

Employing Alternating Magnetic Fields for Biofilm Destruction

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Abstract

Background: Prosthetic Joint Infections (PJI) are a common complication of implant surgery. Due to biofilm formation, treatment is costly, includes weeks of antibiotic therapy, and even total replacement of the prosthesis. However, a non-invasive thermal method of biofilm eradication has recently been developed: using high-frequency alternating magnetic fields (AMF) to destroy biofilm via induction.

Methods: The experiment used *Staphylococcus aureus*, a prototypic pathogen implicated in PJI. Stainless steel rings were used to mimic prosthetic joints. Biofilms of Methicillin-Sensitive *Staphylococcus aureus* (UAMS1-lux) were grown on stainless steel rings in a shaking incubator for 24 hours, 110rpm at 37°C, in Tryptic Soy Broth media. The rings were then resuspended in fresh media and incubated for another 24 hours at 110rpm, 37°C. Untreated rings, Antibiotics only, AMF only, and a combination of AMF + Abx were tested. Biofilms in the latter 2 categories underwent 3s pulses of AMF exposure every 5 minutes for 15, 30, or 60 minutes at a target temperature of 65°C every 12 hours for 24 hours. Ceftriaxone (2.0µg/ml) was used for the Abx conditions. Rings were sonicated at the indicated timepoints and colony-forming units (CFU) were determined.

Results: A synergistic effect between AMF and antibiotics was seen. At 12 hours, the Abx only and AMF only treatments showed regrowth; however, the combination therapy showed a 2.1-log decrease in biofilm CFU. Similarly, at 24 hours, solo AMF treatment showed total regrowth and Abx only treatment showed modest bactericidal effects (2.1 log reduction). However, combination therapy at 24hr showed a 5.35 log reduction and reached the limit of detection of the assay.

Additionally, we are investigating the effects of AMF with Linezolid (2.0 µg/ml). At 24hrs, a 4.3 log reduction in biofilm CFU was observed in the combination treatment, while solo treatments showed total regrowth.

Conclusions: These *in vitro* results serve as a strong basis for future work on AMF utilization in treatment of PJI. AMF and antibiotics are synergistic in reducing biofilm off metal. The observed bactericidal effects combined with this non-invasive means have wide and significant implications in improving the patient's quality of life as well as improving healthcare costs of PJI treatment.

Introduction

Prosthetic Joint Infections (PJI) are a common complication of implant surgery, estimated to affect 1-2% of all prosthetic implant patients in the U.S.^[1] Complications arise from biofilm formation by common Gram-negative pathogens such as *Pseudomonas aeruginosa* and Gram-positive pathogens such as *Staphylococcus aureus*. The annual cost of treatment of PJI in US hospitals increased from \$320 million to \$566 million during a study period from 2001-2009 and was projected to exceed \$1.62 billion by 2020^[2].

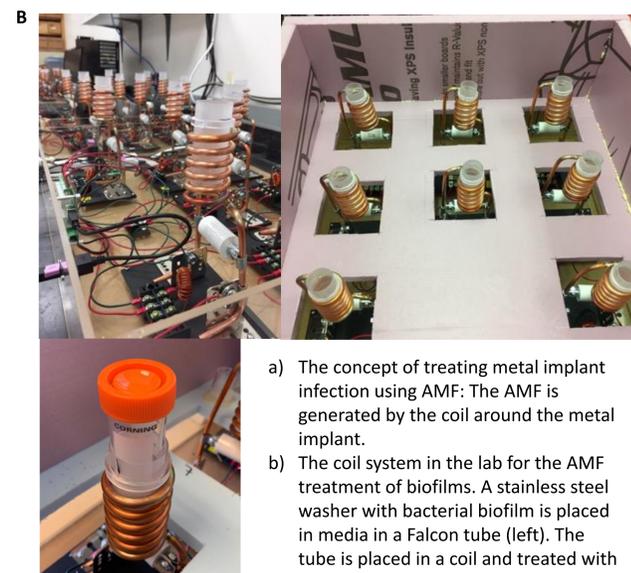
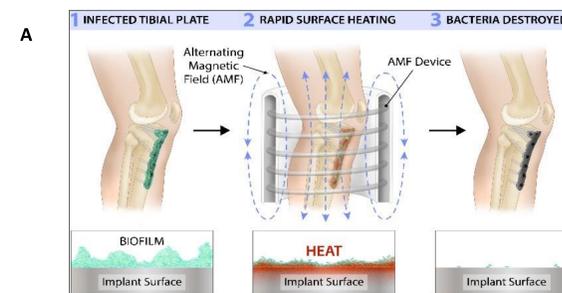
To address this concern, our laboratory is developing a non-invasive thermal method of biofilm eradication using high-frequency alternating magnetic fields (AMF) to destroy biofilm via magnetic induction. While heating alone eradicates biofilm to a certain extent, the most potent form of treatment is achieved in concert with antibiotic therapy. Previously, our group has demonstrated both *in vitro* and *in vivo* how AMF-antibiotic combination therapy can eradicate *P. aeruginosa* biofilm^[3,4]. Here we demonstrate that similar synergistic effects are achieved by AMF and antibiotic treatment of *S. aureus* biofilms on stainless steel washers.

Bibliography

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Treatment of Infection Using Alternating Magnetic Fields

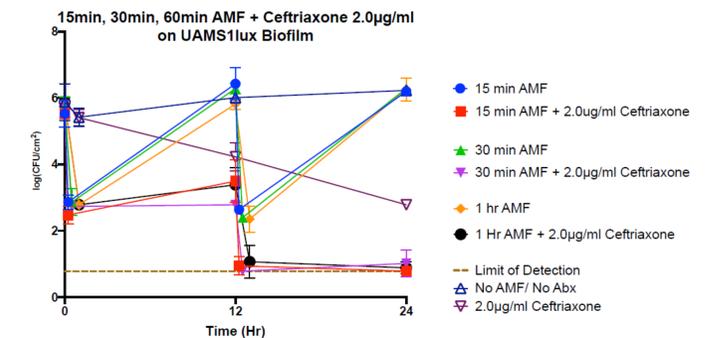
AMF treatment is based on the principle of using magnetic induction to heat the metal prostheses. Heating is localized to the metal surface (the "skin effect"), minimizing surrounding tissue damage.



Methods

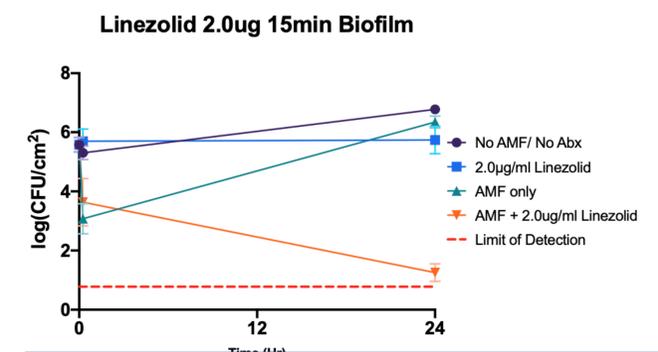
- The experiment utilized methicillin-sensitive *S. aureus* strain UAMS-1 lux
 - Stainless steel washers were used to model the prosthetic joint surface
 - Ceftriaxone and Linezolid were used at a concentration of 2 µg/ml
1. To develop a mature biofilm, grow *S. aureus* biofilms on metal washers in a shaking incubator for 24 h in tryptic soy broth. Remove washer to fresh medium and incubate another 24 h.
 2. In the induction coil apparatus (above), test the following parameters: untreated, AMF only, antibiotics only, and AMF + antibiotics.
 3. Every 5 min for 15, 30, and 60 minutes, pulse AMF exposure for 3 s at a target temperature of 65°C.
 4. Sonicate the washers and determine biofilm viability by colony forming unit (cfu) count

Results



A synergistic effect between AMF and ceftriaxone was observed across all treatment durations (15, 30, 60 min).

Figure 1. Combination treatment with AMF and ceftriaxone shows synergy in biofilm reduction. Experiments were assayed independently three times with two replicates for each condition. Error bars represent standard deviation from the mean.



A synergistic effect between AMF and linezolid was observed at 12 and 24 h.

Figure 2. Combination treatment with AMF and linezolid shows synergy in biofilm reduction. Experiments were assayed independently three times with two replicates for each condition. Error bars represent standard deviation from the mean.

Conclusions

- A synergistic effect of AMF and antibiotic treatment was observed for eradication of *S. aureus* biofilm.
- This study provides a strong basis for future *in vitro* and *in vivo* work using AMF-antibiotic combination therapy
- As a non-invasive treatment, AMF could have a significant impact in improving patient quality of life as well as improving healthcare costs associated with PJI.

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