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The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse

S.S. Glasson †*, M.G. Chambers ‡, W.B. Van Den Berg §, C.B. Little ||

† Pfizer, Tissue Repair, 200 CambridgePark Drive, Cambridge, MA 02140, USA
‡ Eli Lilly and Company, Musculoskeletal Drug hunting Team, Lilly Corporate Center, Building 98C/B, Indianapolis, IN 46285, USA
§ Nijmegen Centre for Molecular Life Sciences, Rheumatology Department, PO Box 9101, 6500 HB, Nijmegen, Netherlands
|| Raymond Purves Bone and Joint Research Laboratories, Kolling Institute of Medical Research, University of Sydney at Royal North Shore Hospital, Level 10 Kolling Building – B6, St Leonards, NSW 2065, Australia

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SUMMARY

Aim: To describe a histologic scoring system for murine osteoarthritis (OA) that can be applied universally to instability, enzymatic, transgenic and spontaneous OA models.
Methods: Scientists with expertise in assessing murine OA histopathology reviewed the merits and drawbacks of methods described in the literature. A semi-quantitative scoring system that could reasonably be employed in any basic cartilage histology laboratory was proposed. This scoring system was applied to a set of 10 images of the medial tibial plateau and femoral condyle to yield 20 scores. These images were scored twice by four experienced scorers (CL, SG, MC, TA), with a minimum time interval of 1 week between scores to obtain intra-observer variability. An additional three novice scorers (CR, CL and MM) with no previous experience evaluated the images to determine the ease of use and reproducibility across laboratories.
Results: The semi-quantitative scoring system was relatively easy to apply for both experienced and novice scorers and the results had low inter- and intra-scorer variability. The variation in scores across both the experienced and novice scorers was low for both tibia and femur, with the tibia always having greater consistency.
Conclusions: The semi-quantitative scoring system recommended here is simple to apply and required no specialized equipment. Scoring of the tibial plateaus was highly reproducible and more consistent than that of the femur due to the much thinner femoral cartilage. This scoring system may be a useful tool for both new and experienced scorers to sensitively evaluate models and OA mechanisms, and also provide a common paradigm for comparative evaluation across the many groups performing these analyses.

Introduction

The histologic evaluation of osteoarthritis (OA) in the mouse has increased exponentially in the past decade with the advent of transgenic animals being used to look for mechanisms involved in the development of OA. The first significant reports of mice developing OA were in the mid-1900s[1–3] and included studies showing that aged C57BL/6 mice developed spontaneous, idiopathic OA. Investigators noted that murine OA exhibited many of the same pathologic features as the approximately 2500-fold heavier human, including loss of proteoglycan (PG) staining, fibrillation, cloning, and erosion of cartilage matrix. Other spontaneous models were intermittently reported over the subsequent decades (STR/ort[4], STR-1 N[5]) and included a number of spontaneous mutations[6,7] that had human counterparts which also developed early OA. The observations that some inbred strains of mice had far greater incidence and severity of OA than others, in early adult-hood as well with advanced-age, were important as they indicated that murine OA was more than a "wear and tear" phenomenon and had a strong genetic basis.

As murine embryonic stem (ES) cells, transgenic, knock out (KO) and knock in (KI) techniques became widely available, mice have been extensively used to replicate the genetic defects and biochemical processes thought to be involved in the development

* Address correspondence and reprint requests to: S. Sonya Glasson Consulting, 2 Pinewood St, Lexington, MA 02421, USA.
E-mail addresses: ssglasson@gmail.com (S.S. Glasson), chambers_mark_geoffrey@lilly.com (M.G. Chambers), W.vandenBerg@reuma.umcn.nl (W.B. Van Den Berg), christopher.little@sydney.edu.au (C.B. Little).

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of human disease. These mice also allow for a better understanding of the mechanisms of OA development and confirm the role of critical molecules, such as degradative enzymes[9], in the disease process. Therefore in the past two decades, the mouse is being used not only to replicate known human diseases, but also to examine the impact of deleting, over-expressing or altering critical enzymes or structural proteins that could impact OA pathogenesis. The replication of human pathology in the mouse following the targeting of the same human genetic mutation validates the utility of mouse models of genetic conditions[8].

A universal system for histologic scoring of murine OA would allow comparison of the severity of cartilage destruction across different spontaneous, enzymatic, chemical or surgically-induced murine OA models. A universal system would also provide a more objective evaluation as to the relative level of disease acceleration or amelioration using a specific treatment or gene-deleted mouse. This could allow prioritization of resources to those targets found to be more critical to OA progression in the mouse. The proposed system is considered sufficiently resilient to be utilized for all the widely used models of murine OA[10], including surgical, intra-articular (IA) collagenase, and spontaneous models.

This paper will restrict itself to the description of histology of the knee only, since the knee is the predominant joint for spontaneous OA development and is sufficiently large for IA access (chemical, enzymatic and cruciate disruption models) and for microsurgery.

Anatomy and joint pathology

The anatomy of the mouse knee resembles that of other species and is only notable from other mammals by its extremely small size. Typical mice weigh only 20–40 g, more than 10-fold less than rats, and 2500-fold less than man. The cartilage of the mouse is only 30 μm thick, which is nearly 10-fold thinner than the rat and approximately 50-fold thinner than man[11]. The layer of calcified cartilage is nearly as thick as the non-calcified cartilage (or even thicker in some joint regions), which is in stark contrast to the thin calcified cartilage layers seen in larger animals and humans. The organization and pathology of cartilage degeneration in the mouse are largely related to the extremely thin cartilage. The cartilage is only several cell layers thick and does not have clearly distinguishable superficial, transitional and radial zones. It is rare to capture the pathology extending through different depths in the non-calcified cartilage, as non-calcified cartilage loss tends to be an all-or-none phenomenon. The pathology of cartilage degeneration tends to progress rapidly from a loss of PG, then mild fibrillation, through focal, extending to broader, full-thickness loss of non-calcified cartilage.

- Mouse cartilage is very thin and rapidly progresses to full-depth fibrillation, which starts as focal regions of non-calcified cartilage loss, and progressively involves larger areas.

Macroscopic scoring of mouse cartilage degeneration

Due to the extremely small size of the mouse, macroscopic staging of cartilage degeneration is difficult and should utilize dissecting microscopes, microsurgical dissection and potentially the use of dyes such as India ink to contrast the lesions. Due to the shallow nature of the lesions, depth information may not be available. We recommend preserving intact mouse joints for histology so that the intact joint can be evaluated without a concern for iatrogenic damage inflicted at dissection and kissing lesions can be appreciated between the tibia and femur. The entire mouse joint is small enough to be captured on a single microscopic section, decreasing sampling bias for histology.

- Macroscopic scoring of mouse OA is not routinely performed.

Microscopic scoring of mouse cartilage degeneration

Specimen preparation

Histology is the gold standard for evaluation of murine OA. Knee joints are dissected free of skin or excess muscle, and placed in a fixation solution. The patella (or other orienting region) may be stained with a tissue marker to aid in orientation to provide consistent embedding. Murine knee joints can undergo frozen sectioning or be embedded in plastic, but in most joints are decalcified and paraffin embedded. The paraffin methodology requires less specialized equipment, is cost-effective and provides high quality slides adequate for most purposes and will be the method described here. Twenty-four hours fixation in 10% formalin is utilized for routine histology, with 4% paraformaldehyde providing extra flexibility for immunohistochemistry. The samples are then transferred to a decalcification solution which may be a formic acid (10% v/v), commercially-available decalcification solution, or 20% ethylenediaminetetraacetic acid (EDTA). Seven days in 20% EDTA on a plate shaker at RT or 48 h in 10% formic acid is generally sufficient to decalcify adult mouse knees depending on the surrounding length of tibia and femur. Longer decalcification times are required for very large and/or aged mice such as the STR/ort mice. Formic acid or other rapid decalcification systems require less time but must be carefully optimized so that excessive decalcification, leading to decreased staining of PGs is avoided. Following decalcification, the samples are thoroughly rinsed and samples processed with graded alcohol dehydration and infiltration with paraffin. The knee joints are then embedded in paraffin blocks. We recommend frontal (coronal) embedding as it allows for concurrent evaluation of the medial and lateral tibio-femoral joints and fewer sections need to be evaluated in the anterior–posterior plane than in the medial to lateral plane to incorporate the whole joint. Since the pathology in novel murine OA models or in genetically modified animals cannot be predicted to occur in only the medial compartment, the evaluation of the entire joint is important. Embedding the joint to provide frontal sections is more difficult than that for sagittal sections. Accurate identification of the femur, tibia and patella is critical so that the patella can either be embedded uppermost or at the bottom of the paraffin mold.

- Joints are usually fixed for 24 h in formalin, decalcified for 7 days in 20% EDTA, then embedded frontally in paraffin.

Sectioning

The method of sectioning in the literature varies greatly. Many groups utilize serial sections through the entire knee[12] while others restrict the evaluation to a focal region. Some studies describe the methodology of sectioning through the entire joint and stipulate the location of lesions[4]. Lapveteläinen *et al.*[13] utilize frontal sections located at the insertion of the anterior cruciate ligament to examine the four quadrants there and at two more 200 μm intervals, to cover approximately the central third of the volume of the knee. In many papers, the location of sections for analysis is not always clearly disclosed.

Since the whole mouse joint is small and harvested intact, rather than a specific gross lesion or joint region, it is feasible to section the entire joint. Sectioning requires a trained histologist to recognize the start and stop landmarks in a joint, usually confirmed on