The Impact of Surgical Fixation on Fracture Healing:
Radiographic Analysis of a Novel Fracture Model in Rats

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Purpose: The effect of inflammation on fracture healing is well recognized by clinicians. In addition, the inflammatory effect of surgery on the trauma patient is known to induce an additional surge of inflammatory mediators (second hit) with known systemic consequences. Currently, our understanding of the effects of inflammation on fracture callus formation and tissue homeostasis are derived from one hit animal models where the experimental fracture is simultaneously fixed. The purpose of this study was to develop a translational animal model to determine the impact of temporized (24 hours) surgical fixation on local inflammatory shifts, tissue homeostasis, and fracture healing.

Methods: A closed middiaphyseal femur fracture was generated in the right femur of 20 young adult, female Sprague Dawley rats using the method described by Bonnarens and Einhorn. Half (n = 10) underwent conventional fixation with retrograde intramedullary pin placement (0.8 mm) prior to the experimental fracture. The remaining rats (n = 10) underwent fracture fixation 24 hours after the index injury. Under fluoroscopic guidance, a 0.05-mm guide pin was utilized to realign the fracture segments. Hypodermic tubing (0.8 mm) was then placed over the pin in a retrograde fashion for final fixation. Fracture healing rates were measured with weekly radiographs and scored independently by three of the authors based on bone bridging across the healing callus (0-4 points). A score of 4 denoted complete healing. Time to healing was assessed using Kaplan-Meier methods, and a two-way repeated-measures analysis of variance (ANOVA) was utilized to determine the effect of immediate and temporized fracture fixation over time. Four-point bend testing was performed to assess mechanical strength after 6 weeks. A P value of <0.05 was set to denote statistical significance. This study was approved by our institutional animal care and use committee.

Results: All animals tolerated the procedures well without any complications. There were no significant differences on the average time to union between groups (5.7 vs 6.0 weeks, $P = 0.063$). However, average radiographic scores were significantly lower in rats that underwent temporized fixation compared to rats that underwent fixation at the time of injury (1.7 ± 1.3 vs. 2.4 ± 1.3, $P = 0.001$). Analysis of simple main effects demonstrated that these differences were only significant at week 3 (1.4 ± 0.8 vs 2.4 ± 0.5, $P = 0.04$) and week 5 (2.7 ± 0.5 vs 3.6 ± 0.5, $P = 0.015$). Average radiographic scores increased from week 1 to week 6 in both groups ($P < 0.001$). In addition, maximum load was significantly lower in the setting of temporized surgical fixation, compared to simultaneous injury and fixation (115.6 ± 42.4 vs 198.4 ± 34.2 N, $P < 0.001$).

Conclusion: This study demonstrates the feasibility and reproducibility of a novel translational animal fracture model that reflects a more realistic clinical scenario. The impact of surgical fixation, as a major inflammatory event, on fracture healing has not been previously considered in animal models. Although there were no significant differences in time

to union, bone bridging across the fracture site was achieved earlier (week 3 and 5) in rats that underwent fixation immediately after injury. In addition, temporized surgical fixation was associated with reduced biomechanical strength at 6 weeks. Given the disparity in radiographic healing and failure load, future studies will focus on the assessment of structural characteristics as well as the longitudinal shifts in inflammatory mediators at the fracture site in this new model. Improving the translation strength of preclinical animal models of fracture healing using delayed fracture fixation may further enhance our ability to derive clinically driven answers from basic science studies. Ultimately, characterizing inflammation at the fracture site will guide the development of biological augmentation strategies and the use of anti-inflammatory medications in the postoperative period with the aim of improving patient outcomes.

Table 1 – Radiographic scores between immediate and delayed fixation

	Immediate Fixation	Radiographic Score (SD)	Delayed (24h) Fixation	Radiographic Score (SD)	p-value
1 wk		0.0±0.0		0.0±0.0	1.0
2 wk		1.6±0.9		0.9±0.7	0.134
3 wk		2.4±0.5		1.4±0.8	**0.040**
4 wk		2.6±0.5		2.0 ±1.3	0.356
5 wk		3.6±0.5		2.7±0.5	**0.015**
6 wk		3.9±.04		3.4±0.9	0.339

See pages 49 - 106 for financial disclosure information.