

## Development and Validation of Large Animal Models for Acute Fracture-Related Infections

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**Introduction:** Fracture-related infections (FRIs) are a major challenge in orthopaedics based on the associated burdens and costs for patients, healthcare teams, and the healthcare system in attempting to effectively resolve them. The reported rate for FRIs in the US is ~20-30% with a subsequent treatment failure (nonunion) rate of up to 11%.<sup>1-3</sup> For complete resolution, FRIs typically require patients to undergo multiple operations that involve irrigation and debridement (I&D) as well as the removal of fracture-fixation implants. Current evidence indicates that only 32% of all acute FRIs can be expected to retain fracture-fixation implants long-term.<sup>4</sup> This need for implant removal is largely due to microbial biofilms that form on these fixation devices. The bacteria embedded in biofilms are difficult to eradicate with conventional antibiotic treatment as the biofilm glycocalyx matrix protects them from chemical and mechanical destruction.<sup>5</sup> Approximately 35% of biofilm-inducing infections are caused by *Staphylococcus aureus* with the vast majority involving an antibiotic-resistant strain (e.g. methicillin-resistant *S. aureus*, MRSA).<sup>6,7</sup> In order to develop and validate more effective strategies for the prevention and treatment of FRIs, animal models that accurately replicate the pathomechanisms and clinical features of this problem are needed. The purpose of this study was to characterize two preclinical canine models for inducing fracture-related infections that included clinical, radiographic, and laboratory evidence of fracture site infection, biofilm formation on fracture-fixation implants, and bone healing complications, supporting the development and testing of novel therapeutic interventions.

**Methods:** Two different canine models were developed to address distinctive features of long-bone FRI: an ulnar defect model and a fibular defect model.

**Ulna model:** A known biofilm-producing strain of *Staphylococcus aureus* (OJ1) was prepared as a suspension of  $1 \times 10^5$  colony-forming units (CFU).<sup>8-10</sup> Fracture-fixation bone plates and screws were incubated in the suspension for 48 hours. Immediately prior to implantation, the plates and screws were washed in phosphate buffer solution to remove free-floating bacteria. Skeletally mature purpose-bred research hounds (n=16) were premedicated, anesthetized, and prepared for aseptic surgery of both forelimbs (n=32). Via caudal approach, a 1 cm segment of the distal ulna was osteotomized ("fracture"). Each ulnar "fracture" was stabilized using a pre-inoculated 6-hole, 2.7 mm bone plate and four 2.7 mm cortical screws (DePuy Synthes). Surgical wounds were closed routinely. After 3 weeks, dogs were again premedicated, anesthetized, and prepared for aseptic I&D of both fracture sites and surgical wounds were closed routinely. At 8 weeks post-I&D, dogs were humanely euthanized and assessed as described below. **Fibula model:** The same biofilm-producing strain of *Staphylococcus aureus* (OJ1) was used for this model and prepared as described above. Skeletally mature purpose-bred research hounds (n=8) were premedicated, anesthetized, and prepared for aseptic surgery of both hindlimbs (n=16) followed by creation of a 1 cm proximal osteotomy ("fracture") of both fibulas. Each fibular "fracture" was stabilized using a pre-inoculated 4-hole, 1.5 mm bone plate and four 1.5 mm cortical screws (DePuy Synthes). Surgical wounds were closed routinely. After 7 days, dogs were again premedicated, anesthetized, and prepared for aseptic surgery of both hindlimbs followed by I&D as described above. At 10 days post-I&D, dogs were humanely euthanized and assessed as follows. **Wound Assessments:** Wounds were assessed daily for clinical signs of infection including swelling/edema, redness, and drainage, which were documented when present. **Diagnostic Imaging:** Craniocaudal and mediolateral radiographs were obtained for each fracture site at study endpoint and assessed by a board-certified veterinary radiologist for findings consistent with osteomyelitis, implant failure, and/or bone healing complications. **Histology:** Two board-certified veterinary pathologists assessed histologic sections of ulnas and fibulas for grading of biofilm on the fracture-fixation implants. **Bacteriology:** Tissue samples from fracture sites were collected for quantitative microbial cultures at the time of I&D and study endpoint. Colony-forming units per gram (CFU/g) of tissue were calculated.

**Results: Ulna model:** At the 3-week I&D timepoint, all surgical wounds had clinical evidence of infection including redness, swelling, edema and scant to moderate serosanguinous drainage, but remained intact without systemic signs of infection. Radiographic findings were consistent with implant failure and bone healing complications (Figure 1). Based on tissue cultures at both time points, all ulnar fracture sites had confirmed FRIs that produced abundant microbial growth of *S. aureus* ranging from  $2.1 \times 10^2$  to  $3.2 \times 10^5$  CFU/g. At study endpoint, histological evaluation confirmed bacteria-laden biofilms present on fracture-fixation implants, graded as mild to moderate (Figure 2). **Fibula model:** At the 7-day I&D timepoint, all surgical wounds had clinical evidence of infection including redness, swelling, edema and mild to moderate serosanguinous or purulent drainage, but remained intact without systemic signs of infection. Radiographic findings were consistent with implant failure and bone healing complications (Figure 1). At the time of I&D, 12 of 16 fibula fracture sites had confirmed FRIs that produced abundant growth of *S. aureus* ranging from  $5.9 \times 10^2$  to  $1.2 \times 10^5$  CFU/g. At the study endpoint, all 16 fracture sites had confirmed FRIs that produced abundant growth of *S. aureus*. At study endpoint, histological evaluation confirmed bacteria-laden biofilms present on fracture-fixation implants, graded as mild to moderate (Figure 2).

**Discussion:** The results of this study validate two preclinical canine models for inducing fracture-related infections that produce clinical, radiographic, and laboratory evidence of fracture site infection, biofilm formation on fracture-fixation implants, and bone healing complications that mimic those seen in human patients with FRIs. For both models, incubation of fracture-fixation implants in  $1 \times 10^5$  CFU of biofilm-producing *Staphylococcus aureus* (OJ1) for 48 hours prior to implantation proved effective for creating acute FRIs that were associated with consistent signs of wound infection, implant failure, and bone healing complications. In addition, abundant growth of *S. aureus* from fracture sites and bacteria-laden biofilms on fracture-fixation implants were documented for all "fractures" by study endpoints. In terms of comparative advantages for each model, the ulnar model provides a more biomechanically challenging model based on its higher level of loading, while the fibular model provides a more biologically challenging model based on its greater degree of soft tissue coverage.

**Significance:** Two canine models for acute fracture-related infections were developed and validated to result in clinical and radiographic signs of wound and fracture site infection, abundant *Staphylococcus aureus* microbial growth, and consistent biofilm formation on fracture-fixation implants. These models allow for clinically relevant development and testing of novel preventative and therapeutic strategies that improve management of this challenging problem in orthopaedics.

**References:** <sup>1</sup>Kanakaris *et al.*, Bone Joint J., 2014; <sup>2</sup>Metsemakers *et al.*, J Trauma., 2011; <sup>3</sup>Bose *et al.*, Bone Joint J., 2015; <sup>4</sup>Rightmire *et al.*, Clin Orthop Relat Res., 2008; <sup>5</sup>Boudarel *et al.*, NPJ Biofilms Microb., 2018; <sup>6</sup>Trampuz *et al.*, Injury, 2006; <sup>7</sup>Morgenstern *et al.*, J Orthop Res., 2016; <sup>8</sup>Zhang *et al.*, Exp Ther Med., 2017; <sup>9</sup>Reizner *et al.*, Eur Cell Mater., 2014; <sup>10</sup>Bottagisio *et al.*, Med Microbiol., 2019



Figure 1 – Diagnostic images of fractured ulna and fibula

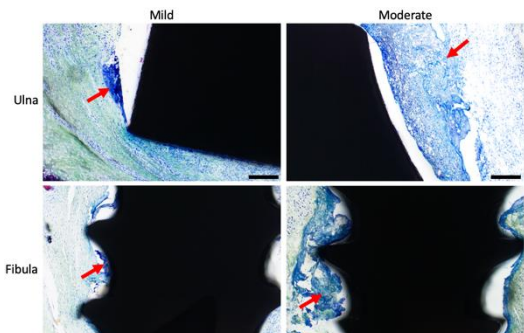


Figure 2 – Histological confirmation of bacteria-laden biofilm (→) using Stevensen's blue stain