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Development of a microcomputed tomography scoring system to characterize disease progression in the Hartley guinea pig model of spontaneous osteoarthritis

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Abstract

Aim: There is potential discrepancy between human and laboratory animal studies of osteoarthritis (OA), as radiographic assessment is the hallmark of the former and histopathology the standard for the latter. This suggests a need to evaluate OA in animal models in a manner similar to that utilized in people. Our study aimed to develop a whole joint grading scheme for microcomputed tomography (microCT) images in Hartley guinea pigs, a strain that recapitulates joint changes highlighted in human spontaneous OA.

Materials and Methods: Knees from animals aged 2, 3, 5, 9, and 15 months were evaluated via whole joint microCT and standard histologic scoring. Quantitative microCT parameters, such as bone volume/total volume were also collected.

Results: Both whole joint microCT and histologic scores increased with advancing age and showed strong correlation ($r = 0.89$, $P < 0.0001$). Histologic scores, which focus on cartilage changes, increased progressively with age. Whole joint microCT scores, which characterize bony changes, followed a stepwise pattern: scores increased between 3 and 5 months of age, stayed consistent between 5 and 9 months, and worsened again between 9 and 15 months.

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Conclusions: This work provides data that advocates the use of a whole joint microCT scoring system in guinea pig studies of OA, as it provides important information regarding bony changes that occur at a different rate than articular cartilage changes. This grading scheme, in conjunction with histology and quantitative microCT measurements, may enhance the translational value of this animal model as it pertains to human work.

Keywords

osteoarthritis; Hartley guinea pig; microcomputed tomography; histology; knee joint

Introduction.

Osteoarthritis (OA) is the most widespread form of arthritis, with estimates that at least 10–15% of those over age 60 suffer from the disease(1). OA is thus a leading cause of pain and disability across the globe(2), with the knee identified as the most commonly affected joint. Unfortunately, the individual and economic burden of OA is expected to increase as people continue to live longer and lifestyle factors that contribute to OA, such as inactivity and obesity, continue to rise(1,3).

OA is characterized by loss of articular cartilage, subchondral bone sclerosis, and synovial hyperplasia and inflammation(4). Eventually, these changes lead to loss of normal joint function, resulting in decreased mobility, pain, and poor quality of life(5). Although great strides have been made in understanding the underlying mechanisms that contribute to OA, its pathogenesis is still not completely understood. In particular, there is a lack of understanding of the inciting and underlying triggers of spontaneous OA, which is the predominant form of disease and occurs without prior trauma to the joint. Other than symptomatic pain relief and total joint replacement, treatment options for OA are limited and an active area of investigation(6–8).

Numerous animal models have been employed to aid in the study of human OA(9–11). These include small animals models, such as mice, rats, guinea pigs, and rabbits, as well as large animal models, including dogs, small ruminants, and horses(9,11). One outbred rodent species with spontaneous OA is the Hartley guinea pig, which has joint pathology, especially in the knee, that mimics that seen in humans(9–12). Other advantages of the Hartley guinea pig are its physiologic requirement for exogenous vitamin C(13), which is identical to people, and the ability to collect larger tissue samples than smaller mouse and rat models. As the Hartley guinea pig is an accepted model for both spontaneous and post-traumatic OA, the Osteoarthritis Research Society International (OARSI) has developed guidelines on the histologic examination of knee joints from these animals(14).

Of note, histopathology is the predominant outcome measure used in animal models of OA, as it is considered the gold standard for laboratory work (10). In contrast, radiographic analysis, typically using X-ray radiographic images, is practical and readily available in the medical community, making it the main descriptor of joint pathology. This presents a potential dichotomy when comparing basic and clinical research, as it is not clinically feasible to do histopathologic assessment of knee joints on human patients outside of total joint replacement surgery.

Two-dimensional (2D) radiographic images of OA patients are interpreted using a variety of clinical features, such as presence and location of osteophytes, subchondral bone changes, and articular bone lysis(15). Of note, 2D radiography is not often employed for animal work and, instead, microcomputed tomography (microCT) is utilized(16). MicroCT, a form of advanced radiography, provides high resolution images and the ability to view both 2D and 3D reconstructions of bony structures. It is primarily used in research settings to quantitatively evaluate bony features such as subchondral bone sclerosis, subchondral bone volume fraction (BV/TV), trabecular thickness, and bone tissue mineral density (TMD), particularly in rodent models such as guinea pigs (17–19). Similar to traditional radiography, microCT can be performed longitudinally *in vivo*, allowing researchers to follow OA progression in live animals and direct appropriate harvest time points in studies(20,21).

While quantitative microCT measurements provide valuable information, it would be complementary to apply a clinically-oriented evaluation of microCT images from these animals in a manner similar to human radiographic findings. Although microCT is not frequently used in the human clinical setting, OA features assessed on radiographs can be readily applied to 2D microCT images. Thus, the main objective of the current study was to create and utilize a new whole joint scoring system to evaluate microCT images from Hartley guinea pigs as they age from 2 to 15 months old. We also compared and contrasted the benefits and limitations of this new grading scheme to both histologic grading and quantitative microCT measurements, such that there is rationale to include this clinically-based grading scheme in our repertoire of tools used to describe OA.

Methods.

Animals.

All procedures were approved by the university's Institutional Animal Care and Use Committee and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Thirty male Hartley guinea pigs were acquired from a commercial vendor (Charles River Laboratories, Wilmington, MA) to allow harvest at the following ages: 2 months (pre-OA), 3 months (OA onset), 5 months (early OA), 9 months (moderate OA), and 15 months (severe OA)(14). Group size and power were determined using the statistical software at www.stat.uiowa.edu/~rlenth/Power. Based upon previous work and pilot studies, histologic assessment of OA was used as the principle outcome. Using a within group error of 0.5 and a detectable contrast of 1.0 in a linear regression model, power associated with a Tukey/HSD post-test ($\alpha = 0.05$) was calculated as 0.9 with a sample size of 6 per experimental group. All animals were purchased one month prior to target ages and maintained at Colorado State University's Laboratory Animal Resources housing facilities. Animals were monitored daily by a veterinarian and provided with a full screening to ensure animals were healthy prior to harvest. All guinea pigs were singly-housed, as is suggested for males, in solid bottom cages. They were provided regular guinea pig chow (Harlan Teklad 7006) fortified with vitamin C (800 mg/kg) and vitamin D (2.4 IU/g). Food and water were provided *ad libitum*.

Specimen collection.

At time of harvest, guinea pigs were anesthetized and maintained with a 1.5 to 3% mixture of isoflurane and oxygen. Thoracic cavities were opened, and blood was collected via direct cardiac puncture using a 20-gauge butterfly catheter for full complete blood count and biochemistry evaluation (data not shown). This data, coupled with a complete necropsy, confirmed that animals did not have concurrent morbidities. Anesthetized animals were then immediately transferred to a carbon dioxide chamber for euthanasia. Left hind limbs were removed at the coxofemoral joint and fixed in 10% neutral buffered formalin for 48 hours. Right hind limbs were used for an unrelated study. After 48 hours, limbs were placed in phosphate buffered saline (PBS), at which time limbs were imaged with microCT.

Quantitative microCT measurements.

Knee joints were scanned in PBS using the Inveon microPET/CT system (Siemens Medical Solutions, Malvern PA), with a voxel size of 18 micrometers, a voltage of 100 kV, and an exposure time of 2356 ms. All quantitative microCT measurements (Table 1) were performed using ImageJ software (Research Service Branch, National Institutes of Health, Bethesda, MD). Thresholds were determined optically using the edge detection method(22). For analysis of tibial subchondral trabecular bone, a region with an area of $1.04 \times 1.04 \times 0.52 \text{ mm}^3$ was selected as the region of interest (ROI), as previously described (17,23). For analysis of femoral subchondral trabecular bone, a cuboid region of trabecular bone with size of $1.5 \times 1.5 \times 0.5 \text{ mm}^3$ was identified, as published (23). Example ImageJ photos with regions of interest highlighted are provided in Supplemental Figure 1. Bone TMD (g/cm^3), BV/TV, trabecular thickness (mm), and trabecular spacing (mm) were quantified for both medial and lateral tibial subchondral trabecular bone, and medial and lateral subchondral trabecular bone in the femoral condyles. Bone TMD was calibrated using phantoms of known hydroxyapatite density embedded in epoxy.

Whole joint scoring system for microCT.

In conjunction with a board-certified veterinary radiologist (AJM), we developed a novel, clinically-oriented scoring system to assess whole joint microCT radiographic images in this species. Key features of this grading scheme (Table 2) are radiographic assessments typically utilized in day-to-day evaluation of human OA, including presence and location of osteophytes, subchondral bone changes, and articular bone lysis(24). Features of the proposed grading system are similar to the Kellgren-Lawrence score, which is the most widely used classification for radiographic analysis of human OA in clinical practice and is commonly applied in research settings(25). In this human scheme, a score of 0 (no OA) to 4 (most severe OA) is assigned and is based on joint margin osteophytes, joint space narrowing, subchondral bone sclerosis, small cyst-like lesions in the subchondral bone, and altered shape of bone ends(25). Similar to the OARSI histologic score (described below), the microCT grading scheme scored whole knee joints in their entirety. This same radiologist then assessed all anatomical structures across all planes and serial sections of 2D microCT image stacks. Images were scored in duplicate in a random order, blinded to age group. A perfect intraclass correlation coefficient of 1.0 for intra-reviewer consistency was calculated.

OARSI recommended histopathologic assessment of OA.

After legs were scanned for microCT, PBS was removed and replaced with 12.5% ethylenediaminetetraacetic acid (EDTA) at pH 7 for decalcification. The EDTA solution was replaced every 3–5 days for 4–6 weeks, based on age, until legs were appropriately decalcified. Coronal slices of the knees at the level of the medial tibial plateau were sectioned, as previously described(14). Samples were paraffin embedded and a 5-micron intact central section was stained with Toluidine Blue, as recommended by the OARSI guidelines(14). Medial and lateral femoral condyles, as well as medial and lateral tibial plateaus, were scored using a semiquantitative grading scheme outlined by OARSI(14). This semiquantitative histopathologic grading scheme is based on articular cartilage structure, proteoglycan content, cellularity, tidemark integrity, and presence of osteophytes. Scores for medial and lateral tibia, as well as medial and lateral femur, were assigned and summed for a total knee joint OA score. Scores pertinent to the medial and lateral aspects of the knee, only, were also calculated. Scores were performed in a blinded fashion by two independent reviewers (LBR and KSS). An intraclass correlation coefficient for inter-reviewer consistency was calculated at 0.92, which was considered excellent. Scores from each of the four anatomic locations were summed to obtain a total knee joint OA score for each guinea pig. Due to rare, imperfect coronal sectioning of the joints, not all compartments at the level of the medial tibial plateau could be adequately scored for every guinea pig. If any single structure was not appropriately sectioned for scoring, that animal was excluded from the analysis for OARSI histology scoring for the total joint score but included in the supplemental materials/figures for medial or lateral compartments.

Statistical analysis.

Data for total body weights, quantitative microCT measurements, microCT OA scores based on the novel grading scheme, and OARSI histology scores were subjected to, and passed, normality testing via the Kolmogorov-Smirnov test. Data were compared between the five age groups using parametric ordinary one-way ANOVA analyses followed by Tukey's multiple comparisons tests to allow for adjusted P values. Pearson's correlation coefficients were calculated between the whole joint microCT scoring system, histology scores, and quantitative microCT measurements. Statistical significance was set at $P < 0.05$. All statistical analyses were performed with GraphPad Prism (La Jolla, CA, USA).

Results.

General description of animals.

Guinea pigs in all groups remained clinically healthy and gained weight as expected for this species when fed ad libitum. Mean weight was 598.9 grams for the 2-month old group, 747.3 grams for the 3-month old group, 901.8 grams for the 5-month old group, 1031 grams for the 9-month old group, and 1016 grams in the 15-month old group. Significant differences in weight and weight ranges are presented (Figure 1A).

Quantitative microCT measurements.

All 6 animals in each age group were included in this aspect of the study. Mean values (and 95% confidence interval) for each of the quantitative microCT measurements can be found in Table 1. Significant differences among the five age groups were found for: BV/TV, trabecular thickness, and trabecular spacing in the medial tibial subchondral trabecular bone; and tibial and femoral cortical thickness. BV/TV decreased with age in the medial tibial compartment (overall ANOVA $P = <0.0001$: $P < 0.05$ for 2 months vs 5 months; $P < 0.01$ for 2 months vs both 9 and 15 months, and 3 months vs 5 months; $P < 0.001$ for 3 months vs both 9 and 15 months). Similar decreasing trends with advancing age were noted for the medial femoral and lateral tibial compartments ($P = 0.0857$ and $P = 0.0555$, respectively). Trabecular thickness in the medial tibial compartment increased from 2 months to 5 months, and then remained stable (overall ANOVA $P = 0.0080$: $P < 0.05$ for 2 months vs both 3 and 5 months). Trabecular spacing in the medial tibial compartment followed the same trend ($P = 0.0492$), although there were no significant differences noted on multiple comparison analysis of the age groups. Cortical thickness of the tibia increased after 2 months of age (overall ANOVA $P = 0.0156$: $P < 0.05$ for 2 months vs both 3 and 5 months). Cortical thickness of the femur followed a similar pattern (overall ANOVA $P = 0.0308$: $P < 0.05$ for 2 months vs 9 months).

Whole joint microCT scoring scheme.

As above, all 6 animals in each age group were included in this analysis. No microCT pathology (score of 0) was noted in the 2 or 3-month old age groups. The mean score was 4.2 (95% confidence interval [CI] 2.6–5.7) for the 5-month old group, 4.5 (95% CI 2.4–6.6) in the 9-month old group, and 11.8 (95% CI 11.4–12.3) in the 15-month old group. Individual scores and statistical differences are found in Figure 1B.

In the 5-month old group, all animals had small osteophytes ($<1\text{mm}$) on either the patella or femur. Two animals in this group had osteophytes present on both the patella and femur. In the 9-month old group, all animals had small osteophytes on the patella and/or femur. Additionally, 3 animals in this group also had radiographic evidence of subchondral bone sclerosis and subchondral bone cystic changes. In the 15-month age group, all 6 animals had large osteophytes ($>1\text{mm}$) present on the patella, femur, and tibia. All 6 animals also had evidence of subchondral bone sclerosis and subchondral bone cystic changes. Five animals exhibited articular bony lysis. Representative sagittal and coronal microCT images from each age group are presented (Figure 2).

OARSI histology score.

All 6 animals in the 2-month old age group were evaluated, but only 5 animals in each of the remaining age groups were included in the total joint analysis due to skew in the sectioning of the joints ($n=26$, total). Data pertinent to animals with individual intact medial and lateral compartments ($n=26$ and $n=27$, respectively) were similar to that for the total joint evaluation (Supplemental Figures 2A and B).

The OARSI total joint scores were significantly different among the age groups ($P = 0.0001$). As expected, mean OA scores progressively increased with age and were

statistically different between every age group, with the exception of the 2 and 3 month old groups (Figure 1C). Mean OARSI scores for all four knee joint compartments were 0.375 (95% CI -0.1 -0.9), 1.2 (95% CI 0.8 -1.6), 10.8 (95% CI 6.9 - 14.6), 26.2 (95% CI 16.9 - 35.5), and 40.4 (95% CI 30.3 - 50.5) for the 2, 3, 5, 9, and 15 month old groups, respectively. In general, scores from the medial compartment were higher than those from the lateral compartment.

Representative lesions from each age group are presented (Figure 3). In both the 2 and 3-month old groups, mild articular surface irregularities and slight proteoglycan loss in the superficial zone were the most common lesions observed. Proteoglycan loss was more severe in the 5-month old group, and occasional fissures were present in the articular cartilage surface. Mild hypocellularity in the superficial zone was also commonly noted in this age group. In the 9-month old group, proteoglycan loss with more accentuated hypocellularity coupled with regions of chondrocyte clustering were noted. Tidemark duplication was also common in the 9-month olds. All of above mentioned lesions were present in the 15-month old group, but to a more severe extent. In some animals, there was complete loss of articular cartilage at this late age.

Correlations between quantitative microCT measurement, the whole joint microCT scoring scheme, and OARSI OA scores.

Medial tibial BV/TV and whole joint microCT scores demonstrated a moderate negative correlation ($r = -0.61$, 95% confidence interval -0.79 to -0.31, $P < 0.0001$). Moderate to strong correlations were present between medial tibia BV/TV and OARSI OA scores ($r = -0.72$, 95% confidence interval -0.86 to -0.46, $P < 0.0001$). No significant correlations were found for remaining quantitative microCT measurement.

The whole joint microCT-based clinical OA scoring system demonstrated strong correlation to OARSI OA grading, with a Pearson coefficient of 0.89 (95% confidence interval 0.76 to 0.95) ($P < 0.0001$). Similar correlations were noted when OARSI scores from only the medial or lateral compartments were compared to the microCT score ($r = 0.88$ and 0.82 , respectively).

Discussion.

To the authors' knowledge, this is the first study to utilize a clinically-based, whole joint microCT grading scheme in a rodent model of OA, with a focus on the Hartley guinea pig model of spontaneous disease. Other studies have examined quantitative microCT measurements and histology simultaneously in guinea pigs(17-19,23), but we aimed to implement a translational approach to disease assessment. To this end, we have demonstrated that our grading scheme is a useful tool that provides important and complementary data to existing outcome measures in the evaluation of OA. Indeed, this new scoring system strongly correlates to OARSI-based histologic grading. Increasing severity of osteophytes, subchondral bone sclerosis and cystic changes, and articular bone lysis detected by microCT were associated with increasing severity of articular cartilage surface lesions and proteoglycan loss seen on histology.

Advantages of microCT include 3-dimensional visualization of the joint (vs. 2-dimensional views on histology), the ability to evaluate living specimens in a longitudinal fashion, and more sensitive detection of subtle bony lesions that tend to occur early in OA disease progression(26,27). As discussed further, below, it is advantageous that these multiplanar reconstructions of the intact organ can be made in an endless number of angles for complete evaluation of the entire joint.

One particular benefit of microCT highlighted in the current study is the increased ability to detect osteophytes. With whole joint microCT, all animals in the 5, 9, and 15-month old groups (18 animals total) had visible osteophytes. However, only one animal had visible osteophytes detected by histology. Osteophytes are an optional category to include in OARSI scoring of knee joints, as they may be missed depending on the plane of section obtained(14). Because sectioning for OA grading focuses on coronal sectioning through the medial tibial plateau, osteophytes present elsewhere in the joint, particularly on the patella, may be missed. Also, imperfect trimming of knee joint tissue may result in histologic sections lacking representative lesions from all compartments of the joint (both medial and lateral tibial and femoral regions), as occurred with a few animals in the current study. Furthermore, coronal sectioning precludes histologic sagittal examination of the same joints. Finally, because the joint remains intact for microCT, many different views from the same animal can be evaluated. In particular, this allows assessment of the patella, a structure often excluded from histologic OA grading but considered relevant in clinical evaluation of human patients(28). Of note, evaluation of the patella can provide important clinical information in regards to overall knee OA, including presence of osteophytes, sclerosis, and chronic remodeling(28). Here, we have demonstrated that the patella became severely affected in the progression of OA in this guinea pig strain, which provides yet another similarity between this species and humans (28).

As expected, OA total knee joint scores based on the OARSI scale increased with advancing age in the Hartley guinea pigs. Very mild changes, such as minor articular surface undulations or minimal proteoglycan loss, were noted at 2 months in a few animals. Histologic evidence of OA was consistently present by 3 months of age, as has been described in other studies examining age-dependent changes in articular cartilage in Hartleys(18,19,29). Others have reported no obvious cartilage damage by 3–4 months of age(17,30), while another study saw no significant difference in OA scores in Hartley guinea pigs between 4 and 5 months of age(31). In our project, dramatic increases in severity of histologic OA were noted between the 5 and 9-month, as well as the 9 and 15-month, groups. Investigating additional time points between these ages would more clearly highlight when these changes occur, as well as the specific changes that contribute to the overall OARSI score. Of note, the current work is strengthened by the fact that all four knee joint compartments were scored according to OARSI recommendations, and the sum of the four regions was used as a total knee joint OA score. Other studies vary on whether only a single compartment is scored (typically the medial tibial region) or if a subset are scored and combined. It is recognized, however, that dedicating an entire joint to histology is not always practical in studies where tissue is needed for other assays.

Interestingly, statistical differences in histologic OA scores were found between every group, while whole joint microCT OA scores did not worsen between 5 and 9 months of age. It is possible that bony changes are most striking during both the early and later phases of OA, with cartilage changes occurring continuously throughout disease progression. While both are typically present in patients with end-stage OA, cartilage degeneration and bony changes are considered separate mechanistic events(32). Thus, as emphasized in our work, it is important to simultaneously examine cartilage and bone changes in animal models of OA to gain a complete picture of the disease.

Most OA studies employing microCT describe quantitative morphometric measurements, such as BV/TV, bone TMD, and subchondral bone trabecular thickness and spacing, as major outcome measures. These quantitative measurements were performed in the current study, with the majority of statistical differences focused on the medial tibial compartment. Overall, BV/TV in the medial tibial subchondral trabecular bone decreased with age and increasing OA severity. In the 3-month age group, BV/TV of the medial tibial subchondral trabecular bone was higher than that of the lateral tibial subchondral trabecular bone, a finding previously reported in 3-month Hartley guinea pigs of undesignated sex(17). Another study examining OA in female Hartley guinea pigs at 1, 3, 6, and 9 months of age found that BV/TV of the tibia was highest at 3 months of age and then remained constant(18). It is not known, however, if this particular study examined the tibia in its entirety, or if medial and lateral compartments were considered separately. Rat and rabbit studies of secondary, trauma-induced OA also showed that BV/TV decreased in both the femur and tibia in injured legs (with more severe OA) compared to sham control legs(33,34). As bony remodeling results in weakened bone with reduced bone volume – especially in the medial tibia – in both spontaneous and secondary OA in animal models(33,34), the decreasing BV/TV in this study is not surprising. Conversely, however, a recent human study of 14 tibial samples showed that subchondral bone volume increased with advancing OARSI histologic score for cartilage degeneration(35). These discrepant results may reflect differences in BV/TV based on the stage of OA evaluated, as microCT is typically only performed on samples from end-stage human patients undergoing knee replacement surgery.

Many OA studies using microCT as a major outcome measure have also focused on bone TMD. Of note, manuscripts vary on whether TMD is measured in the tibial subchondral plate or tibial subchondral trabecular bone. Further, microCT methodologies and analysis software are often different among studies and this may explain the lack of consensus amongst studies regarding TMD and OA. No statistical differences were detected for TMD in any of the four knee joint compartments as guinea pigs aged in the present study; however, highest TMD for all four compartments were present at 3 months of age, tended to decrease at 5 months, and then remained stable thereafter. A previous study examining TMD changes in female Hartley guinea pigs at various ages found that tibial subchondral TMD increased with age and was stable by 9 months(29). Another study comparing male Hartley guinea pigs to the OA-resistant GOHI/SPF strain found femoral subchondral bone TMD was lower in the Hartleys(23). A different study comparing male and female Hartleys to female OA-resistant Strain 13s found tibial subchondral TMD was similar between male Hartleys and female Strain 13s, yet was increased compared to female Hartleys(36). Likewise, female Hartleys have higher tibial subchondral plate TMD than OA-resistant Bristol Strain 2 guinea

pigs(17). In a rabbit injury-induced model of OA, both tibial and femoral TMD were decreased in the injured limb(34), while no differences in TMD were found in a rat model of injury-induced OA(33). Standardization of methods and location within the bone (subchondral trabecular bone vs subchondral plate) for TMD measurement may allow for more meaningful comparisons amongst studies.

One hurdle of the current study was the inability to assess weight-bearing joint space narrowing, a key radiographic feature assessed clinically in human OA patients(25). Fixation of limbs prior to microCT scanning results in artificial shrinkage of the joint space, making this measurement invalid. Furthermore, even when *in vivo* microCT is available, laboratory animals must be anesthetized, which precludes normal weight bearing of the knee joint. Thus, joint space narrowing is especially challenging, if not impossible, to obtain accurately in many animal models of OA. Another limitation of the proposed whole joint microCT grading scheme is the inability to assess soft tissue structures of the knee joint, such as cartilage and menisci. As such, it is important to evaluate joints using multiple modalities, including microCT and histology, that provide complementary data.

Of note, there are advanced imaging methods available that can provide information regarding both bone and soft tissue changes, bridging the gap between microCT and histology. In particular, Equilibrium Partitioning of an Ionic Contrast (EPIC) and other related forms of contrast-enhanced microCT exist, but are not yet as commonly used as more traditional microCT. These modalities utilize ionic contrast agents that electrochemically interact with cartilage matrix components, resulting in a nonuniform partitioning of the agent throughout the cartilage. This partitioning is then visualized and is reflective of articular cartilage defects and proteoglycan content (37–39). Contrast-enhanced microCT should be used in future studies, as the lack of cartilage visualization is a major drawback of traditional microCT in OA assessment. Assessment of cartilage in conjunction with bony changes using a single imaging modality may also strengthen our proposed whole joint microCT score.

In summary, we have shown that a whole joint microCT OA scoring system provides data complementary to OARSI histologic OA scores and quantitative microCT parameters in a guinea pig model of spontaneous OA. Although not yet demonstrated, this scheme may also be applicable in other species used to study OA. This clinically-oriented microCT grading scheme, particularly in conjunction with histology, may also be more comprehensive than individual microCT or histologic outcome measurements, alone. Future work would include an evaluation of additional species and models utilized in OA research, as well as both male and female animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Total body weight (in grams, g) for each age group of guinea pigs. (B) Comparison of total knee joint OA scores using the novel microCT grading scheme. (C) Comparison of total knee joint OARSI OA scores using toluidine blue-stained histology slides among the five age groups. Red lines indicate the mean value for each group. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ **** $P < 0.0001$.

Figure 2.

Representative photos from microCT evaluation of knee joints using the novel scoring system. (A) Dorsal and (B) sagittal reconstructions from a 2 month old Hartley guinea pig with no clinically significant OA lesions. This animal received a microCT OA score of 0. (C) Dorsal reconstruction from a 5 month old Hartley guinea pig. Sclerosis and small osteophytes are present on the medial femoral condyle (red arrows), and mild sclerosis of the central tibial plateau (D) Sagittal reconstruction from the same 5 month old guinea pig. Mild sclerosis of the cranial aspect of the patella and caudal tibial condyle is present. The small articular cystic changes are artifact due to the angle of the multiplanar reconstruction, which was selected to highlight the entire patella. This animal received a microCT OA score of 4. (E) Dorsal reconstruction from a 9 month old Hartley guinea pig. There is sclerosis and small osteophytes present on the medial femoral condyle (red arrow). (F) Sagittal reconstruction from the same 9 month old guinea pig. There is sclerosis along the cranial margin of the patella and at the proximal caudal end of the patella (red arrows). A large osteophyte is present on the patella (blue arrow). There are 2 cystic areas in the tibia that extend to the articular surface (green arrows). This animal received a microCT OA score of 5. (G) Dorsal reconstruction from a 15 month old Hartley guinea pig. There are 3 large osteophytes (red arrow) on the medial and lateral tibial plateau and on the medial femoral condyle. (H) Sagittal section from the same guinea pig. There is moderate subchondral sclerosis (outlined by red arrows) along the caudal tibia. (I) Dorsal reconstruction from a different plane of section from the same 15 month old guinea pig. Osteophytes on both medial and lateral tibial plateau and medial femoral condyle are still visible. There is cystic change with articular lysis (red arrow) in the tibial plateau. Subchondral bone sclerosis is also evident in the tibial plateau. (J) Sagittal reconstruction from the same animal. There is marked articular lysis of the tibial plateau (red arrow). This animal received a microCT OA score of 12.

Figure 3.

Representative photos of histologic lesions at the medial tibial surface from each age group using Toluidine blue stain. A) 2 month old with medial tibial OARSI score of 0; B) 3 month old with mild surface undulation, medial tibial OARSI OA score of 0; C) 5 month old demonstrating superficial proteoglycan loss, medial tibial OARSI OA score of 4; D) 5 month old showing articular cartilage fibrillation, medial tibial OARSI OA score 5; E) 9 month old showing proteoglycan loss into the middle zone and loss of superficial cartilage, medial tibial OARSI OA score 8; F) 9 month old demonstrating more diffuse proteoglycan loss into the deep layer with tidemark duplication, medial tibial OARSI OA score 10; G) 15 month old showing complete loss of articular cartilage, medial tibial OARSI OA score of 17; H) Another 15 month old demonstrating loss of articular cartilage and proteoglycan loss, medial tibial OARSI OA score of 18.

Table 1.

Mean values (with 95% confidence interval) for quantitative microCT measurements from each age group. Values are derived from the medial and lateral tibial and femoral subchondral trabecular bone.

		2 month	3 month	5 month	9 month	15 month	P-value
Bone Volume/Total Volume	MT	0.6318 (0.5794,0.6843)	0.6737 (0.5723,0.7750)	0.5013 (0.3882,0.6145)	0.447 (0.3939,0.4954)	0.4643 (0.4049,0.5237)	<0.0001
	LT	0.6057 (0.4190,0.7923)	0.5422 (0.4211,0.6633)	0.4567 (0.3604,0.5529)	0.4353 (0.3262,0.5445)	0.4155 (0.3210,0.5100)	0.0555
	MF	0.5292 (0.4533,0.6050)	0.5438 (0.4633,0.6244)	0.5385 (0.4695,0.6075)	0.4587 (0.4125,0.5049)	0.4443 (0.3299,0.5588)	0.0857
	LF	0.4507 (0.3722,0.5291)	0.4852 (0.4415,0.5288)	0.4887 (0.4087,0.5687)	0.4373 (0.4187,0.4560)	0.4175 (0.3330,0.5020)	0.2561
Bone Tissue Mineral Density (g/cm ³)	MT	2.682 (2.121,3.243)	2.653 (1.923,3.383)	2.550 (2.461,2.638)	2.209 (2.061,2.357)	2.494 (2.416,2.572)	0.2868
	LT	2.671 (2.126,3.217)	2.650 (1.925,3.375)	2.508 (2.384,2.632)	2.206 (2.036,2.364)	2.511 (2.411,2.639)	0.2946
	MF	2.681 (2.104,3.258)	2.648 (1.910,3.386)	2.527 (2.384,2.669)	2.200 (2.143,2.292)	2.525 (2.461,2.603)	0.3057
	LF	2.692 (2.115,3.270)	2.634 (1.902,3.365)	2.517 (2.386,2.648)	2.223 (2.061,2.385)	2.521 (2.435,2.606)	0.3460
Trabecular Thickness (mm)	MT	0.1775 (0.1546,0.2004)	0.2133 (0.1948,0.2319)	0.2123 (0.1860,0.2387)	0.1825 (0.1694,0.1956)	0.1902 (0.1706,0.2097)	0.0080
	LT	0.1707 (0.1626,0.1787)	0.1765 (0.1653,0.1877)	0.1847 (0.1623,0.2070)	0.1770 (0.1543,0.1997)	0.1755 (0.1479,0.2031)	0.7887
	MF	0.1905 (0.1679,0.2131)	0.2117 (0.1905,0.2328)	0.2303 (0.1963,0.2644)	0.1935 (0.1766,0.2104)	0.2055 (0.1497,0.2613)	0.2246
	LF	0.1683 (0.1501,0.1865)	0.1873 (0.1721,0.2026)	0.2118 (0.1838,0.2399)	0.2085 (0.1586,0.2584)	0.1997 (0.1474,0.2520)	0.2106
Trabecular Spacing (mm)	MT	0.2427 (0.2043,0.2811)	0.2305 (0.1997,0.2613)	0.3038 (0.2238,0.3839)	0.2800 (0.2480,0.3120)	0.3052 (0.2394,0.3709)	0.0492

		2 month	3 month	5 month	9 month	15 month	P-value
	LT	0.2150	0.2518	0.2813	0.2608	0.2485	0.0634
		(0.1808,0.2492)	(0.2247,0.2790)	(0.2401,0.3226)	(0.2083,0.3184)	(0.2151,0.2819)	
	MF	0.2430	0.2467	0.2710	0.2640	0.3682	0.3973
		(0.2206,0.2654)	(0.2217,0.2716)	(0.2422,0.2998)	(0.2460,0.2820)	(0.08492,0.6514)	
	LF	0.2705	0.2715	0.2877	0.2993	0.2735	0.5006
		(0.2294,0.3116)	(0.2460,0.2970)	(0.2591,0.3162)	(0.2627,0.3359)	(0.2334,0.3136)	
Cortical Thickness (mm)	T	0.6206	0.7934	0.7733	0.7532	0.6965	0.0156
		(0.5001,0.7410)	(0.7093,0.8776)	(0.6650,0.8816)	(0.6761,0.8304)	(0.6377,0.7552)	
	F	0.6368	0.7665	0.8035	0.8278	0.7239	0.0308
		(0.5014,0.7723)	(0.6828,0.8502)	(0.6675,0.9395)	(0.7201,0.9355)	(0.6640,0.7838)	

MT, medial tibia; LT, lateral tibia; MF, medial femur; LF, lateral femur; T, tibia; F, femur. In the P-value column, bolded numbers are statistically significant. P-value represents significance of overall one-way ANOVA analysis.

Table 2.

Guinea Pig Stifle Osteoarthritis Scoring System based on MicroCT analysis.

MicroCT Finding	Score
Presence of osteophytes	0 = none 1 = small osteophyte (< 1mm) 3 = large osteophyte (≥ 1 mm)
Location of osteophytes	1 = medial and/or lateral tibia 2 = patella 3 = medial and/or lateral femur
Subchondral bone cystic changes	0 = no 1 = yes
Subchondral bone sclerosis	0 = no 1 = yes
Articular bone lysis	0 = none 1 = yes
Intra-articular soft tissue	0 = normal 1 = increased