Characterization of fracture healing pathways following different fixation methods: Developing a bilateral femoral fracture model in Sprague-Dawley Rats

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Introduction
It has been long observed that fractures in the diaphyseal bone treated with rigid fixation achieve healing through a different mechanism than fracture treated less rigidly. Those fixed rigidly undergo primary ossification; direct cortical healing without callus formation. Primary fixation is made possible by compression plating. It requires anatomic reduction and rigid fixation, with no motion at the fracture site. In contrast less rigid fixation, intramedullary nailing and external fixation devices, achieve healing by formation of a large, stabilizing callus. Bridging occurs primarily through endochondral ossification. Anatomic reduction is not required, however, there must be stable fixation with a tolerance for some amount of interfragmentary motion.

While these differences are known, little has been demonstrated about the comparative differences between internal fixation and external fixation present in the same animal under similar conditions. Our goal was to establish an animal model that would represent each pathway of bone repair and would allow for further investigation. We suspect that various exogenous/environmental factors have varying effects on each healing pathway, and this will allow us to directly compare primary and secondary healing mechanisms, eventually examining the differential effects of various factors on each pathway. For example we plan to utilize this model to test the impact of fixation methods on fracture repair in rats exposed to cigarette smoke. Ultimately, this would potentially contribute to the establishment of preferred fixation methods for different patients (e.g., smokers versus non-smokers). This would be more realistically served by an optimization of healing technique than a reliance on an unlikely change of behavior.

We are developing a bilateral fracture model for facilitation of direct comparison of fixation method on quality of repair.

Methods
Animal Model: We use a bilateral femoral osteotomy performed in Sprague_Dawley rats (SDR) to examine the mechanism of healing with rigid plate fixation on one limb and less rigid external fixator on the opposite limb.

The surgical procedure followed a previously established lateral approach to expose the femur. A lateral incision from the greater trochanter to 1.5 cm proximal to the knee joint. An intermuscular plane was developed between the vastus lateralis and biceps muscle exposing the anterolateral portion of the femur. A blunt osteotomy was creating utilizing a ¼ inch osteotome. To ensure a clean fracture six holes were drilled with three passes in the plane to the desired fracture pattern. External fixation was achieved using four (4) 1.6 mm Kirchner wires and an external fixation device established with the assistance of a drill hole guide. Internal fixation was achieved with the use of a five (5) hole plate held in place by four (4) 1.5 mm cortical screws.

Using this model twenty-four (24) SDR underwent the surgical osteotomy procedure. Following surgery the animals were randomly assigned to one of six time points. Two rats were assigned to 3, 10 and 14 days and six rats to 3, 6 and 12 weeks. Radiographs were taken at two week intervals to evaluate the healing process. Subjects were euthanized at their assigned time points. Femurs were harvested immediately post-mortem and fixed for prepared for histologic examination. All samples underwent staining with Masson’s Trichrome and Safranin O and are awaiting histomorphometric quantitation of healing.

This protocol was pre-approved by the Institutional Animal Care and Use Committee of the University of Maryland.

Results
Radiographic Analysis: Anterior and lateral radiographs of the fractures femurs were taken every 2 weeks from the date of surgery to each animals predetermined time point. The films were assessed callus formation, remodeling, and bridging one formation. The femurs treated with external fixation demonstrated significant callus formation around the fracture. The plated femurs formed no detectable callus at the site of fracture. As expected the amount of bridging increased over time.

Discussion
The external fixation and plate fixation techniques to this point have provided a predictable path to healing. This model is unique in that by using different fracture fixation treatments in a bilateral fashion the animal will serve as its own control. This is important because there been an observed variability between individual rats with regards to the response to fracture healing.

Preliminary observations of our completed animals supports that our fixation method dictates the mechanism of repair in the animal. External fixation proceeds through callus formation with bone formation through a fibrocartilage pathway. Plate fixation achieves direct cortical healing without callus. The success of this model is important because it will allow for further investigation into the effects that various exogenous/ environmental factors have on primary versus secondary pathways of healing.

Biomechanical Studies will be undertaken on four of the six subjects in each of the later time points, 3, 6 and 12 weeks. Four point bending test will be applied by a Mechanical Testing and Simulation machine. Histomorphometry analysis will be undertaken on two subject from each of the six time points. Sections will be analyzed using the Osteometrics Program.

Figure 1 lateral x-ray post-op day 42 – evidence of fracture healing. a) plated fracture b) externally fixed fracture

Figure 2 histology sections at fracture site at six weeks post osteotomy in the coronal plane stained with Safranin O (red – cartilage). a) plated fracture – plate fixed to left femur, minimal callus b) externally fixed side – abundant callus incl. endochondral ossification. c) original cortex, fr) location of transverse fracture

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