

# Probe-Based Ultrasonication Effectively Removes Biofilm from Clinically Relevant Orthopedic Materials: An In-vitro proof of concept

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## INTRODUCTION:

Periprosthetic joint infections (PJI) remain a major clinical challenge, with biofilm formation on implant surfaces contributing to treatment failure. Standard debridement techniques such as DAIR (debridement, antibiotics, and implant retention) often fail to eliminate biofilm. Ultrasonication has shown promise in other industries for disrupting biofilms, but its efficacy on orthopedic implant materials remains unproven. We hypothesized that submerged, probe-based ultrasonication could effectively disrupt biofilms formed on clinically relevant orthopedic materials.

## METHODS:

This in vitro study utilized methicillin-sensitive *Staphylococcus aureus* (Xen40) cultured on four orthopedic materials: bone cement (BC), polyethylene (PE), rough metal (Grit-blasted titanium) (RM), and smooth metal (Polishes cobalt-chrome) (SM). After 72-hour incubation, discs underwent one of three ultrasonication protocols (pulse, 20-sec continuous, 60-sec continuous) using a submerged piezoelectric probe. Biofilm presence was quantified by crystal violet staining, optical density of sonication fluid, and colony-forming units (CFUs). Scanning electron microscopy (SEM) was used to visualize structural biofilm changes. Data were analyzed using 2-way ANOVA with Tukey's post-hoc testing.

## RESULTS:

All ultrasonication protocols significantly reduced biofilm on all materials to levels statistically indistinguishable from negative controls ( $p < 0.001$ ). There was no significant difference in efficacy among the three protocols ( $p > 0.92$ ). Optical density and CFU measurements confirmed robust bacterial detachment, and Cohen's D effect size analysis (minimum  $D = 2.46$ ) demonstrated large treatment effects. SEM imaging revealed that biofilm matrices were eliminated post-treatment, with only sparse, non-matrix-associated bacteria remaining—likely planktonic cells susceptible to antibiotics or irrigation.

## DISCUSSION AND CONCLUSION:

Submerged probe-based ultrasonication effectively disrupted mature *S. aureus* biofilms from multiple orthopedic materials in vitro. The data strongly support its potential as an adjunctive technique for managing acute and chronic PJI. Future in vivo studies are warranted to assess its translational applicability in surgical settings.

