

Propionibacterium Acnes Colonization Impairs Fracture Healing in a Rat Model of an Open Femur Fracture Treated with Intramedullary Fixation

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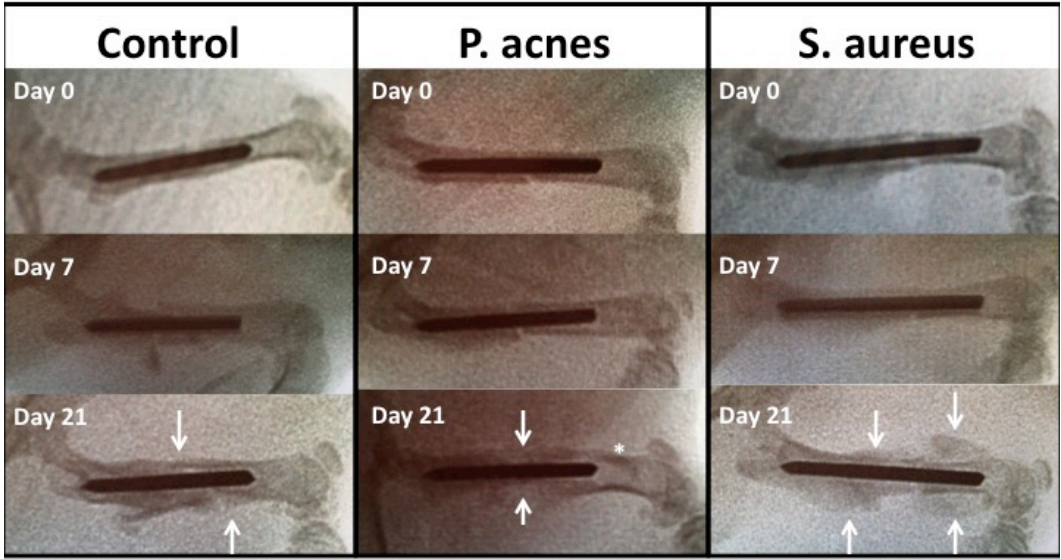
Background/Purpose: Bacterial biofilms play an important role in failed orthopaedic surgeries, notably joint arthroplasty and fracture nonunion. Novel molecular diagnostics have led to multiple studies characterizing biofilms in orthopaedic infections. Such studies suggest these infections are usually polyclonal, and often, but not always, associated with negative outcomes. Furthermore, these studies demonstrate that some species, which are often missed or discarded as lab contaminants, are in fact colonizers of bones or orthopaedic implants. One prominent example is *Propionibacterium acnes*, a gram-positive anaerobic bacillus traditionally considered nonpathogenic. It is commonly found colonizing the skin, upper respiratory tract, and / or gastroenteric mucosa. *P. acnes* is often overlooked in clinical diagnostics as it is an anaerobe and not easily detected in standard culture. Multiple recent studies using highly sensitive molecular diagnostics have detected *P. acnes* in orthopaedic infections supporting the hypothesis that *P. acnes* plays a pathogenic role. We hypothesize that *P. acnes* has a pathogenic role in the formation of biofilms and will impair fracture healing after intramedullary (IM) fixation of a femur fracture in a rat model.

Methods: Once IACUC (Institutional Animal Care and Use Committee) approval was obtained, a pilot study was completed to ensure the efficacy of our novel surgical technique. 84 male Sprague Dawley rats then underwent IM fixation of an open femur fracture created under sterile conditions and were inoculated with (1) sterile saline (control), (2) *Staphylococcus aureus*, or (3) *P. acnes*. 24 rats were excluded from the study; 9 did not recover from anesthesia, 9 wounds dehisced, 4 fractures were comminuted and not amenable to IM fixation, and 2 had loss of fixation. The rats were followed for either 7 or 21 days, for a total of 10 rats in each group. At the end point, a lateral radiograph of the hindlimb was obtained, and necropsy was performed to evaluate for signs of infection and fracture healing. The lateral radiograph of the femur was evaluated for osteolysis, soft-tissue swelling, periosteal reaction, general impression, and deformity and scored as (0) absent, (1) mild, (2) moderate, or (3) severe. Also, 1 additional point was added for either sequestra formation, or spontaneous fracture for a maximum score of 17. Radiographs were scored independently by two orthopaedic surgeons. The groups were compared using Student's t test for statistically significant differences ($P < 0.05$).

Results: At 7 days, the average radiographic score was 2.7 ± 1.3 , 3.8 ± 1.4 , and 5.2 ± 2.7 for the control, *S. aureus*, and *P. acnes* groups respectively. At 21 days, the average radiographic score was 4.8 ± 2.4 , 7.9 ± 3.2 , and 7.2 ± 2.4 for the control, *S. aureus*, and *P. acnes* groups respectively. There was a significant difference between the control and *P. acnes* groups at 7 days ($P = 0.02$) and at 21 days ($P = 0.04$). There was also a significant difference between

the control and *S. aureus* groups at 21 days ($P = 0.025$). There was no significant difference between the *P. acnes* and *S. aureus* groups at 7 ($P = 0.16$) or 21 days ($P = 0.59$). At necropsy, the control group showed signs of early callus formation at 7 days and most were healed by 21 days. In several of the *P. acnes* and *S. aureus* rats, there were obvious signs of infection with frank purulence at the fracture site and minimal signs of healing.

Figure 1



Control: No loss of fixation, bridging callous across fracture site at 21 days (arrows)
***P. acnes*:** At day 21: loss of fixation in distal fragment, osteolysis at fracture site (arrows) and within distal canal (*)
***S. aureus*:** At day 21: Substantial periosteal reaction (arrows), correlated clinically with sequestra formation

Conclusion: *P. acnes* inoculation of a rat femur fracture treated with IM fixation impairs fracture healing and leads to radiographic changes similar to those seen with *S. aureus* inoculation at 7 and 21 days (Fig. 1). The use of a control group and a known pathogenic comparison group enabled a thorough evaluation of the radiographic changes that occur in a rat model of an open femur fracture treated with IM fixation. The possibility of contamination during the surgical procedure is a risk, as with any surgical procedure, although our best efforts to maintain sterility were utilized and the fact that none of our control groups had signs of infection at necropsy is encouraging. Future studies utilizing molecular diagnostics to identify the bacterial species at necropsy and confocal microscopy to evaluate for the formation of biofilms at the fracture sites are planned. The primary limitation of the study is the small number of animals in each group, although we were able to achieve significance.

The FDA has stated that it is the responsibility of the physician to determine the FDA clearance status of each drug or medical device he or she wishes to use in clinical practice.