

Abstract

Prosthetic joint infections (PJIs) are serious complications of total joint arthroplasty, affecting approximately 1–2% of procedures, depending on the joint involved. Treatment is challenging because microbial biofilms on implant surfaces limit the effectiveness of antimicrobial therapy and contribute to persistent infection. Management often requires two-stage revision surgery and prolonged antimicrobial treatment, yet recurrence remains a concern. The associated morbidity, mortality, and repeated surgeries substantially reduce patients' quality of life. PJIs also impose a considerable financial burden, with annual healthcare costs exceeding \$1 billion in the United States. Fungi account for approximately 1–3% of PJIs, with *Candida albicans* reported as the causative pathogen in approximately 85% of fungal cases. These infections are particularly difficult to treat, highlighting the need for additional strategies that target implant-associated fungal biofilms. Alternating magnetic fields (AMF) induce heating of metallic surfaces and have been shown to reduce bacterial biofilms, but their effects on fungal biofilms remain unclear. We investigated the effects of AMF on *C. albicans* biofilms and evaluated whether combining AMF with antifungal therapy improves biofilm reduction.

C. albicans biofilms were grown on stainless steel rings to model fungal growth on metallic implant surfaces. Rings were incubated in Sabouraud medium at 37 °C with shaking, and the culture medium was replaced daily to support continued biofilm development. In the first series of experiments, biofilms were grown for 48 or 72 hours before receiving a single 120-second AMF exposure reaching target temperatures of 50, 60, 70, or 80 °C. This temperature series evaluated the relationship between AMF-induced heating and fungal survival within the biofilm. Rings were processed one hour after treatment, and adherent organisms were recovered by sonication in phosphate-buffered saline. Recovered suspensions were diluted and plated, and viable fungal burden was quantified using colony-forming unit counts. This approach measured surviving culturable organisms rather than relying solely on changes in total biofilm biomass. AMF produced a significant temperature-dependent reduction in viable biofilm burden, with the greatest reductions observed at temperatures above 70 °C.

We next evaluated AMF in combination with antifungal therapy using biofilms grown for seven days before treatment. This experiment extended the evaluation to more established biofilms and examined treatment over multiple days. Rings underwent three days of treatment under four conditions: untreated control, AMF alone at 65 °C, antifungal therapy alone, or combined AMF and antifungal therapy. The untreated group provided a reference for biofilm persistence, while the individual treatment groups allowed assessment of the added benefit of combination therapy. Viable biofilm burden was assessed through recovery and culture of adherent organisms. Combined AMF and antifungal treatment reduced biofilm burden more than either intervention alone, demonstrating that the addition of AMF improved the effects of antifungal therapy at a target temperature of 65 °C. These findings demonstrate that AMF reduces viable *C. albicans* biofilm burden on stainless steel surfaces and enhances the effects of antifungal therapy in established biofilms. The single-exposure experiments identified a temperature-dependent treatment response, while the multiday experiments demonstrated the benefit of combining AMF with antifungal therapy. Together, these results provide experimental support for further development of AMF as an adjunctive treatment targeting fungal biofilms in PJI.

Methods

Figure 1:

C. albicans biofilms are cultured on stainless steel rings for 48 hours on a 37 °C shaking incubator. Samples are treated under AMF conditions corresponding to temperatures of 50, 60, 70, and 80 °C, with a control. The remaining biofilm cultures are given fresh media and treated and harvested on subsequent days. Each experiment had biological and technical replicates. After treatment with AMF, the samples are sonicated in PBS, serially diluted, and plated on blood agar plates to calculate the biofilm burden in log CFU/mL.

A Biofilm Growth

C. albicans biofilms were grown on stainless steel rings inside 50 mL conical tubes. Cultures were incubated for 48 hours in a 37°C shaking incubator.

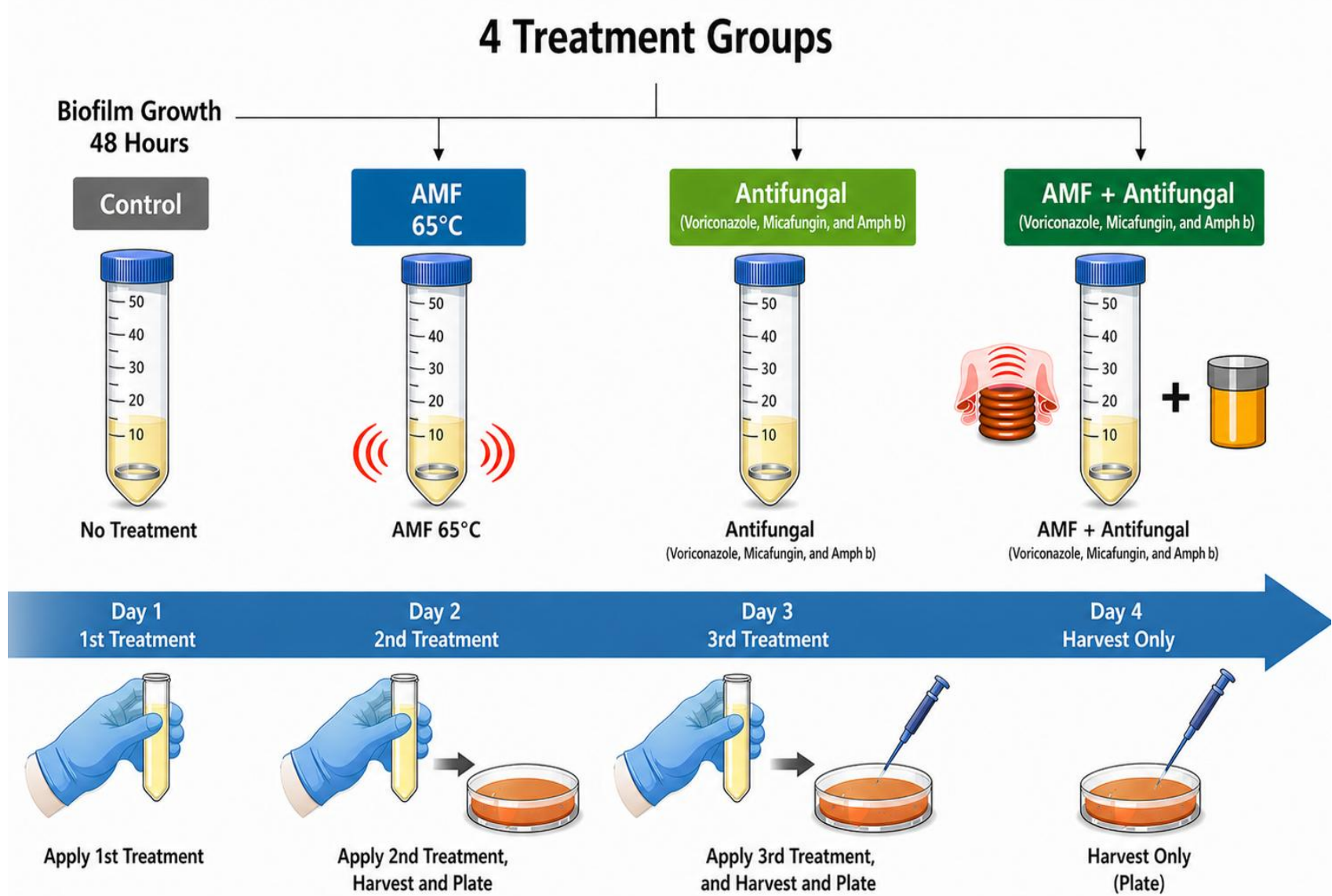


B AMF Temperature Treatment

After 48 hours of growth, a subset of the cultures were treated under AMF conditions corresponding to temperatures of 50, 60, 70, and 80°C. The diagram shows a sequence of tubes being treated at 50°C, 60°C, 70°C, and 80°C, followed by a control tube.

Figure 2:

C. albicans biofilms were grown for 7 days. Following this incubation period, samples were assigned to one of four treatment groups and treated daily for three consecutive days. The treatment groups included: (1) control, (2) alternating magnetic fields (AMF) alone at 65 °C, (3) Antifungal alone (Voriconazole, Amphotericin B, Micafungin), and (4) a combination of AMF with Antifungal. Each day, samples from each group were harvested to evaluate changes in biofilm burden over time. Harvested biofilms were sonicated in phosphate-buffered saline (PBS), serially diluted, and plated on blood agar plates for colony quantification.



Results

Figure 3:

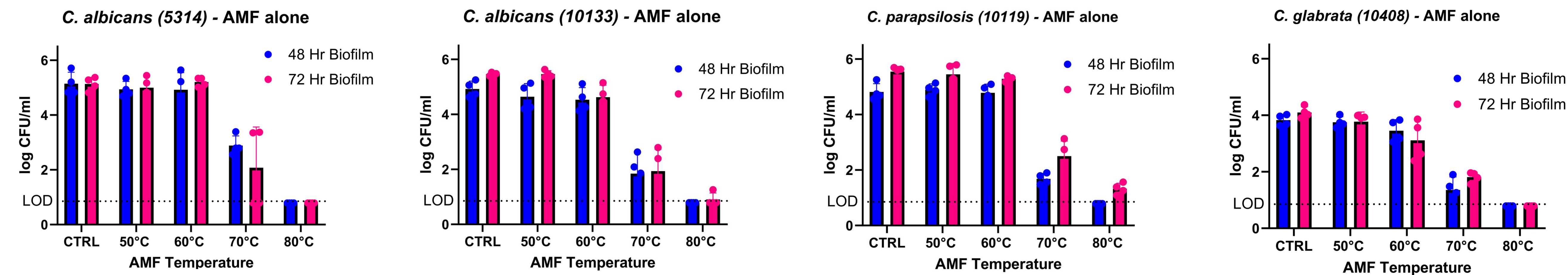


Figure 3. Measures of biofilm burden from *C. albicans*, *C. parapsilosis*, and *C. glabrata* strains grown for 48 hours and then treated solely with AMF for 120 seconds at various temperatures. The dashed line represents the limit of detection of the assay

Figure 4:

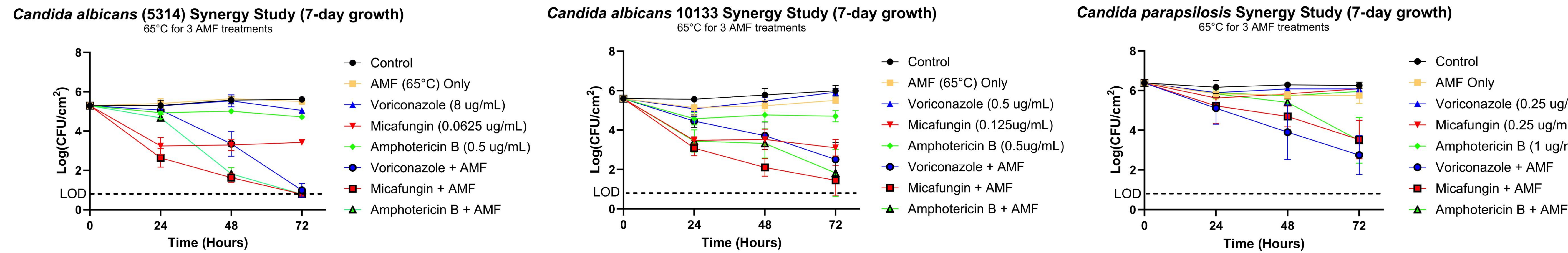


Figure 4. Measures of biofilm burden from *C. albicans*, *C. parapsilosis*, and *C. glabrata* strains. Strains were grown for 7 days and then subjected to various treatments (Control, AMF for 120 seconds, Voriconazole, Amphotericin B, Micafungin, and Combination treatments). Treatment was started at time 0 on the graph and consisted of daily treatment for three days

Figure 5:

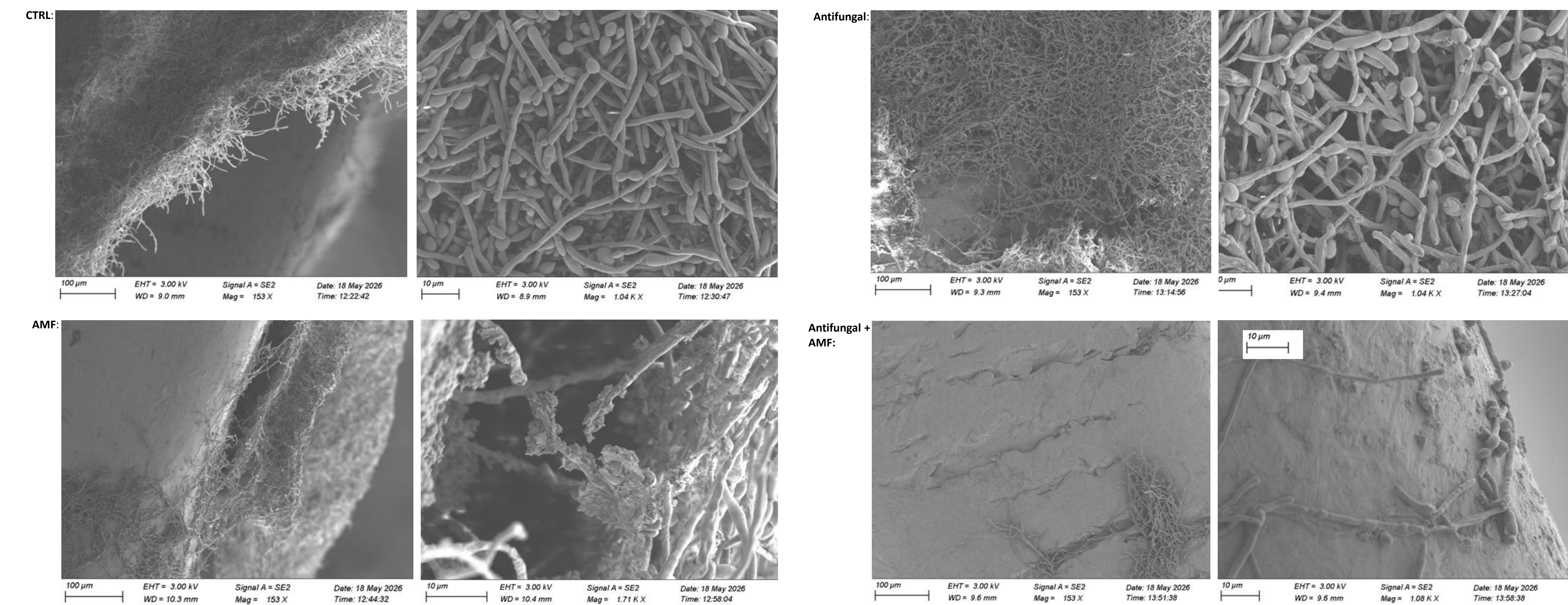
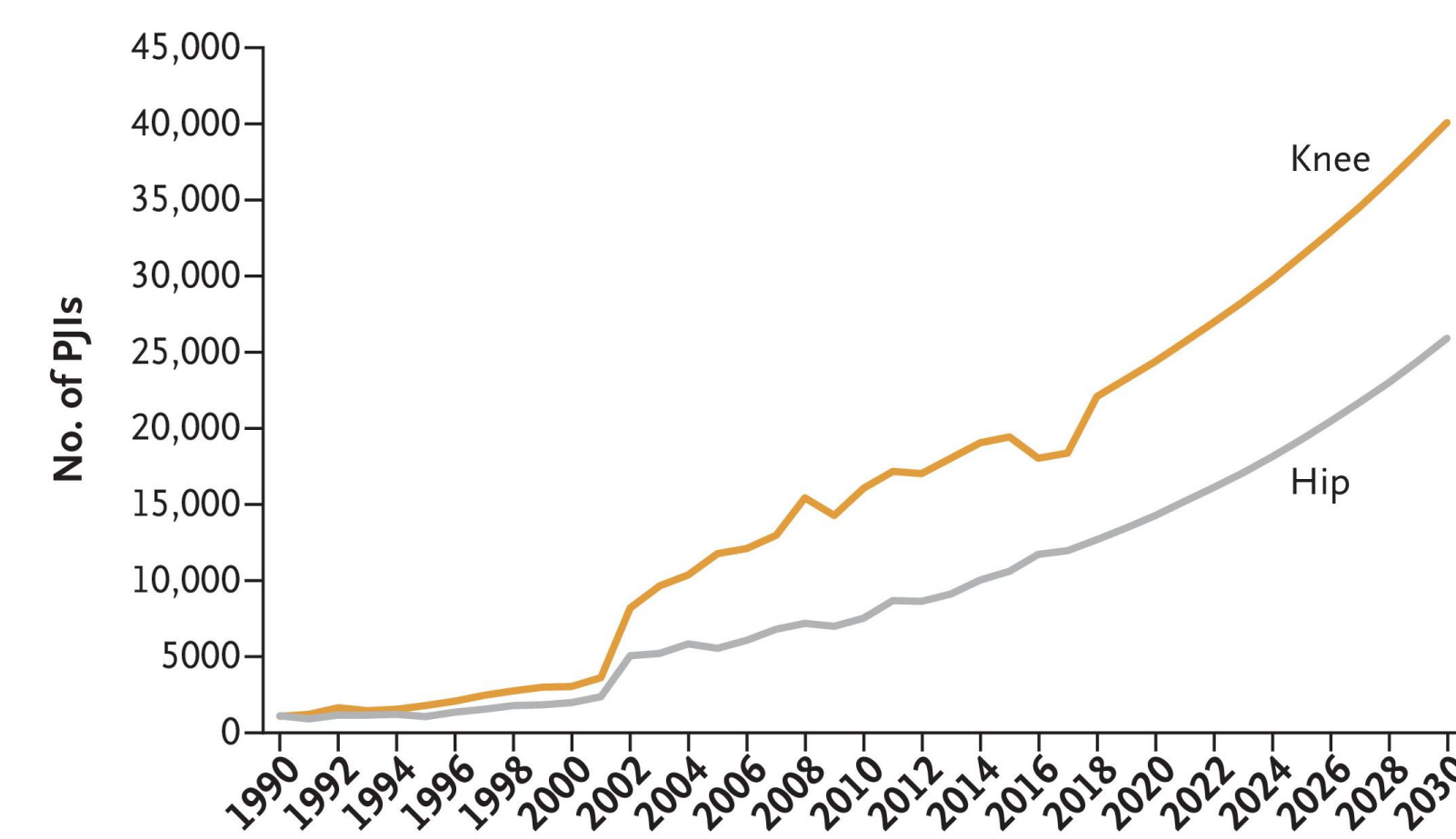
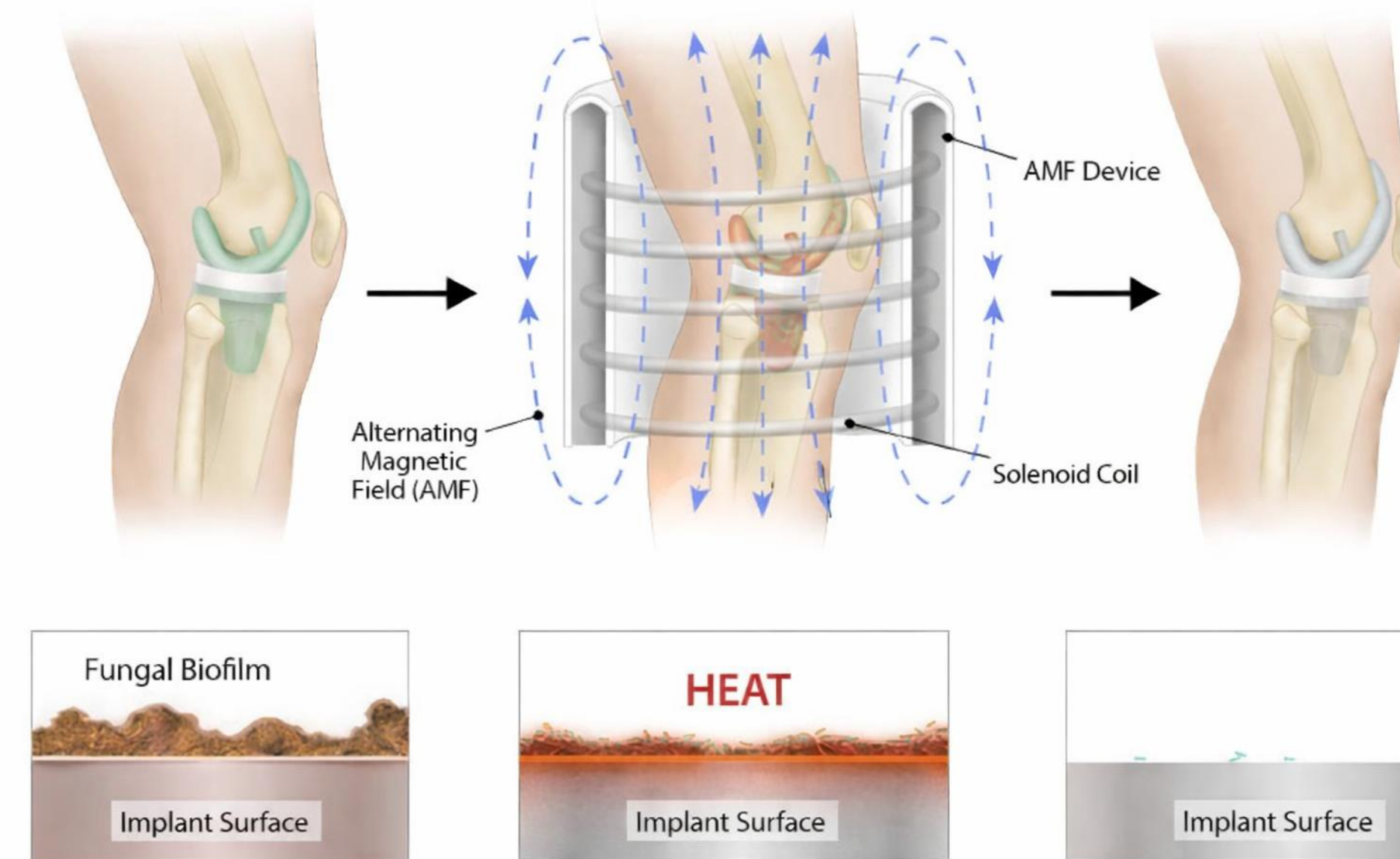


Figure 5. Scanning electron microscopy: *C. albicans* was grown for 7 days on metal rings and then subjected to one of four different treatments over a three-day period (Control, AMF for 120 seconds, Amphotericin B only, and Combination of AMF and Amphotericin B)

Importance and Clinical Relevance



1 INFECTED PROSTHETIC JOINT 2 RAPID SURFACE HEATING 3 BIOFILM DISRUPTED



Conclusions

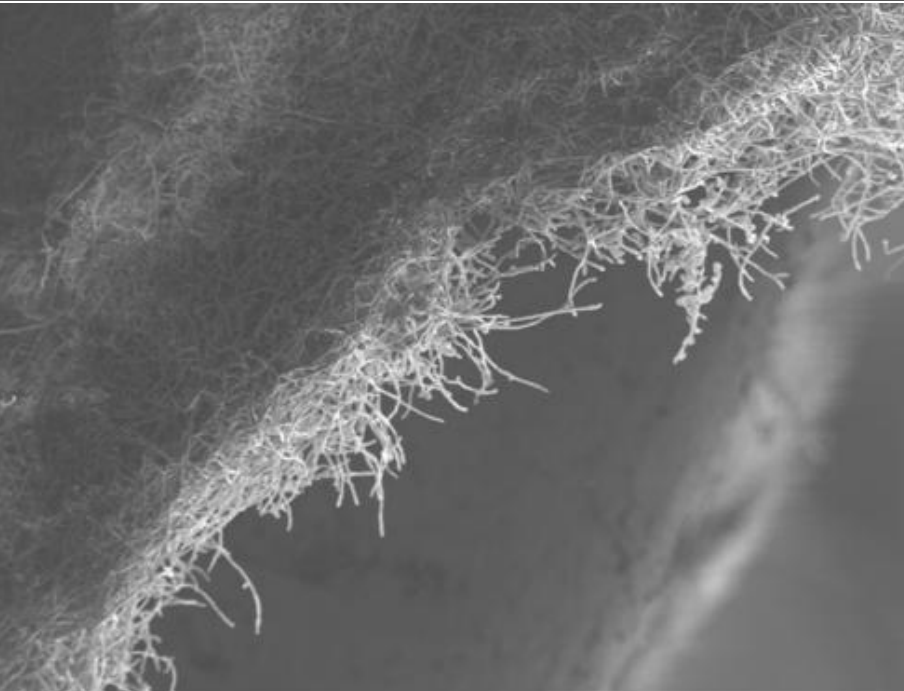
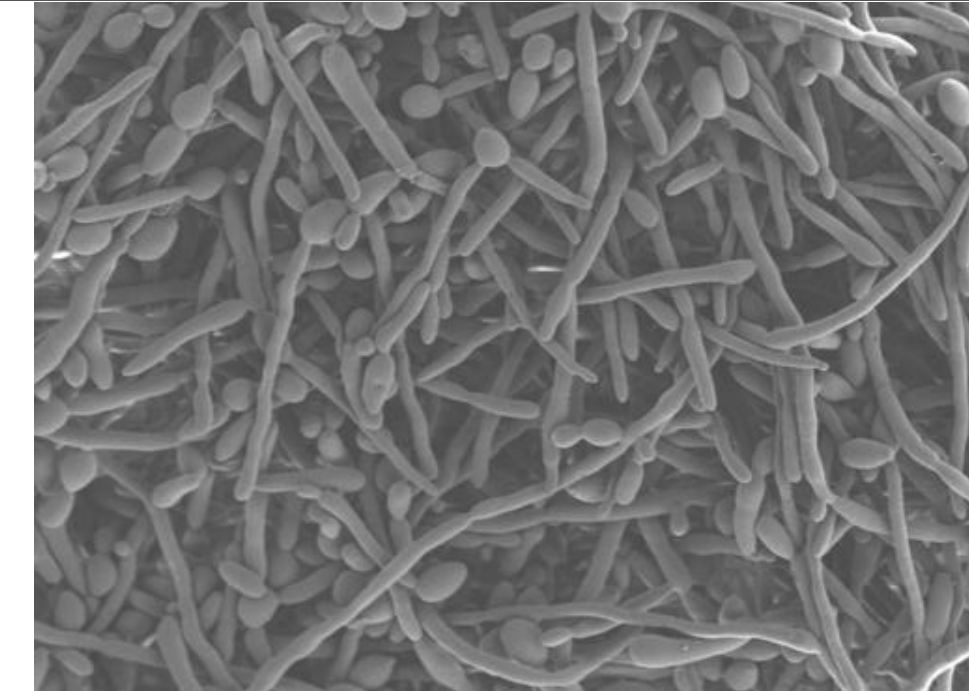
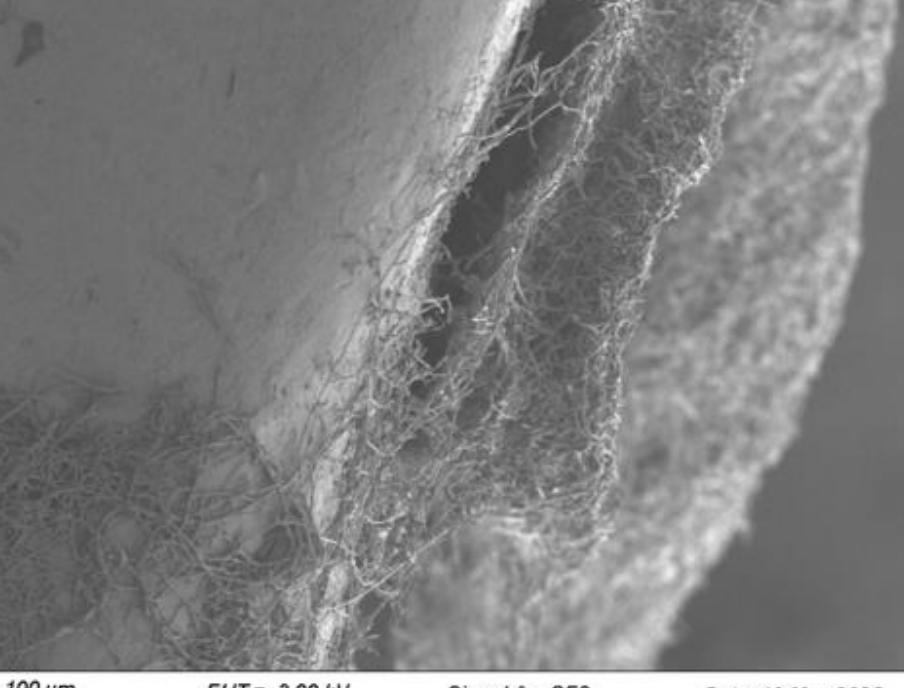
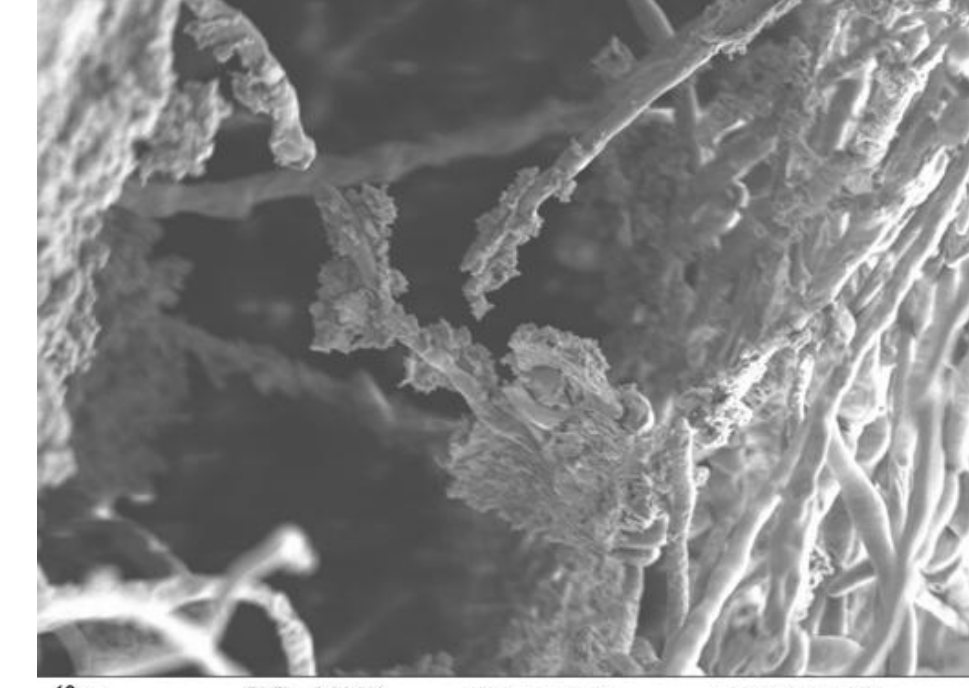
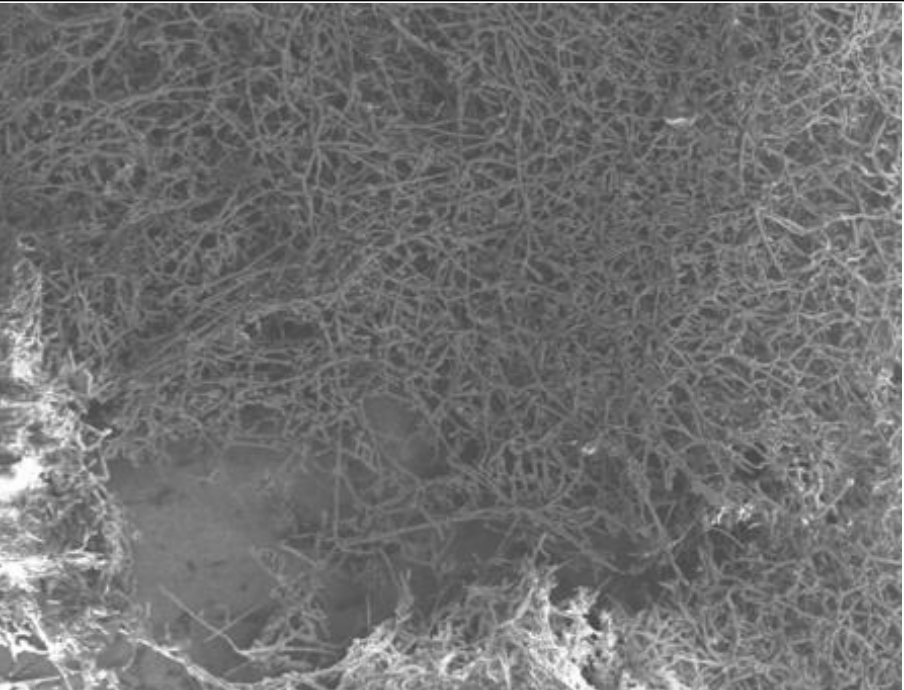
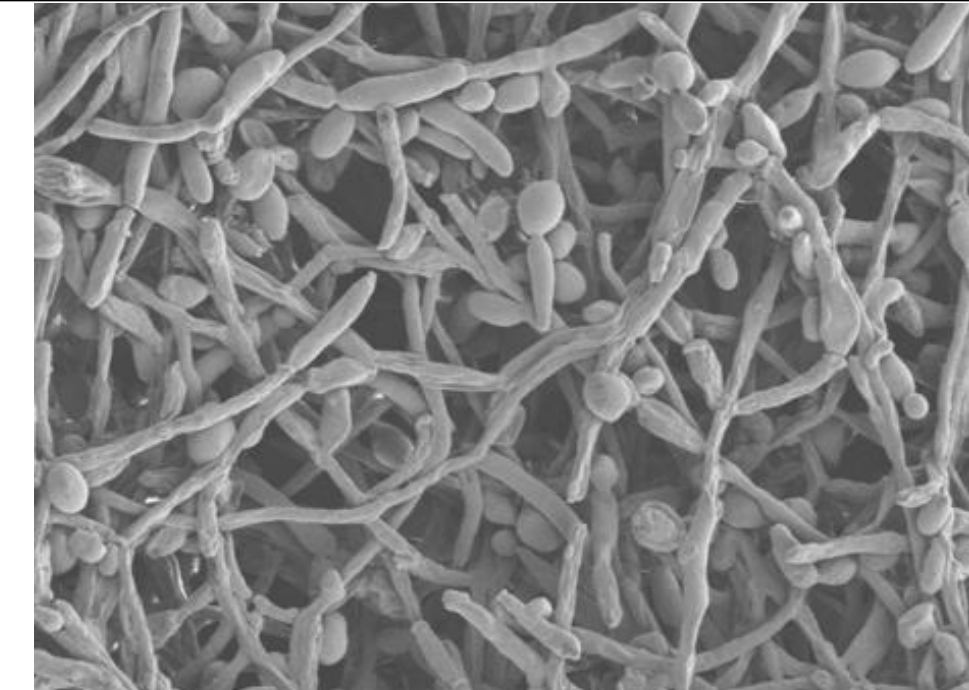
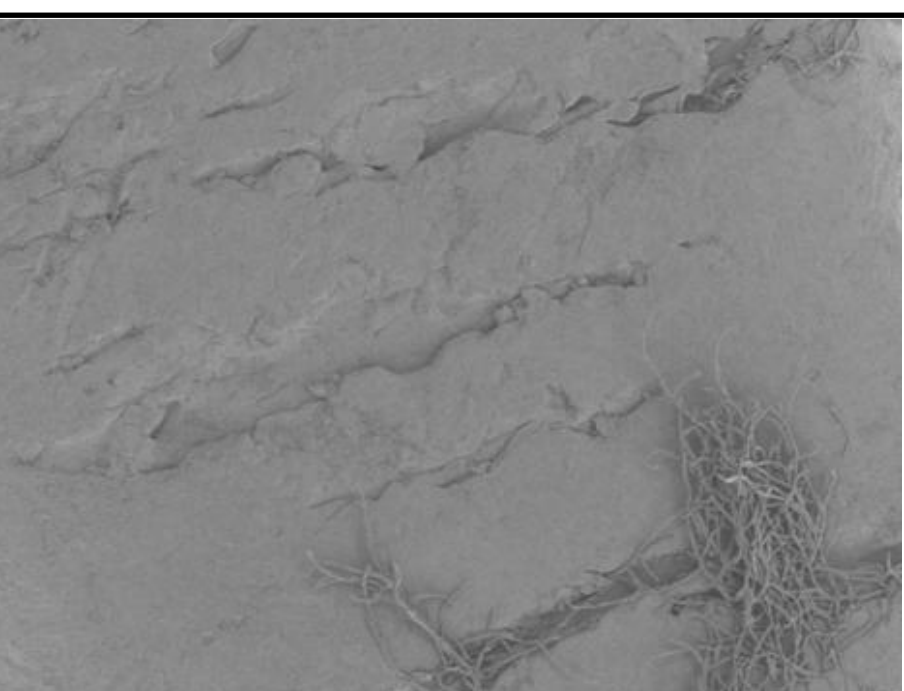
- These experiments demonstrate a temperature-dependent reduction of fungal biofilm after AMF treatment with a multi-log decrease seen between 60 and 70 degrees.
- Application of alternating magnetic fields (AMF) in combination with antifungals resulted in synergistic biofilm reduction, exceeding the effects observed with AMF alone, antifungal alone, or untreated controls.
- This potential synergy suggests that AMF may enhance antifungal efficacy, possibly by increasing biofilm permeability, inducing thermal stress, or altering fungal cell integrity.
- Collectively, these findings support AMF as a promising adjunctive strategy to conventional antifungal therapy for biofilm-associated infections, including prosthetic joint infection models.
- Future experiments will expand on these results by testing additional antifungal agents and evaluating *Candida auris* species to assess reproducibility, species-specific responses, and translational potential.

Acknowledgments

Funding was provided through a sponsored-research agreement from Solenic Medical. Dr. Greenberg is an inventor on multiple AMF-related patents and the CMO of Solenic Medical.

References

- Chopra R, Shaikh S, Chatzinoff Y, Munaweera I, Cheng B, Daly SM, Xi Y, Bing C, Burns D, Greenberg DE. Employing high-frequency alternating magnetic fields for the non-invasive treatment of prosthetic joint infections.
- Patel R. Periprosthetic Joint Infection. N Engl J Med. 2023 Jan 19;388(3):251-262. doi: 10.1056/NEJMr2203477. PMID: 36652356.
- Chat GPT for the creation of images for Experiments 1 and 2

<p>CTRL:</p> <p>Biofilms were grown for 7 days on a metal washer, then received no treatment for 3 straight days</p>	 <p>100 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 12:22:42 WD = 8.0 mm Mag = 153 X</p>	 <p>10 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 12:30:47 WD = 8.8 mm Mag = 1.04 K X</p>
<p>AMF:</p> <p>Biofilms were grown for 7 days on a metal washer, then received AMF treatment at 65 °C for 3 straight days</p>	 <p>100 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 12:44:32 WD = 10.3 mm Mag = 153 X</p>	 <p>10 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 12:58:04 WD = 10.4 mm Mag = 1.71 K X</p>
<p>Antifungal:</p> <p>Biofilms were grown for 7 days on a metal washer, then received Amphotericin B treatment for 3 straight days</p>	 <p>100 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 13:14:58 WD = 9.3 mm Mag = 153 X</p>	 <p>10 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 13:27:04 WD = 9.4 mm Mag = 1.04 K X</p>
<p>Antifungal + AMF:</p> <p>Biofilms were grown for 7 days on a metal washer, then received AMF treatment at 65 °C, plus Amphotericin B treatment for 3 straight days</p>	 <p>100 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 13:51:58 WD = 9.6 mm Mag = 153 X</p>	 <p>10 μm EHT = 3.00 kV Signal A = SE2 Date: 18 May 2026 Time: 13:58:38 WD = 9.6 mm Mag = 1.08 K X</p>