INTRODUCTION: Post-traumatic arthritis is one of the most frequent causes of disability following trauma involving weight-bearing joints. It is estimated that 10% of the 21 million Americans suffering from osteoarthritis are post-traumatic. The development of post-traumatic arthritis may follow a variety of joint injuries but most commonly and predictably develops subsequent to fracture of the articular surface.

The mechanisms leading to the progression of post-traumatic arthritis following articular fracture are not well understood, and few experimental models have incorporated all the elements of articular fractures, including blunt trauma, fracture of the cartilage and underlying subchondral bone, and displacement of the articular surface. The majority of current animal models for studying post-traumatic arthritis open the joint capsule either for an open osteotomy, or direct cartilage impaction. The physical disruption of the joint capsule alone without an associated trauma induces an inflammatory response that may affect the healing process. A model of closed articular fracture would allow for a more clinically relevant evaluation of the cartilage and subchondral bone healing following trauma without the confounding elements of the joint response to disruption of the capsule and synovium.

The objective of this study was to develop a novel model of closed articular fracture in the mouse knee joint in order to quantify the temporal sequence of post-traumatic healing and the progression of post-traumatic osteoarthritis.

METHODS: Closed articular fractures were created in the left tibial plateau of 12 adult mice (28-35 g, male, C57BL/6) in accordance with an IACUC approved protocol. Mice were sedated, and the proximal tibia was loaded in compression to 55N at a rate of 20N/s in load-control on a materials testing system (EnduraTEC, ELF3200). Fractures were immediately characterized by AP and lateral radiographs using high resolution digital x-ray (Model MX-20 Digital, Faxitron). Animals were allowed immediate full weight bearing with unlimited range of motion. Tibial plateau fractures were classified from digital radiographs using the Orthopaedic Trauma Association (OTA) classification system. The energy of fracture was calculated from load-displacement curves for each fracture. Healing fracture was followed radiographically at 2, 4 and 8 weeks.

Four animals were randomly assigned and sacrificed at either 2, 4 or 8 weeks following surgery. Experimental and contralateral control limbs were harvested and fixed in formalin in a jig that maintained their neutral position. Following fixation, joints underwent micro-computed tomography evaluation (microCT 40, Scanco Medical AG) followed by histology. MicroCT evaluation was performed in the first 160µm (10 slices) of the tibial plateau immediately distal to the subchondral plate. Bone volume (mm³) and bone density (mg HA/ccm) were reported. Two dimensional coronal and sagittal images of the bone were generated in order to determine the subchondral thickness for the lateral and medial femoral condyles, and lateral and medial loaded regions of the tibial plateau. Within each location, ten thickness measurements were taken across the region, and the average was reported.

Histological sections of the entire knee joints from both the experimental and contralateral control limbs were prepared for Safranin-O and fast green staining from the limbs also scanned with microCT. Sections were taken in the coronal plane at the A/P location of normal loading. A modified Mankin scoring system (maximum score 30) was used to evaluate any degenerative joint changes in the mouse cartilage. Five locations in the knee joint were graded separately: lateral femur, medial femur, lateral tibia, medial tibia, and trochlear groove.

Multifactorial analysis of variance (ANOVA) was utilized to examine statistical differences between experimental and control limbs for all listed parameters along with the effect of healing time with significance reported at the 95% confidence level.

RESULTS: Articular fractures were induced in 11 of 12 mice resulting in a 92% success rate. Animals ambulated well within 4 hours of fracture induction, and we reported no incidences of infection or self-mutilation of the affected joint. The energy of fractures (range 33 to 173J) correlated with the type of fracture as classified using the OTA system (Figure 1). Higher energy was associated with more comminuted fractures. From microCT analysis, the tibial plateau demonstrated no difference in bone volume compared to the contralateral control but did show a decrease in bone density (p<0.05). Subchondral thickening was observed in the fractured limb in all locations of the knee joint compared to the contralateral control limb (Figure 2). No significant changes were observed with time post surgery (p>0.05).

From histology sections, the fractured experimental limbs demonstrated structural changes in the articular cartilage and a loss of proteoglycans as indicated by a loss of Safranin-O staining. Modified Mankin scores showed significant degenerative changes (p<0.05) between the fractured and control limb in the articular cartilage in all locations except the trochlear groove. Additionally, greater degenerative changes were observed at 4 and 8 weeks than at 2 weeks (p<0.05).

DISCUSSION: This study introduces a novel model of closed articular fracture in the mouse knee that demonstrates degenerative changes representative of post-traumatic arthritis. The model produces articular fractures similar to clinically observed fractures described by the OTA classification system in a reproducible manner (>90% success rate). MicroCT and histological analyses demonstrate osteoarthritic-like changes, consisting of subchondral bone thinning and degenerative changes in the cartilage that progresses out 8 weeks. In addition to these changes, microCT also revealed changes in bone density in the tibial plateau immediately distal to the subchondral plate. This finding suggests that although healing of the fracture occurs, the bone may be less structurally sound. These findings are consistent with those reported for osteoarthritic human tibia. This model provides a novel platform from which the pathology of post-traumatic arthritis can be studied in a physiologically relevant setting in a small animal model. Most notably, this model permits the use of genetically modified mice to investigate the role of specific genes implicated in the pathologic function of articular cartilage and subsequent pathologic development of post-traumatic arthritis.


ACKNOWLEDGMENTS: Supported by grants from the NIH and the Duke Orthopaedic Research Scholarship for Medical Students. We also thank Steve Johnson for his technical support.

Figure 1. Digital x-ray images of intra-articular fracture of mouse tibia (arrow). Severity of fracture correlated with energy of fracture.

Figure 2. Thickness of subchondral bone for experimental (fractured) and control limbs at all locations (*p<0.05).

Figure 3. Modified Mankin score for experimental (fractured) and control limb.

Table: Comparison of subchondral thickness

<table>
<thead>
<tr>
<th>Location</th>
<th>Average Subchondral Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral femur</td>
<td>0.35</td>
</tr>
<tr>
<td>Medial femur</td>
<td>0.25</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>0.45</td>
</tr>
<tr>
<td>Medial tibia</td>
<td>0.30</td>
</tr>
<tr>
<td>Trochlear groove</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Experimental vs Control: p<0.05