16,18,37]. Although the femoral pseudocyst is initially the larger, the rate of increase of the tibial pseudocyst is greater [16]. Pseudocysts are not observed at other locations within the joint [16]. The volume of cysts (mm^3) in epiphyseal bone has been quantified as a parameter of OA [101].

III. Evaluation of sources of variability

III. a. Intra- and interobserver validation study

Inter-rater reliability of histological grading with the recommended histochemical-histological scoring system—A set of 20 guinea pig knee joint histological sections were graded by 12 independent observers using the recommended scoring scheme described in Table 4. The intra-class correlation coefficients (ICCs) depicting the inter-rater reliability of grading the features of the scoring system is shown in Table 7. Two graders (JH, VBK) were co-authors of this review and considered, for the purposes of this validation exercise, to be paired expert guinea pig histology graders, as they had graded sections together for many years. An additional 7 graders (“trained novices”) underwent a 30-minute training session, and then were asked to grade the same 20 sections for the three main features of cartilage structure, proteoglycan content, and osteophyte. Training consisted of a group introduction and review (in this order) of Figures 5, 4a, 1, and 6, followed by a review of the appropriate panels in Figures 7a-c. The three “untrained” experts are experienced histologists who did not undergo a group training session together. Two of these three graders however work together and their scores are shown in the “untrained” paired experts column; one of these two is an experienced joint histologist and the other a skin histopathologist who was briefly instructed on how to score the sections. The ICCs were highest for cartilage structure and

proteoglycan content and highest for paired graders who have some common understanding of the grading scheme. Total novices performed surprisingly well after only a very brief training session. The inter-rater reliability of novices would likely be improved with a discussion period following the first training, followed by regarding for purposes of training. Of note, the greatest variability was found for grading of cellularity and tidemark, the features with the lowest ICC values among expert graders.

III. b. Variability due to animal housing

Effects of animal housing on semi-quantitative histological outcome—To evaluate potential effects of single versus multiple housing on histological severity of knee OA in the Hartley guinea pig, the histological results of four independent studies were analyzed. Two studies involved individual housing of animals (n=26), and two studies involved paired housing of animals (n=14). All animals were assessed for histological severity of knee OA at 12 months of age using the recommended histochemical-histological scoring system. Separate scores were computed for medial tibial cartilage structure and proteoglycan content, medial compartment cartilage structure and proteoglycan content, and total histological severity of OA (sum of cartilage structure and proteoglycan content scores for all four compartments). For each parameter, paired housing resulted in mean histological scores of approximately 2.5 units higher than the same parameter assessed in singly housed animals (Figure 9). This difference was significant for all histological scores of the medial tibia ($p<0.05$) and medial compartment ($p<0.05$). The presumed reason for the difference of OA in paired versus singly housed animals is due to differences in physical activity levels. For this reason, when feasible, we recommend housing in groups.

Discussion

The process of recommending a particular histological scoring system for common use must take into consideration several factors: 1) ease of use and inter-rater and intra-rater reliability, 2) ready adoption or familiarity by investigators in the field of musculoskeletal and joint diseases, 3) potential utility across various animal systems, and 4) demonstrated validity through a correlation of histological findings with a wholly different outcome measure, such as synovial fluid biomarker concentrations, which provide reassurance that the histological joint scoring is accurate and fully representative of the pathological process. The guinea pig provides a model system in which synovial fluid harvest is feasible and in which synovial fluid biomarker measurements can be performed. Correlations have been demonstrated between severity of histological OA assessed by the recommended scoring system (sum of cartilage structure and proteoglycan content scores) and several synovial fluid biomarkers, namely cartilage oligomeric matrix protein and keratan sulfate [6,14,81]. Our estimations of the number of animals required in a clinical trial differs somewhat by outcome measure. For instance, to provide 80% power to detect a significant change in response to a hypothetical treatment capable of achieving a 30% treatment difference compared to untreated animals, with statistical significance of $p=0.05$, histological scoring requires 16 animals per group, synovial fluid cartilage oligomeric matrix protein requires 12 animals per group, and synovial fluid keratan sulfate requires 11 animals per group [103]. Thus, it is apparent that in this model system, a significant treatment effect can be realized using a relatively small number of animals. In summary, we recommend the OARSI-HISTOgp histochemical-histological scoring system in Table 4 for semi-quantitative histological assessment of OA in the guinea pig, due to its already widespread adoption, ease of use, the achievable high inter-rater reliability, similarity to the scoring system most often used to assess OA in humans, and its demonstrated correlation with synovial fluid biomarker concentrations. In summary, as demonstrated by this review, a wealth of tools exist to facilitate the semi-quantitative and quantitative assessment of OA in the guinea pig to achieve a comprehensive characterization of the whole joint organ.

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Central Histological Section of a 12-Month-Old Hartley Guinea pig Tibiofemoral Joint

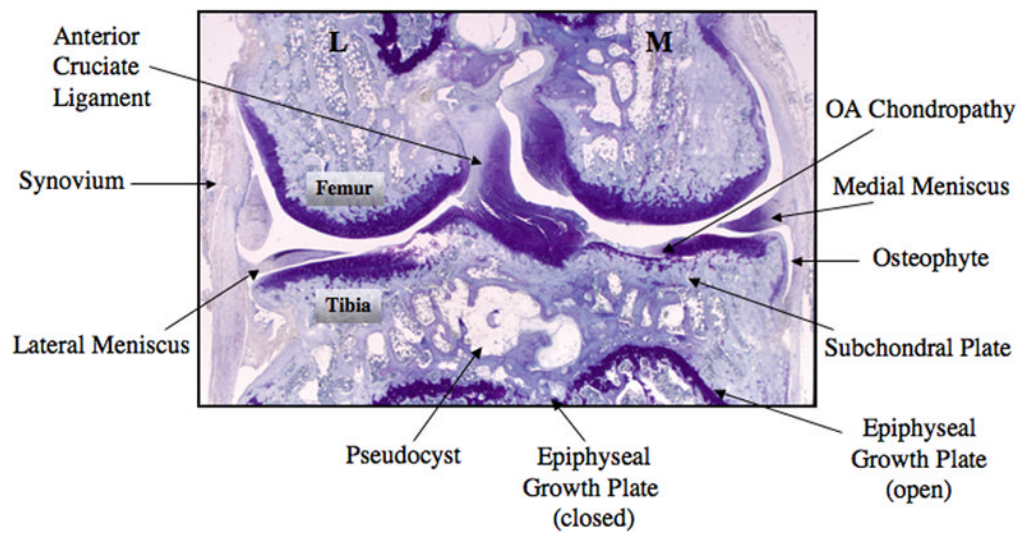


Figure 1. Guinea pig knee joint structures

A frontal section through a 12-month-old guinea pig knee joint to demonstrate its structures and the landmarks and features typical of advanced OA at the center of the tibiofemoral joint (M=medial; L=lateral).

Figure 2a

Cross-sectional Histological Sections of Medial Tibial Plateau from 2-18 Months of Age

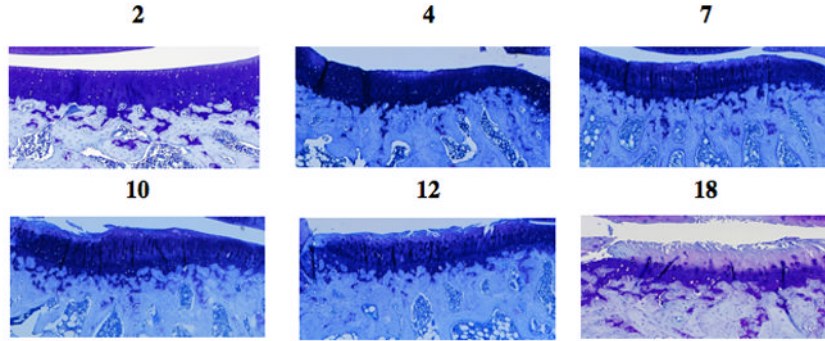


Figure 2b

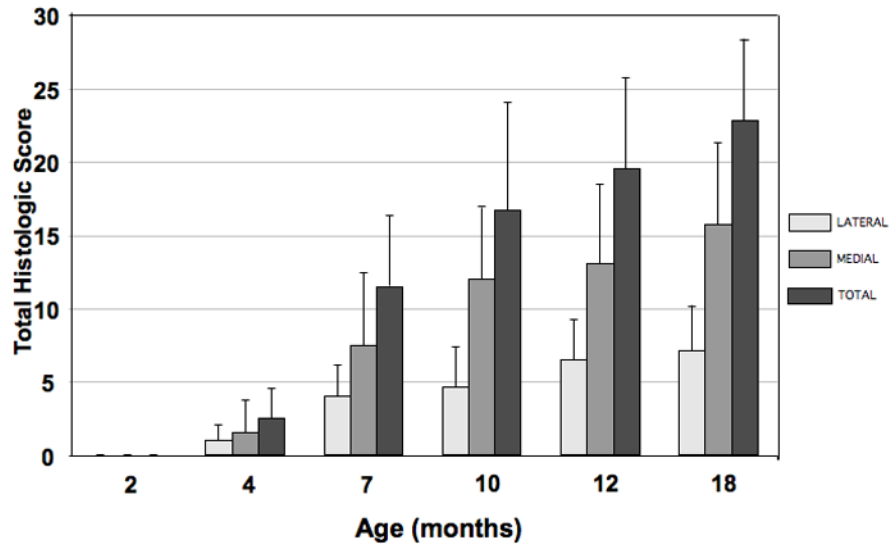


Figure 2. The timecourse of spontaneous histological OA in the Hartley guinea pig knee
 a) Representative sections of the central medial tibial plateau stained with toluidine blue for each age group (ages 2 to 18 months). Scores of cartilage structure (CS) and proteoglycan content (PG) using the recommended scoring system for the regions shown is as follows: 2 months CS=0/PG=0; 4 months CS=2/PG=1; 7 months CS=5/PG=4; 10 months CS=5/PG=5; 12 months CS=8/PG=5; and 18 months CS=8/PG=6.
 b) Mean histological scores of the knee demonstrating increasing OA severity with age in this model system (N=6 for each group ages 2–12 months and N=10 for 18 month age group). Error bars = SD.

Figure 3a

Gross Image of Hartley Guinea Pig Knee

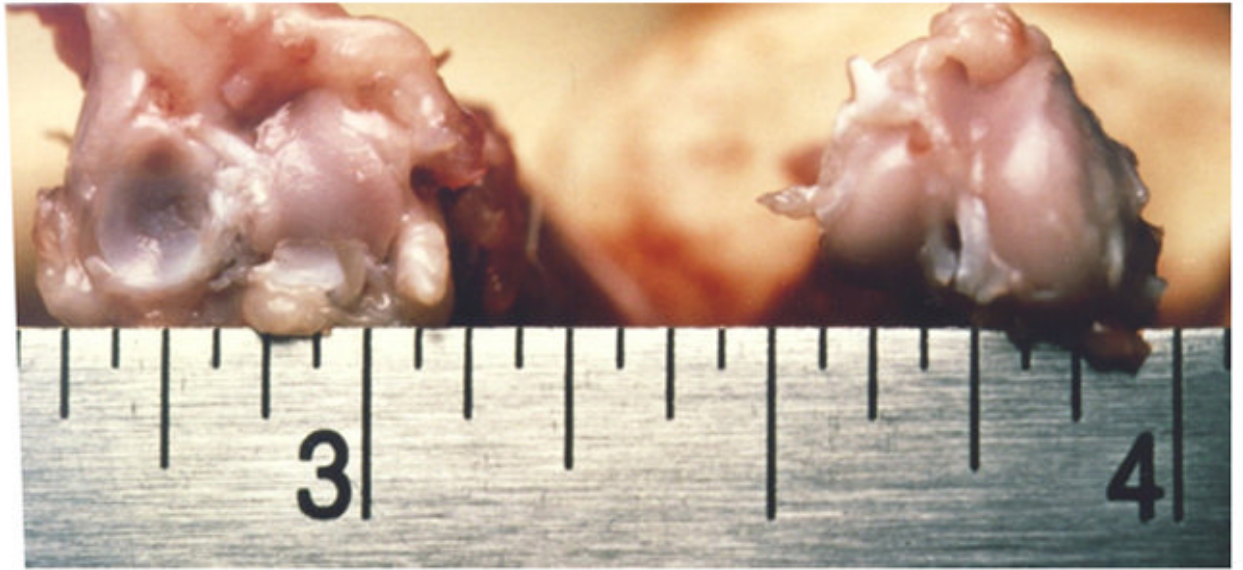


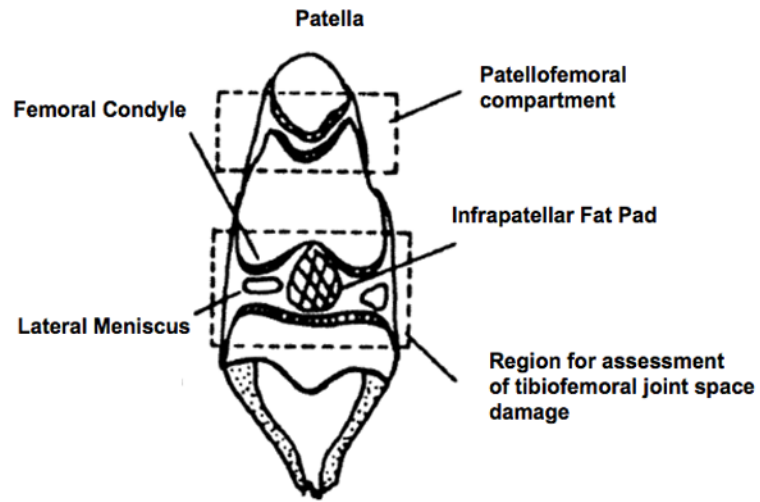
Figure 3b



Figure 3. Macroscopic images of the guinea pig knee joint

- a) Guinea pig knee joint demonstrating normal dimensions of approximately 1 cm x 1 cm (width x depth);
- b) Aerial views of the tibial plateau oriented (medial on left, lateral on right) with macroscopic lesions of increasing severity from no lesions (left panel), to moderate surface fissuring (middle panel), and severe full depth erosion to bone (right panel); the diseased areas in the middle and right panels are indicated by the dotted demarcated region.

Figure 4a



From Cashin, British Journal of Rheumatology 1987;26(4):251-258.

Figure 4b

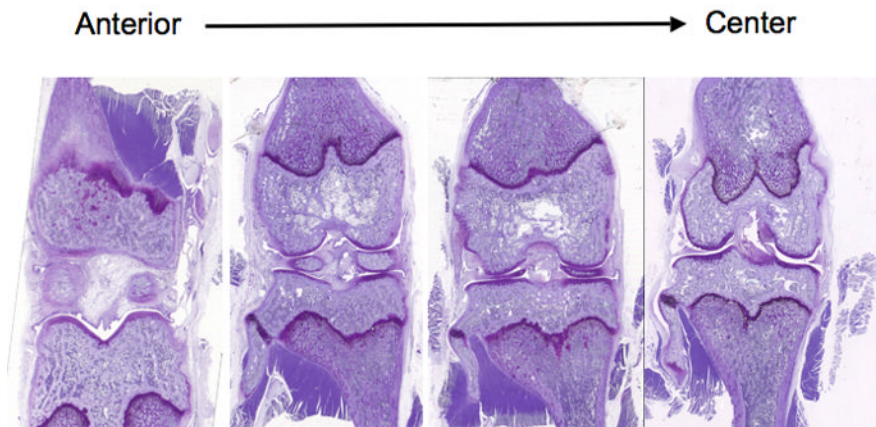
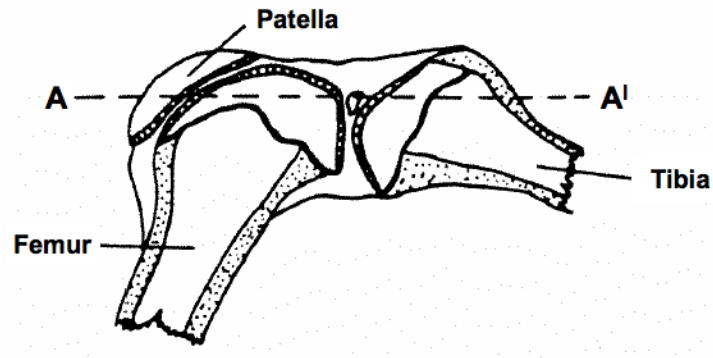


Figure 4. Landmarks of the central region of the guinea pig tibiofemoral joint

- a) A schematic representation of a centrally sectioned guinea pig knee joint based upon Cashin [35] and reproduced by permission of Oxford University Press;
- b) A series of 4 sections taken at 200 μm intervals in the coronal plane, spanning the anterior of the joint through to the center. Note the elongated appearance of the lateral meniscus (on left) and the more rounded appearance of the medial meniscus (on right) when the center of the knee joint is attained.

Lateral Aspect of Guinea Pig Knee Showing Plane of Sectioning



From Cashin, British Journal of Rheumatology 1987;26(4):251-258.

Figure 5. Plane of sectioning of the guinea pig knee joint

The lateral aspect of the guinea pig knee showing the plane of sectioning (A-A') based on Cashin [35] and reproduced by permission of Oxford University Press. Note that the knee is in slight flexion.

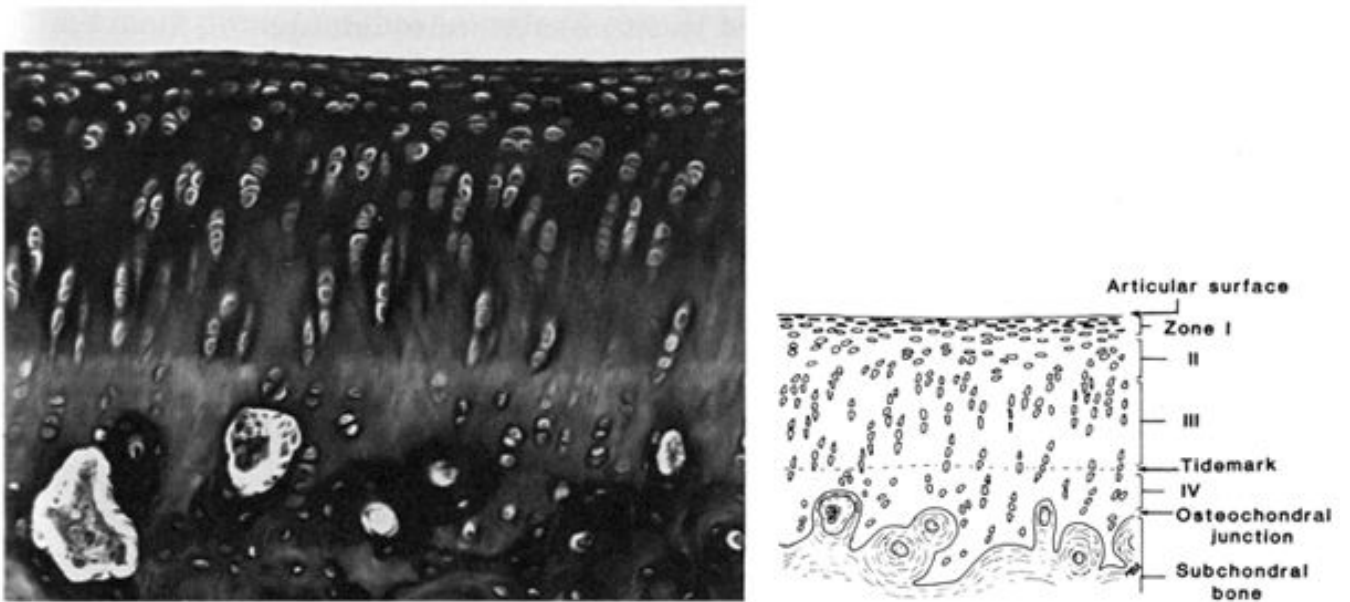
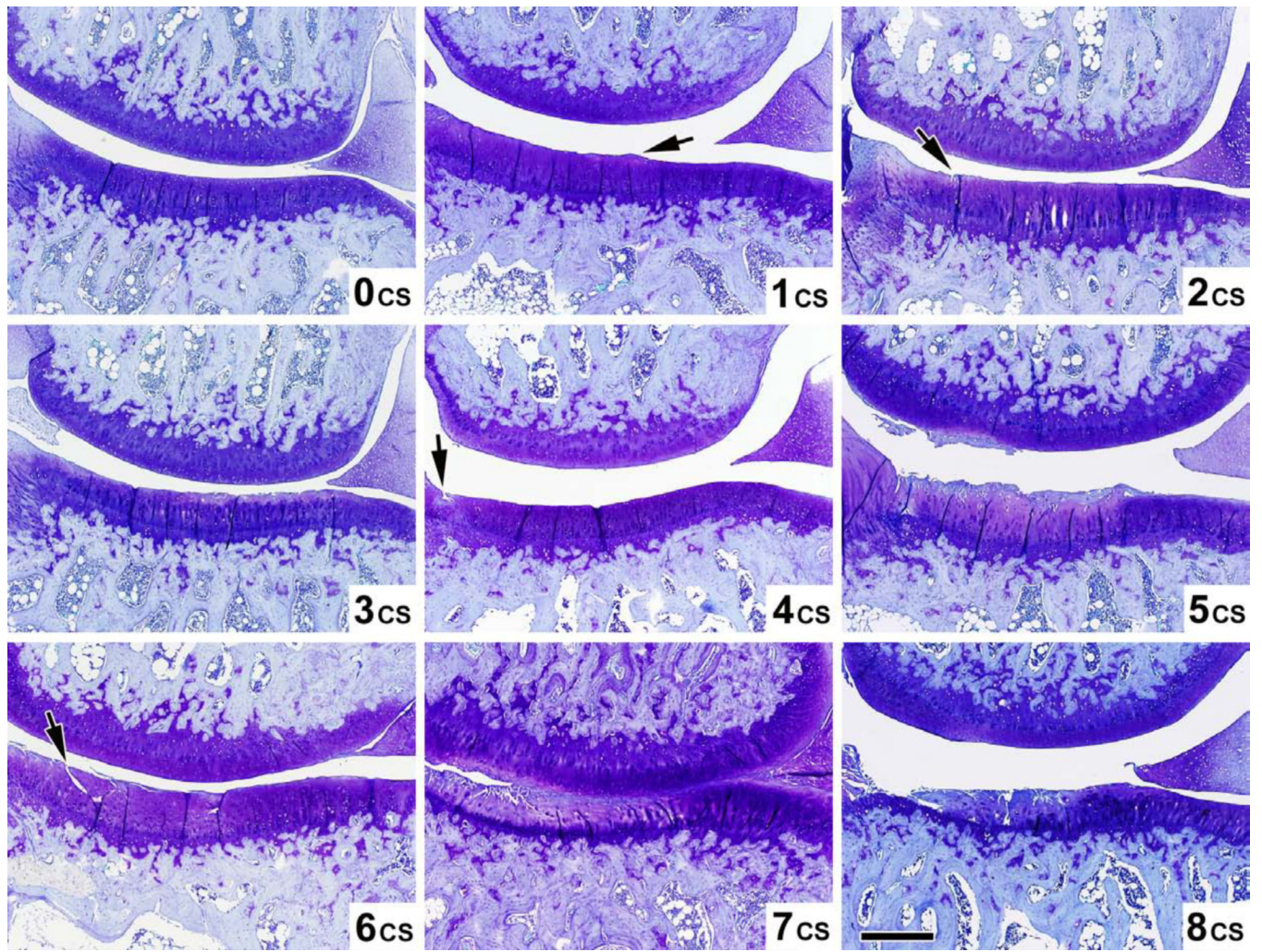
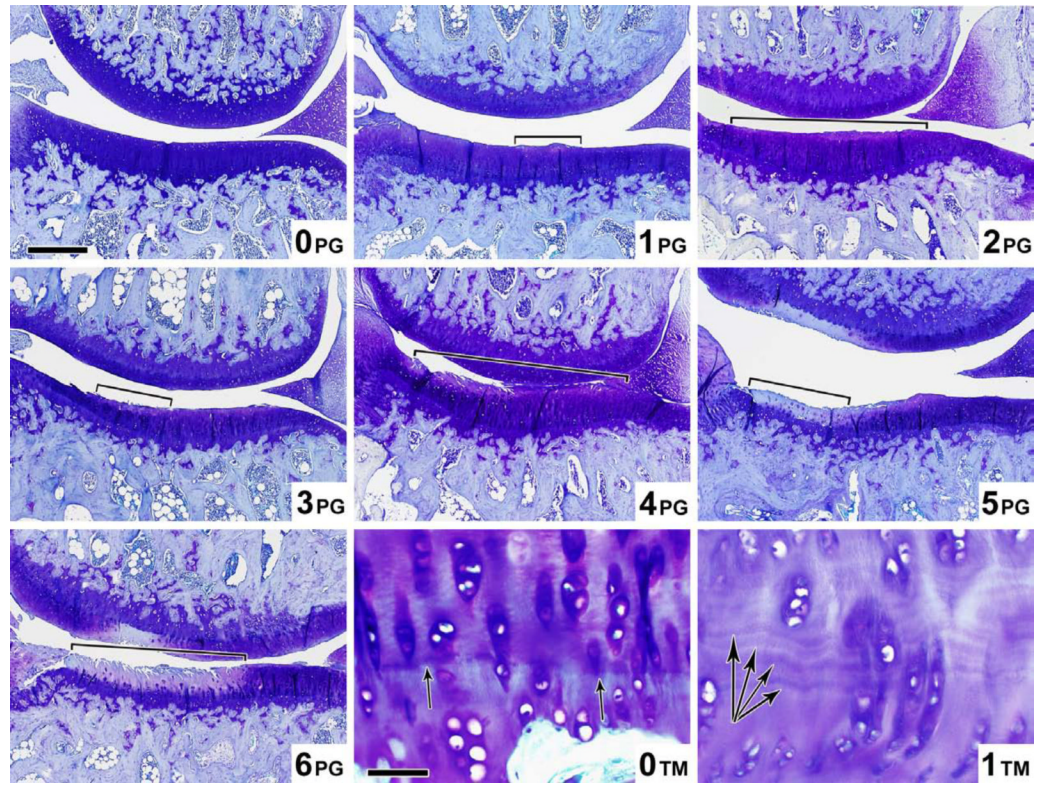


Figure 6. Zones of articular cartilage

Accurate scoring requires the ability to distinguish the four zones of articular cartilage as depicted here: I-superficial zone, II-middle zone, III-deep zone, and IV-calcified cartilage zone, and the tidemark separating zones III and IV. Reproduced by permission of Dr. James Williams.





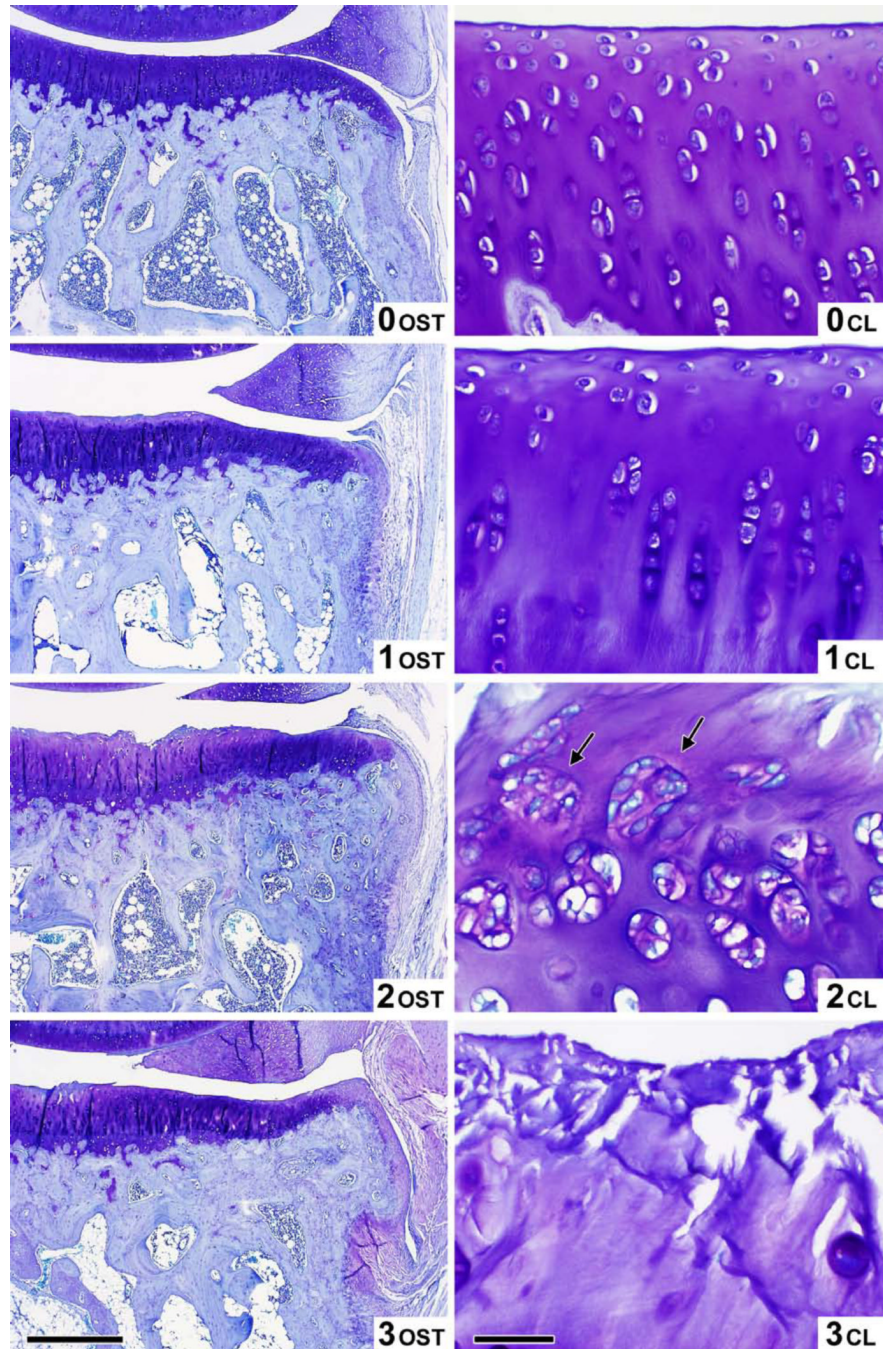


Figure 7. Representative images of guinea pig knees (aged 2–18 months) demonstrating varying severities of histological lesions

Examples of lesions scored by the recommended histochemical-histological scoring scheme. The mean score generated by two experts are shown in the bottom right of each panel in Figures 7a-c.

Figure 7a. Cartilage structure (CS) scoring atlas. Microscopic toluidine blue stained images of the guinea pig knee joint demonstrating the 9 levels (scores 0–8) of cartilage structure (CS) corresponding to the criteria in Table 4. Magnification bar 500 μ m.

Figure 7b. Proteoglycan content (PG) and Tidemark™ scoring atlas. Microscopic toluidine blue stained images of the guinea pig knee joint demonstrating the 7 levels (scores

0–6) of proteoglycan content (PG) (Magnification bar = 500 μm), and the 2 levels (scores 0–1) of Tidemark TM integrity (Magnification bar = 50 μm) corresponding to the criteria in Table 4.

Figure 7c. Osteophyte (OST) and Cellularity (CL) scoring atlas. Microscopic toluidine blue stained images of the guinea pig knee joint demonstrating the 4 levels (scores 0–3) of osteophyte (OST) (Magnification bar = 250 μm), and the 4 levels (scores 0–3) of Cellularity (CL) (Magnification bar = 50 μm) corresponding to the criteria in Table 4.

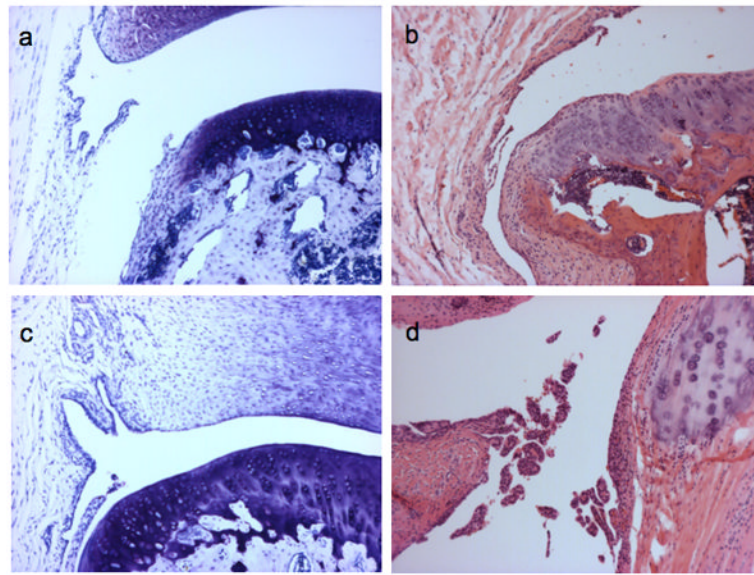


Figure 8. Examples of synovitis associated with spontaneous OA in the Hartley guinea pig knee joint

Representative images (100x) of synovitis scored according to the recommended scoring system in Table 6. Sections stained with toluidine blue (a and c) or Hematoxylin and Eosin (b and d). Normal synovium from a 60 day old animal (a); Synovitis scores of 3 in a 145 day old animal (b) and a 180 day old animal (c) based on scoring synovial hyperplasia=1 (3–5 layers), villous hyperplasia=1, and scattered infiltrate=1. Synovitis score of 7 in a 2 year old animal (d) (100x) based on scoring synovial hyperplasia=2 (≥ 6 layers), villous hyperplasia=3, and infiltrate=2.

Effect of Caging on Histological Score

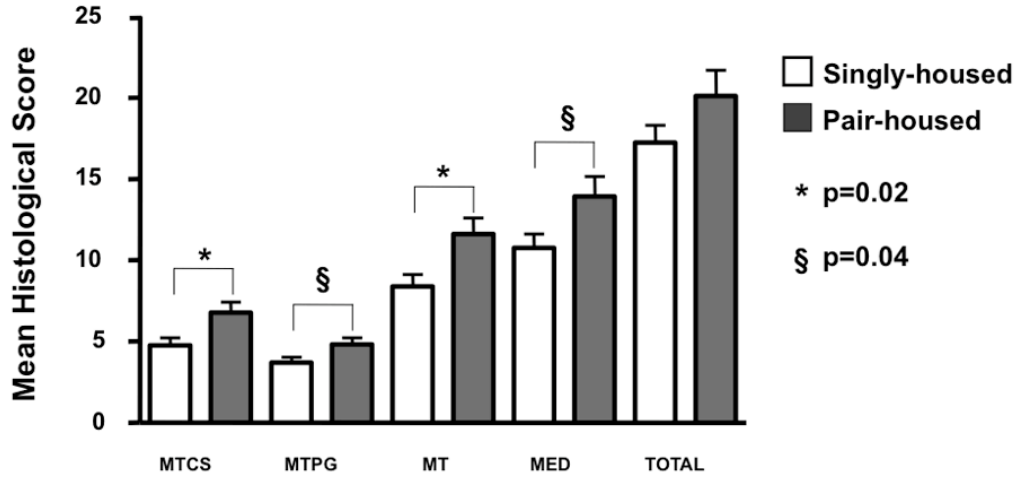


Figure 9. Effect of caging on guinea pig knee joint histological OA severity. Animals were housed either singly (n=26, white bars), or in pairs (n=14, dark bars) from 2 to 12 months of age. Paraffin central sections of these knee joints (aged 12 months) were scored using the recommended scoring scheme to assess cartilage structure (CS) and proteoglycan content (PG) or a combination of both (Total), of the medial tibia (MT) or entire medial compartment (MED). A comparison of paired housing with single housing by the non-parametric Mann Whitney test, was associated with slight but significantly greater histological scores in the paired-housed animals compared with single-housed animals (*p=0.02; § p =0.04, respectively). Error bars = SEM.

Table 1

Studies of osteoarthritis in the guinea pig that included microscopic or macroscopic evaluations.

Types of OA Models	Intervention	References
Spontaneous	None (Spontaneous joint disease in OA prone Dunkin-Hartley compared with OA resistant Strains 2, 13 or GOHI)	[1,3–6,12,15–19,25,37,38,56,60,72,77,78,85,94–97,101,104–113] [7,8,14,20,26–28,30,31,45,47,57,58,69,71,79–82,91,98,99,102,114–118]
Surgically Induced	Medial Meniscectomy	[2,5,21,40,42,43,61,62,74,93,119]
	Anterior cruciate ligament transection	[36]
	Anterior cruciate ligament transection, medial collateral ligament section and partial medial meniscectomy	[120,121]
	Anterior cruciate ligament transection and partial medial meniscectomy	[122,123]
	Osteotomy	[13,39]
	Ovariectomy	[10,11]
	Patellar tendonotomy and patellectomy	[54]
	Lateral and/or medial collateral ligament section	[5]
	Myectomy +/- tendonotomy	[23,76,124]
	Unilateral sciatic neurectomy +/- meniscectomy	[125]
Combination Surgically and Chemically Induced	Medial meniscectomy or Collateral ligament transection +/- iodoacetate	[125]
Enzymatic/Chemically Induced	Iodoacetate (IA)	[126–128]
	Papain (IA)	[33,34,129]
	Copper II bisglycinate (IA)	[35]
	Lipopolysaccharide (IA)	[130]
	Chondromucoprotein (IA) - reported to be antigenic and caused arthritis to develop but this study was performed prior to knowledge of the spontaneous development of OA in this animal	[131]
	Chymotrypsin (IA) <i>ex situ</i>	[46]
Impact Models	Impact Loading	[132]
Other	Intravenous injection of mycobacteria	[32]
	Intraplantar injection of adjuvant (heat killed mycobacterium tuberculosis)	[55]
	Cationic amidated bovine serum albumin (IA) (pI approximately 9.2) into the knee joint of immunized guinea pigs	[53]
	Lymphokine (IA)	[52]
Genetically Induced		None

IA=intra-articular injection

Table 2

Semiquantitative macroscopic scoring of cartilage.

Parameter	Parameter (per OARSI Atlas recommended nomenclature)	Grade	Original Outerbridge Description [64]	Recommended method: Description by Pelletier [66]
Normal	Normal	0	Normal appearance	Normal appearing surface
Fibrillation	Fissures-into superficial zone	1	Softening and swelling	Minimal fibrillation (fraying, fissuring or flaking) or a slight yellowish discoloration of the surface
Erosion-Superficial or Middle	Erosion-into superficial or middle zones	2	Fragmentation and fissuring involving an area of less than half an inch (~1.25 cm) in diameter	Erosion extending into superficial or middle layers
Erosion-Deep	Erosion-into deep zone	3	Fragmentation and fissuring in an area greater than half an inch (~1.25 cm) in diameter	Erosion extending into the deep layers
Bone Exposure	Erosion-full depth	4	Erosion of cartilage down to bone	Erosion extending to the subchondral bone
Lesion Area *				Estimated surface or measured area (mm ²)
Total Joint Score				Sum of (grade x lesion area) for all involved and scored areas

* The scoring of lesion depth (grade 0–4) is performed with the aid of a dissecting microscope. The scoring of lesion area is performed with the aid of calipers (or image analysis software on digital images).

Table 3

Semi-quantitative methods of histological assessment of knee OA in the guinea pig.

Author (Reference)	Description
Modified Mankin [75] with modifications proposed by Carlson [83], Fini [69], and Kobayashi [61]	See Table 4
Modified Colombo method [119] developed by Colombo [133] and modified by Kikuchi [134]	Range 0–24: loss of superficial layer 0–4, erosion of cartilage 0–4, fibrillation and/or fissures 0–4, loss of stainable proteoglycan 0–4, disorganization of chondrocytes 0–4, loss of chondrocytes 0–4
Modified Williams method developed by Williams et al [87] and modified and applied to guinea pig knee OA by Han et al [56]	Range 0–27: articular surface fibrillation 0–4; cellularity 1–4; Safranin-O staining 1–5; osteophyte formation 0–5; brood clusters 1–5; synovial membrane 0–4). A score ≤ 6 for any one surface (medial femoral condyle, medial tibial plateau, distal tibia, and talus) was considered normal.
Lapvetelainen [73] method for mice adopted for guinea pig by Hyttinen et al [72]	Range 0–16 for all 4 condyles combined: cartilage intact and articular surfaces smooth = grade 0; superficial fibrillation of the cartilage = grade 1; lesion extends into the deep zone of uncalcified cartilage = grade 2; lesion surpasses the tidemark and affects the calcified cartilage, in the superficial zone a loss of cells and in the deep zone formation of clones observed = grade 3; no cartilage tissue left between the two adjacent bone ends = grade 4.
Bendele [54]	Cartilage alterations ranging from none, to minimal, focal, moderate, marked or severe scored 0–5; this score was multiplied by the depth of the lesion scored on the basis of involving one-third, two-thirds, or all of the surface score 1–3; osteophytes small, medium or large scored 1–3; synovial membrane proliferation minimal, focal, moderate or marked scored 1–4.

Table 4

Recommended semiquantitative histochemical-histologic scoring scheme for knee joint evaluation in guinea pigs.

Parameter	Grade	Description
Articular Cartilage Structure	0	Normal, smooth, uninterrupted surface
	1	Mild surface irregularities (undulations)
	2	Irregular surface, 1–3 superficial clefts (fissures)
	3	>3 fissures and/or loss of cartilage in the superficial zone
	4	1–3 fissures extending into the middle zone
	5	>3 fissures and/or loss of cartilage extending into the middle zone
	6	1–3 fissures extending into the deep zone
	7	>3 fissures extending into the deep zone and/or loss of cartilage to deep zone
	8	Fissures or loss of cartilage extending to the zone of calcified cartilage
Proteoglycan Content (staining by toluidine blue)	0	Uniform throughout articular cartilage
	1	Decreased in superficial zone only and for <half the length of the condyle or plateau
	2	Decreased in superficial zone for half the length or greater of the condyle or plateau
	3	Decreased in superficial and middle zones for <half the length of the condyle or plateau
	4	Decreased in superficial and middle zones for half the length or greater of the condyle or plateau
	5	Decreased in all 3 zones for <half the length of the condyle or plateau
	6	Decreased in all 3 zones for half the length or greater of the condyle or plateau
Cellularity	0	Normal (1/2 cells/lacuna)
	1	Diffuse/slight hypercellularity
	2	Regions of hypercellularity and clustering
	3	Diffuse hypocellularity
Tidemark Integrity	0	Intact/single tidemark
	1	Crossed by vessels/duplication of tidemark
Additional Features*		
Osteophyte	0	No osteophyte present
	1	Small osteophyte
	2	Medium-sized osteophyte
	3	Large osteophyte
Osteophytes are tallied for three joint margins (medial and lateral tibial plateau, and lateral femoral condyle)		
Total score 0–9		

Examples of varying severity for each of the parameters above are shown in Figures 7a-c.

Scoring system modified from one originally introduced by Mankin [75] whose original scoring system ranged from 0–14 (for structure 0–6, cells 0–3, safranin O staining 0–4, and tidemark integrity 0–1; where 0 has normal cartilage and >10 has severe cartilage degeneration). The semiquantitative histological scoring scheme in this Table was developed by Carlson [83] for studies in primates but has been used successfully by numerous investigators to evaluate guinea pig knee OA. It has been used in truncated form, assessing cartilage structure and proteoglycan content only [84], and in full form, assessing cellularity and tidemark integrity in addition to structure and proteoglycan content [69]. Scale: range 0–18 (for structure 0–8,

proteoglycan content 0–6, cellularity 0–3, tidemark integrity 0–1). A further modification has been employed by Kobayashi et al [61]: range 0–12 (for cartilage structure 0–5, proteoglycan content 0–4, cellularity 0–3). This modified scheme with osteophyte scoring has been used by Kraus et al [81]. In the original usage, fissures were referred to as clefts.

* These features supplemental the scoring system modified from the one first introduced by Mankin.

Table 5

Histomorphometry of cartilage and bone.

Parameter	Description	References
Cartilage		
Cartilage thickness	μm at magnification 20x although measurements at 80x more readily yielded significant differences in the study by Pastoureau et al, 2003	[69,74,91,119]
Average cartilage thickness	Ratio of cartilage volume divided by the projection of the corresponding surface area	[71]
Cartilage surface fibrillation index	Ratio of the length of the cartilage surface border divided by the length of a standardized measured area x 100 (%) at a magnification of 80x	[39,69,74,85,91,101]
Cartilage volume	Point counting of hematoxylin and eosin stained paraffin section at a magnification of x50 with a conventional grid	[85]
Height of calcified cartilage	Measured on 6–8 non-decalcified plastic-embedded step sections of the central compartment stained with toluidine blue	[101]
Proteoglycan content ratio	Ratio of superficial zone to deep zone intensity (optical density of greyscale image) of safranin O staining in a standardized measured area x 100 (%) at a magnification of 80x	[74]
Proteoglycan content by Critical Electrolyte Method (CEC)	Stain sections with alcian blue 8 GS in the presence of increasing molar concentrations of MgCl_2 (0, 0.10, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75, 0.85, and 1.00), which compete with the dye solution (0.05 % alcian blue in 0.025 M acetate buffer, pH 5.6) for anionic sites until all dye is displaced from the substrate. The value of the CEC is the mean of the two concentrations of MgCl_2 between which the alcian blue staining disappears.	[34,86]
Chondrocyte density	Manual count of cells divided by the area of the standardized region at a magnification of 200x	[74]
Subchondral Bone Plate		
Plate volume fraction	Bone volume per total plate volume (%) per Ding; bone volume in standardized region in the upper half of the epiphysis per Pastoureau	[26,27,74,92]
Plate thickness	(μm); measured from cartilage bone interface to the top of the epiphyseal marrow space--mean of 10 measurements perpendicular to the articular surface per Huebner et al; thickness (μm) in a standardized region in the upper half of the epiphysis per Pastoureau	[6,26,27,30,69,74,91] based on definition of Carlson [83]
Plate surface density	Plate surface to total plate volume (mm^{-1})	[26,27]
Plate perforation	Mean pore size (μm)	[26,27]
Surface density of capillaries	mm^2/mm^3 subchondral bone	[101]
Subchondral Cancellous Bone*		
Bone volume fraction	Bone voxel per total specimen voxel (%)	[26,27,30,69,71,91]
Trabecular thickness	Measured in μm	[26,27,30,69,91]
Structure model index	How plate-like, rod-like or combination the structure is	[26,27,30]
Architectural anisotropy	Ratio of the eigenvalue of the primary direction divided by the eigenvalue of the tertiary direction	[26,27,30]
Connectivity density	Number of multiple connected trabeculae per volume (mm^{-3})	[26,27,30]
Bone surface density	Bone surface area per total volume of specimen (mm^{-1})	[26,27,30,31]
3-D trabecular spacing or separation	(μm), at a magnification of 50x	[26,27,30,31,69,91]
Trabecular number	Number per mm	[69,91]
Fractal dimensionality	Trabecular texture analysis	[31]
Subchondral Cortical Bone		

Parameter	Description	References
Bone volume fraction	Bone volume per total specimen volume (%)	[26,27]
Cortical thickness	(μm)	[26,27,30,31]
Bone surface density	Bone surface area per total volume of specimen (mm^{-1})	[26,27]
Cortical bone porosity	Cavity/perforation (%)	[26,27]
Cross-sectional area of cortex	Mean value obtained from subchondral cortical bone (mm^2)	[26,27,30]

Microarchitectural properties of bone based upon Ding et al [27].

Fini et al [69] performed bone and cartilage histomorphometry on 6 central sections (5 μm polymethylmethacrylate embedded) spaced 300 μm apart.

Total, medial and lateral parameters graded.

* Alternatively the epiphyseal trabecular bone underlying the subchondral bone plate is evaluated--defined as the superior half of the epiphysis from the end of the subchondral bone plate to a distance of $450 \pm 50 \mu\text{m}$ from it as described by Fini et al [69] based on Pastoureau [74].

Table 6

Semi-quantitative methods of grading synovitis in the guinea pig.

Parameter	Scoring System	Comments	References
Recommended Scoring System			
Severity of Synovitis	Range 0–10: <u>synovial lining cell hyperplasia</u> 0 = 1–2 layers of cells 1 = 3–5 layers of cells 2 = ≥ 6 layers of cells; <u>villous hyperplasia</u> 0 = not present 1 = few, scattered and short 2 = marked (finger-like projections) 3 = marked and diffuse; <u>degree of cellular infiltration by perivascular lymphocytes and mononuclear cells</u> 0 = normal 5 = marked cellular infiltration mixed with lymphoid follicles).	Based on rabbit joints and the Pond Nuki dog model of OA. Grading performed on hematoxylin and eosin stained paraffin (5 μm) sections. (5 μm) sections. Two synovial membrane specimens were examined for each compartment. The highest score from each compartment was recorded and the average score for the two compartments considered the whole knee score.	Pelletier [66,88]; And Mapp [57]
Other Scoring Systems			
Cellularity Score	Range 0–4: 1 = solitary 2 = small number 3 = moderate number 4 = large number	Grading performed on frozen sections of 3×3 mm specimens of synovial membrane and the highest grade in any section of the synovial membrane was recorded for the joint.	Kopp [33]; and Mejersjo [34]
Synovial Membrane Hyperplasia	Range 0–4: 0 = Decrease in the number of lining cells 1 = normal 2 = slight increase in number of layers (4–6) 3 = > 6 layers of lining cells 4 = Increase of lining cells with inflammatory cells present	Developed in dog and used in the guinea pig by Han, 2002. Grading performed on hematoxylin and eosin stained paraffin (8 μm) sections. Scoring done separately for medial femoral condylar and medial tibial plateau compartments.	Williams [87]; and Han [56]
Severity of Proliferative Changes	Range 0–4: 1 = minimal 2 = mild 3 = moderate 4 = marked	Grading performed on hematoxylin and eosin stained paraffin (8 μm) sections.	Bendele [12,54]

Table 7

Inter-rater reliability of guinea pig histological grading using the recommended scoring system (Intraclass Correlation Coefficients^{*}).

Histological Feature	Guinea Pig Experts (n=2)	Trained Novices (n=7)	“Untrained” Experts (n=3)	“Untrained” Paired Experts (n=2)
Cartilage Structure	0.95 (0.88, 0.98)	0.79 (0.69, 0.91)	0.59 (0.32, 0.79)	0.92 (0.81, 0.97)
Proteoglycan Content	0.91 (0.79, 0.96)	0.74 (0.60, 0.87)	0.49 (0.22, 0.73)	0.90 (0.76, 0.96)
Osteophytes	0.85 (0.66, 0.94)	0.66 (0.50, 0.82)	0.80 (0.55, 0.91)	0.80 (0.55, 0.91)
Cellularity	0.47 (0.05, 0.75)	ND	0.59 (0.34, 0.79)	0.82 (0.60, 0.92)
Tidemark	0.63 (0.27, 0.84)	ND	0.23 (-0.04, 0.53)	0.24 (-0.22, 0.61)

Values are Intraclass Correlation Coefficients (ICCs) for multiple graders of 20 histological sections each.

* 95% Confidence intervals for each ICC are listed below in parentheses.

ND=not determined