Acute Synovial Inflammation is Associated with Increased Intra-Articular Fracture Severity in the Mouse Knee
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INTRODUCTION: Post-traumatic arthritis (PTA) is a frequent cause of disability and occurs most commonly and predictably after articular fracture [1-2]. Importantly, more severe fractures, characterized by greater displacement of the articular surface and greater degree of comminution, have a worse prognosis [3]. Thus energy of injury and subsequent intra-articular fracture severity may play a fundamental role in the pathologic progression of PTA and also serve as an important prognostic indicator for clinical outcomes [3-4]. The effect of articular fracture on synovial inflammation remains unknown, although synovial pathology is critical in the development of various forms of arthritis [5-6]. The objective of this study was to examine the effect of increasing fracture severity on acute joint pathology in a novel murine model of intra-articular fracture. We hypothesized that increased energy of fracture would increase liberated cortical bone surface area and would be associated with acute increases in synovial pathology.

METHODS: All procedures were performed in accordance with an IACUC approved protocol. Twenty adult mice (28-35g, male, C57BL/6) were divided into two groups: low energy (n=10) and high energy fractures (n=10). Fractures of the proximal tibial plateau were created in anesthetized mice as previously described [7]. We reported a correlation (R² = 0.86) between indenter displacement and energy of fracture in this model. We used a 3.2mm indenter displacement limit for creating low energy fractures and no displacement limit for high energy fractures. The contra-lateral (right) hind limb was used as a non-fractured control. Mice from each group were sacrificed at 0 and 7 days after fracture. Hind limbs from all animals were dissected, placed in formalin, and imaged using microCT (microCT 40, Scanco). Fractures were characterized for severity by measuring liberated surface area [4] from microCT images. Semi-automated edge detection software employing a Canny filter (Mathematica 6.0, Wolfram) was used to outline cortical bone using spline curves in axial images with a voxel size of 16x16x16µm (Figure 1A). The splines were then used to create a 3D model of each joint. The fractured joint model was then aligned with its contralateral control using an iterative closest point technique [8] and the change in surface area between the two models was calculated. The liberated surface area was then plotted as a function of applied fracture energy [4], as measured from the force displacement curve of the load frame. The effect of fracture energy classification (low versus high energy) on liberated surface area was also assessed. Statistical analysis of fracture energy and liberated surface area were performed using a student t-test with significance reported at the 95% confidence level and correlations were performed using regression analysis.

Histology sections (FFPE, 5µm thick) of all limbs were taken in the coronal plane, and H&E stained. One animal from the low energy group was excluded due to histology processing issues. The synovial insertion of the lateral tibia (LT), lateral femur (LF), medial tibia (MT), and medial femur (MF) were evaluated separately using a modified form of an established standardized synovitis score [9] for changes in synovial lining thickness and cellular density in the synovial stroma (maximum score 6). The correlation between degree of synovitis and applied energy of fracture was significantly different between the low and high energy fractures (p<0.05). A linear relationship was observed between liberated surface area and energy absorbed in fracture (R²=0.70, p=0.01, Figure 1B).

RESULTS: Fracture Energy and Liberated Bone Surface Area: The applied energy of fracture was significantly different between the low (90±33mJ, p=0.001) and high energy (177±25mJ) fractures. High energy fractures had a greater degree of liberated cortical bone surface area (7.2±2.1mm²) than low energy fractures (3.3±0.0mm²; p=0.03). A linear relationship was observed between liberated surface area and energy absorbed in fracture (R²=0.70, p=0.01, Figure 1B). Synovitis Score: At day 0, there were no statistically significant differences in synovitis scores between fractured and contralateral control limbs for either low or high energy fractures (p>0.05). At day 7, low energy fractures demonstrated significantly greater synovitis scores in fractured limbs compared to non-fractured control limb at the lateral tibia, lateral femur, and medial tibia (Figure 2). At day 7, high energy fractures had significantly greater synovitis scores in fractured limbs compared to non-fractured control limbs at all sites (Figure 2). Applied fracture energy was correlated with synovitis score in the synovial lining layer at the medial tibia only (p<0.05). There were no other significant correlations between synovitis scores and either applied fracture energy or liberated surface area.

DISCUSSION: Our results indicate that, in this closed-joint murine model of intra-articular fracture, the applied energy of fracture was significantly correlated with degree of liberated cortical bone surface area. These data suggest that the use of observed energy of fracture is an appropriate measure of injury severity, providing a less labor-intensive technique as compared to the measurement of liberated surface area. Furthermore, this study demonstrates that fracture severity was associated with increased synovial pathology and increased synovitis throughout the entire joint. Both low and high energy fractures were associated with lateral joint pathology, but only high energy fractures resulted in inflammation medially, with higher energy fractures demonstrating more global joint synovitis. Although the role of synovial inflammation in the progression of both OA and PTA is not fully elucidated, synovial inflammation is a factor that likely contributes to dysregulation of chondrocyte function and leads to degenerative changes in articular cartilage [10]. Further characterization of the early events following trauma could aid in the treatment and prevention of PTA following articular fracture.


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