Exploring a Novel, Non-Invasive Treatment for Prosthetic Joint Infection

Ajay Narayanan[1], Qi Wang[2], Christine Pybus[1], Sumbul Shaikh[2], Imalka Munaweera[2], Carolyn Sturge[1], Rajiv Chopra[2], David Greenberg[1]

Department of Internal Medicine[1] and Department of Radiology[2], UT Southwestern Medical Center

UTSouthwestern Medical Center

ajay.narayanan@utsouthwestern.edu
Phone: (254) 931-9555

Abstract

Periprosthetic joint infection (PJI) is a very prevalent consequence of implant surgery. The surface of the prosthesis provides a favorable environment for the growth of bacterial biofilms, which are notorious for being resistant to conventional antibiotics. The current treatment for PJI involves re-opening the surgical site and replacing the prosthesis, a very costly procedure that diminishes patient quality of life. Recently, a non-invasive procedure has been developed that utilizes high frequency alternating magnetic fields (AMF) to destroy biofilms via induction heating. Our research was focused on both optimizing and further characterizing the cytotoxicity of this treatment method on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two biofilm-forming pathogens commonly implicated in PJI.

The organisms used for these experiments were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biofilms were grown on stainless steel rings or washers, to model the surface of implanted prosthetics. *P. aeruginosa* was grown statically in MH2 media at 37°C for 48 hours. *S. aureus* was grown statically in Tryptic Soy Broth media supplemented with 0.5% glucose and 3.0% NaCl at 37°C for 48 hours. Soaking the metal ring/washer in a 20% Human Plasma solution overnight at 4°C greatly enhanced *S. aureus* biofilm formation. AMF continuous dosing was performed at 20 watts, up to 15 minutes. AMF intermittent dosing was performed using 1 second duration, 670 watt pulses every 10 minutes, up to 6 hours.

The results indicated that *S. aureus* biofilms were eradicated more effectively than *P. aeruginosa* biofilms when treated with intermittent AMF exposure. Specifically, there was a 2.6-log reduction in *S. aureus* biofilm CFU after 30 minutes of AMF exposure, with CFUs reaching the limit of detection after 3 hours. Corresponding studies in *P. aeruginosa* showed a 1.3-log reduction in biofilm CFU after 30 minutes of AMF exposure, with CFUs not reaching the limit of detection after 6 hours. In an ongoing study, ciprofloxacin was administered alongside AMF exposure to investigate any potential synergistic effects on *P. aeruginosa* biofilm eradication.

While the data produced this summer was exclusively in vitro, the results give insight on how AMF might be applied in the clinical treatment of PJI. The observed cytotoxicity combined with the non-invasive nature of AMF suggest significant promise for a much more desired method of PJI treatment for common pathogens.

Introduction

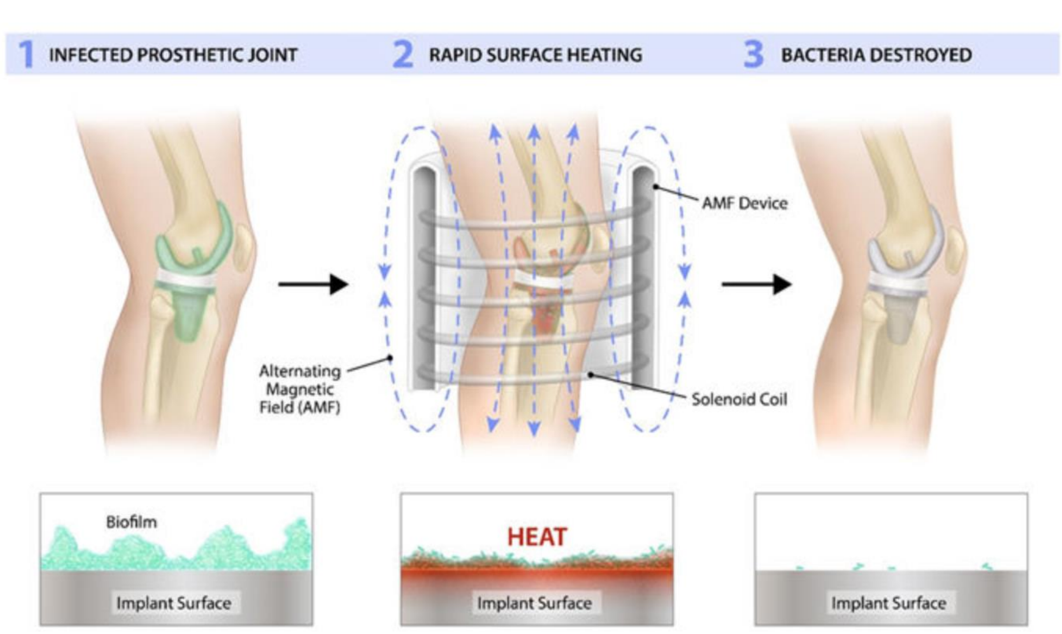

Figure 1. Targeting biofilms located on the surface of metallic implants with high-frequency alternating magnetic fields (AMF). (1) The presence of biofilm on an infected prosthetic usually necessitates surgical replacement as a curative treatment due to an increased resistance of these bacteria to antibiotics. (2) Rapid and non-invasive surface heating of a metallic implant can be achieved using a high-frequency alternating magnetic field (AMF). (3) Cytotoxic levels of heating can be generated by the AMF exposures as a means to destroy bacteria and remove extracellular polymeric substances (EPS) associated with the biofilm.

Methods

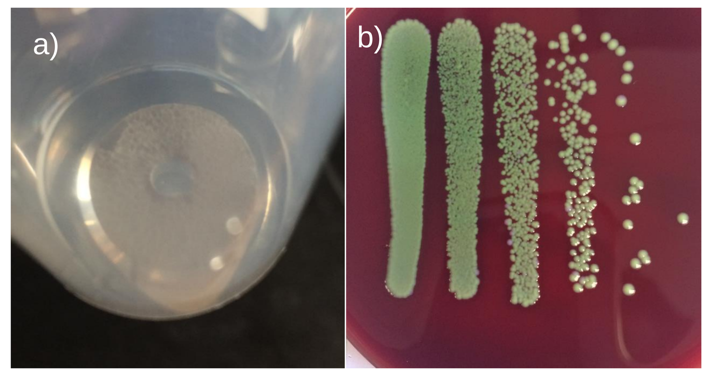

Figure 2. Method of biofilm growth and quantification. (**a**): Biofilms were grown on stainless steel washers/rings under various conditions, depending on the organism. (**b**): Following growth, biofilms were vortexed off the washer/ring into a 4 mL PBS solution and plated onto Sheep Blood Agar in order to obtain CFU counts.

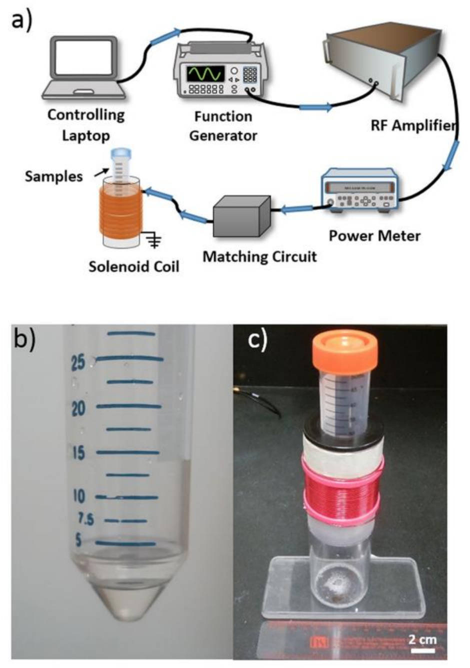

Figure 3. A custom-built solenoid was used to expose metal washers/rings with biofilm to an alternating magnetic field (AMF). (**a**) Under PC control, 20 W was delivered to a solenoid coil at a frequency of 500 kHz. (**b**) Stainless steel washers/rings with biofilm were placed at the bottom of a 50 ml centrifuge tube with 5 ml of PBS. (**c**) The centrifuge tubes were inserted into the center of the solenoid and exposed to an AMF for different durations.

Results

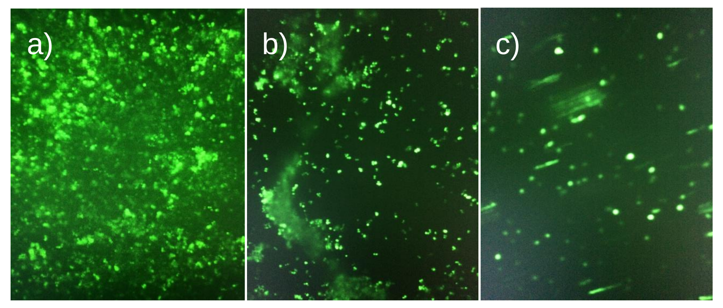

Figure 4. Confocal microscopy studies confirm that the optimal biofilm growth conditions for *S. aureus* is 37°C static incubation on a washer soaked overnight in a 20% Human Plasma solution. (**a, b, c**): Biofilms composed of *S. aureus* UAMS-1 (containing a GFP plasmid) were grown for 36 hours in 3 different environments. (**a**): Washer soaked overnight in a 20% Human Plasma solution + 37°C static incubation. (**b**): Washer soaked overnight in a 20% Human Plasma solution + 37°C shaking incubation. (**c**) Washer soaked overnight in a collagen solution + 37°C static incubation. Biofilms were fixed using a 5% glutaraldehyde solution and viewed using confocal microscopy techniques.

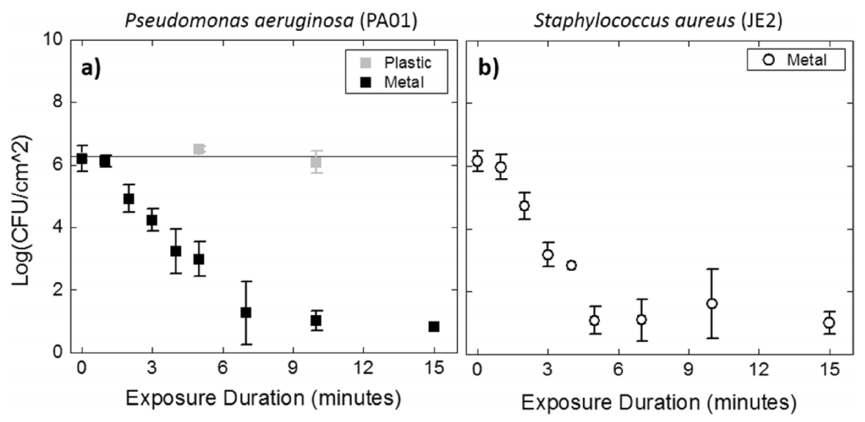

Figure 5. Continuous AMF exposures eradicate *P. aeruginosa* and *S. aureus* biofilm. (**a,b**): the bactericidal effect of a 20 W AMF as a function of exposure duration is shown for two organisms' biofilm grown on a metal washer. A significant reduction in bacterial number is observed for exposures greater than 1 minute in duration, with a greater than 3-log reduction in the number of bacteria between 5 and 7 minutes. The results for a control experiment that involved AMF exposures of a similarly sized plastic washer (gray squares in **a**) confirm the effect is due to direct heating of the metal washer.

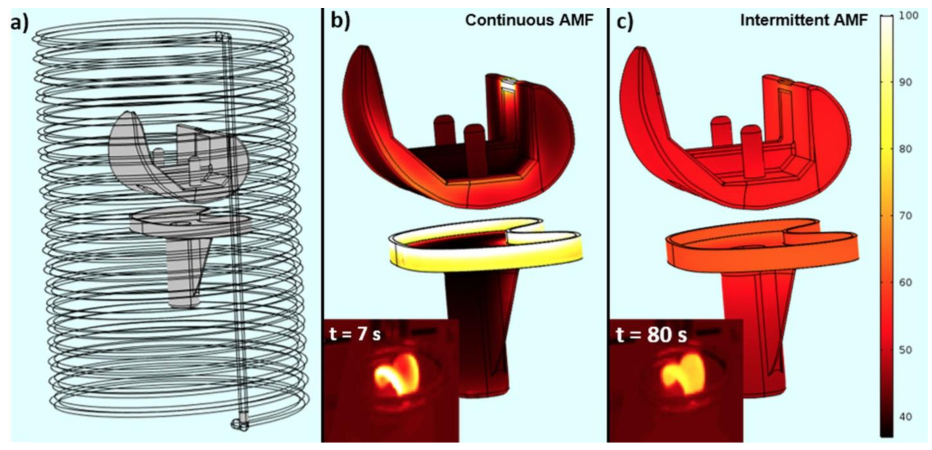

Figure 6. FEA modeling and experiments demonstrate that intermittent AMF exposure can achieve uniform heating across the surface of a prosthetic knee implant. (**a**) Using COMSOL®, a 3D representation of a prosthetic knee implant inside a solenoid coil can be defined. (**b**) The surface temperature distribution after 3 seconds of continuous 600W AMF exposure is very non-uniform (over 60 °C range across surface). (**c**) Using an intermittent AMF exposure (1500W for 1 second, every 50 seconds), a uniform surface temperature distribution is achieved after 1200 seconds (20 exposures).

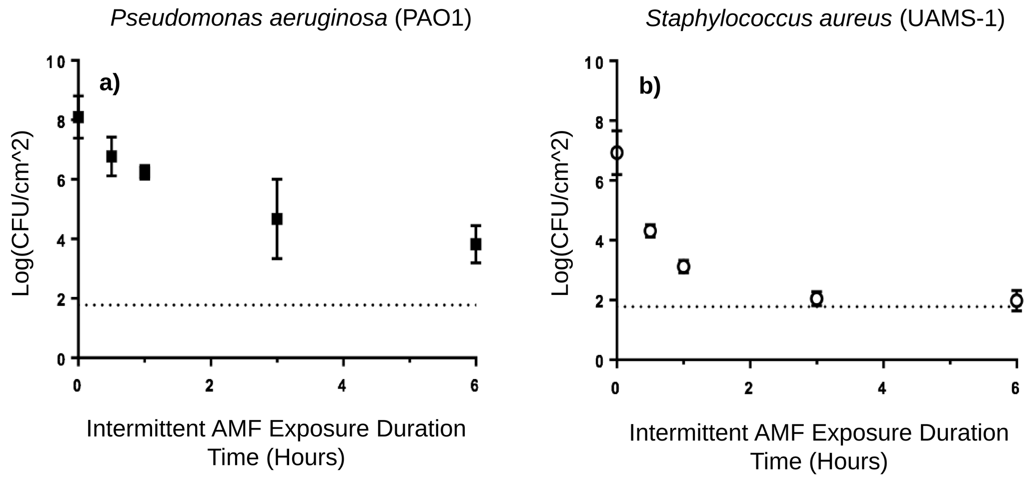

Figure 7. Intermittent AMF exposures eradicate *P. aeruginosa* and *S. aureus* biofilm with differing efficacies. The bactericidal effect of 1 second, 670 W pulses of AMF delivered every 10 minutes as a function of exposure duration is shown for two organisms' biofilm growth on a metal ring. **a)** A 1.3-log reduction in *P. aeruginosa* biofilm CFU was observed after 30 minutes of intermittent AMF exposure, with CFUs not reaching the limit of detection after 6 hours. **b)** A 2.6-log reduction in *S. aureus* biofilm CFU was observed after 30 minutes of intermittent AMF exposure, with CFUs reaching the limit of detection after 3 hours.

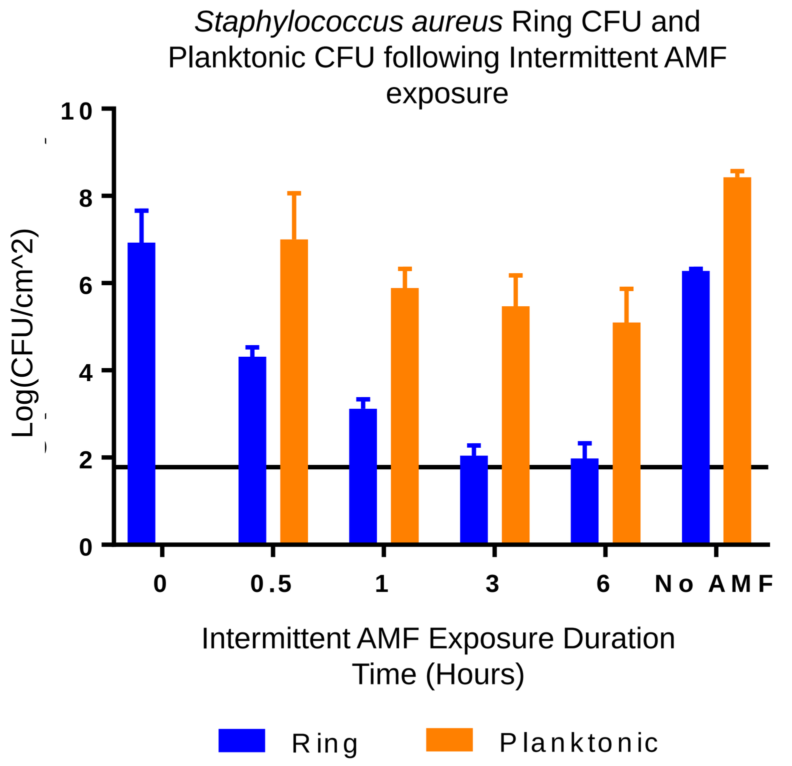

Figure 8. The increased eradication of *S. aureus* biofilm seen in intermittent AMF exposure (Figure 7) is not a result of "knocking off" the organism into planktonic culture. CFUs for the 6-hour intermittent AMF-treated planktonic culture were greater than 3-logs less than those of the 6-hour positive control. This indicates that *S. aureus* is most likely being eradicated by heat induction, as opposed to simply finding a more habitable, cooler, environment.

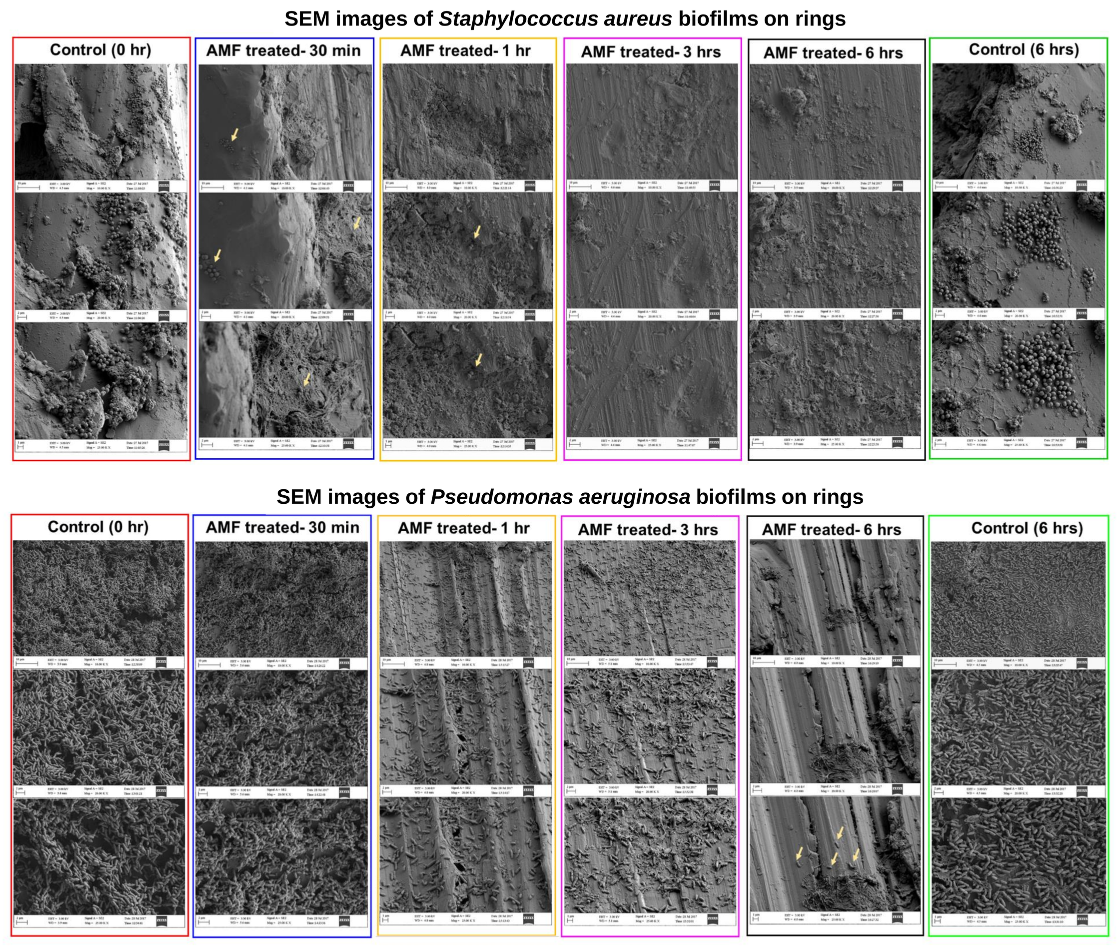

Figure 9. Scanning Electron Microscopy demonstrates the time-dependent bactericidal effect that intermittent AMF exposure has on *S. aureus* and *P. aeruginosa* biofilm. Two positive controls are shown – one at zero hours and one at six hours.

Conclusions

- The optimal growth conditions for *S. aureus* biofilm have been defined as 36-48 hours on a 20% Human Plasma coated stainless steel washer/ring in a static, 37°C environment.
- The optimal growth conditions for *P. aeruginosa* biofilm have been defined as 36-48 hours on an uncoated stainless steel washer/ring in a static, 37°C environment.
- Intermittent AMF exposure produces a more even heating distribution compared to continuous AMF exposure.
- Biofilms composed of *S. aureus* and *P. aeruginosa* are both susceptible to eradication by AMF exposure. When subject to intermittent exposure, *S. aureus* is likely eradicated more effectively than *P. aeruginosa*.

Future Studies

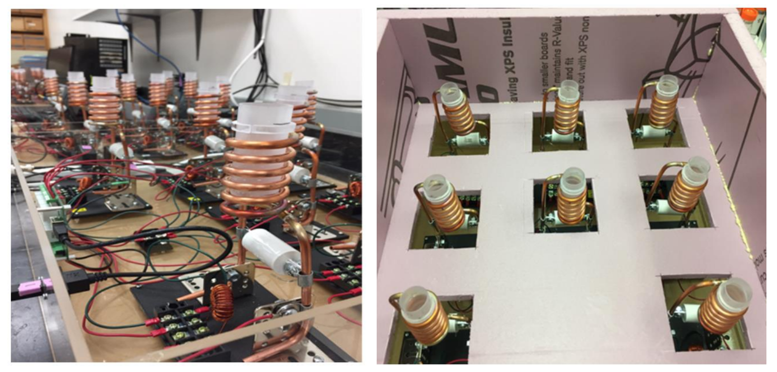

Figure 10. A 32-coil apparatus has recently been constructed for the purpose of high-throughput AMF experimentation.

Acknowledgements

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References

Chopra, R., et al. (2017). Employing high-frequency alternating magnetic fields for the non-invasive treatment of prosthetic joint infections. *Scientific Reports, 7*. doi:10.1038/s41598-017-07321-6