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INTRODUCTION

- Biofilm formation on implant surfaces is a major clinical challenge in periprosthetic joint infections
- Ultrasonication has shown promise for disrupting biofilms, but efficacy on orthopaedic implant materials requires investigation
- Presented here:
 1. Quantification of ultrasonication removal of biofilm grown *in vitro* on orthopaedic materials
 2. Qualitative observations of *ex vivo* biofilm removal using a novel mammalian model for staphylococcal knee PJI

METHODS

The *in vitro* study:

- *Staphylococcus aureus* cultured on four orthopaedic materials: bone cement (BC), polyethylene (PE), rough metal (RM, titanium alloy), and smooth metal (SM, polished cobalt chromium) (Fig. 1A-H)
- Three ultrasonication protocols (pulse, 20-sec continuous, 60-sec continuous) with a submerged piezoelectric probe (Fig. 1I)
- Biofilm presence/removal quantified by crystal violet absorbance assay
- Colony forming units (CFU) remaining quantified by water bath sonication in culture media, serial dilution, agar plate culture, and colony counting
- Statistics: Two-way ANOVAs with Tukey's multiple comparisons and 95% confidence intervals were performed in GraphPad Prism 11.0.1.
- Scanning electron microscopy (SEM) visualized biofilm changes

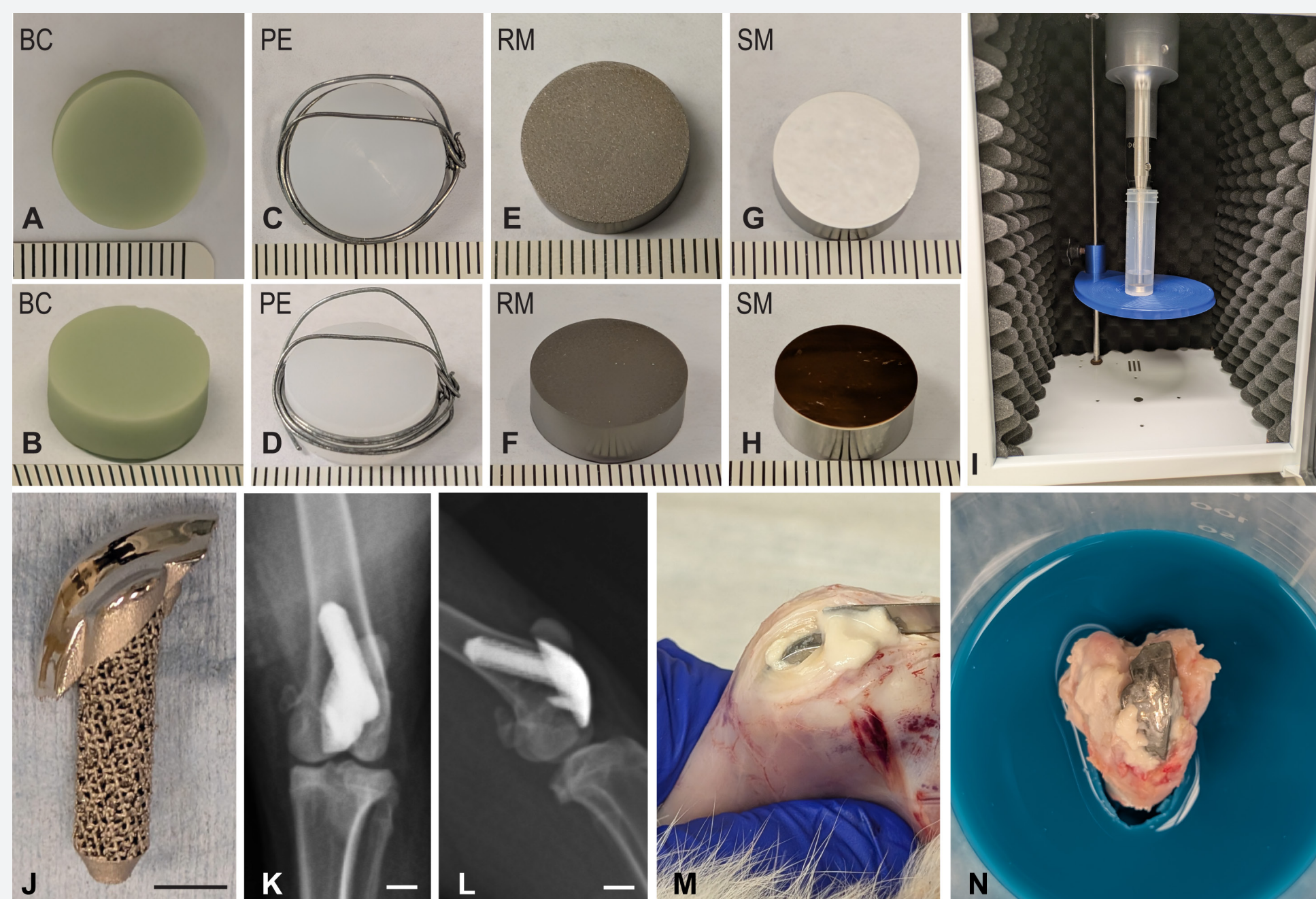


Figure 1. Materials and methods

The *in vivo* study:

- Novel rabbit model for knee PJI with a cementless 3D-printed porous titanium femoral unicondylar arthroplasty (Fig. 1J-L) [PMID: 42393525]
- Intraarticular inoculation 2 weeks post-implant surgery with 2×10^5 CFU *S. aureus*
- Biofilm developed *in vivo* for 2 weeks, distal end of the femur containing the implant removed (Fig. 1M) and placed in a sterile silicone mold (Fig. 1N), sterile PBS added, submerged piezoelectric probe ultrasonication performed, and SEM acquired
 - Negative control: no inoculation, no sonication
 - Positive control: inoculation, no sonication
 - Ex vivo biofilm removal: inoculation, 60 sec sonication

RESULTS

Quantitative *in vitro* assays

- Ultrasonication reduced biofilm matrix on all materials to negative control levels ($p < 0.0001$, minimum Cohen's D effect size=3.1) with no difference between sonication protocols (Fig. 2A)
- Ultrasonication reduced viable CFU on all materials ($p < 0.0001$, minimum Cohen's D effect size=2.29) with no difference between sonication protocols (Fig. 2B)
- Ultrasonication decreased the bacterial burden on BC discs by 2 orders of magnitude and on the PE, RM, and SM discs by 3 orders of magnitude (Fig. 2B)

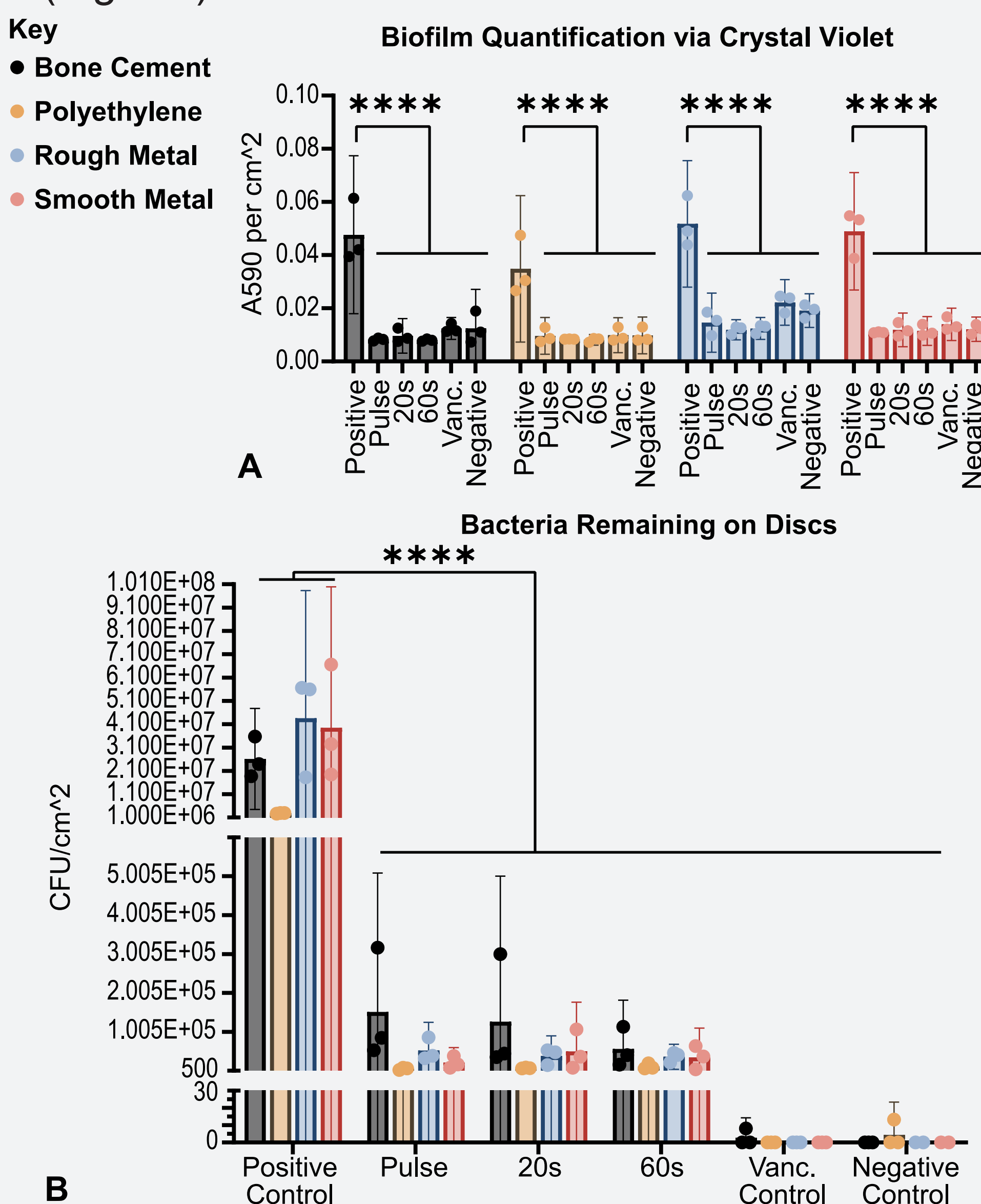


Figure 2. Quantitative *in vitro* biofilm removal via ultrasonication

Qualitative *in vitro* assay - SEM imaging

- Robust biofilm observed on all materials without sonication, with abundant bacteria and biofilm extracellular matrix (ECM) (Fig. 3)
- Biofilm matrices were eliminated on all materials post-treatment, with only sparse bacteria (black arrows) and ECM debris (white arrowheads) remaining (Fig. 3)

Qualitative *ex vivo* assay - SEM imaging

- Uninoculated negative control exhibited host cell adhesion (Fig. 4A-B)
- Untreated biofilm positive control showed robust purulent material and the presence of bacteria (black arrows) (Fig. 4C-D)
- Ultrasonication demonstrated effective removal of biofilm and purulent material (Fig. 4E-F)
- The ultrasonication probe was stationary, with differences in effect observed based on distance from the probe
 - Center of ultrasonic field: full biofilm and purulent material removal (Fig. 4G)
 - Further from probe: a layer of ECM (Fig. 4H-I)
 - ~8 mm off-center: dense purulent material (Fig. 4J)

RESULTS

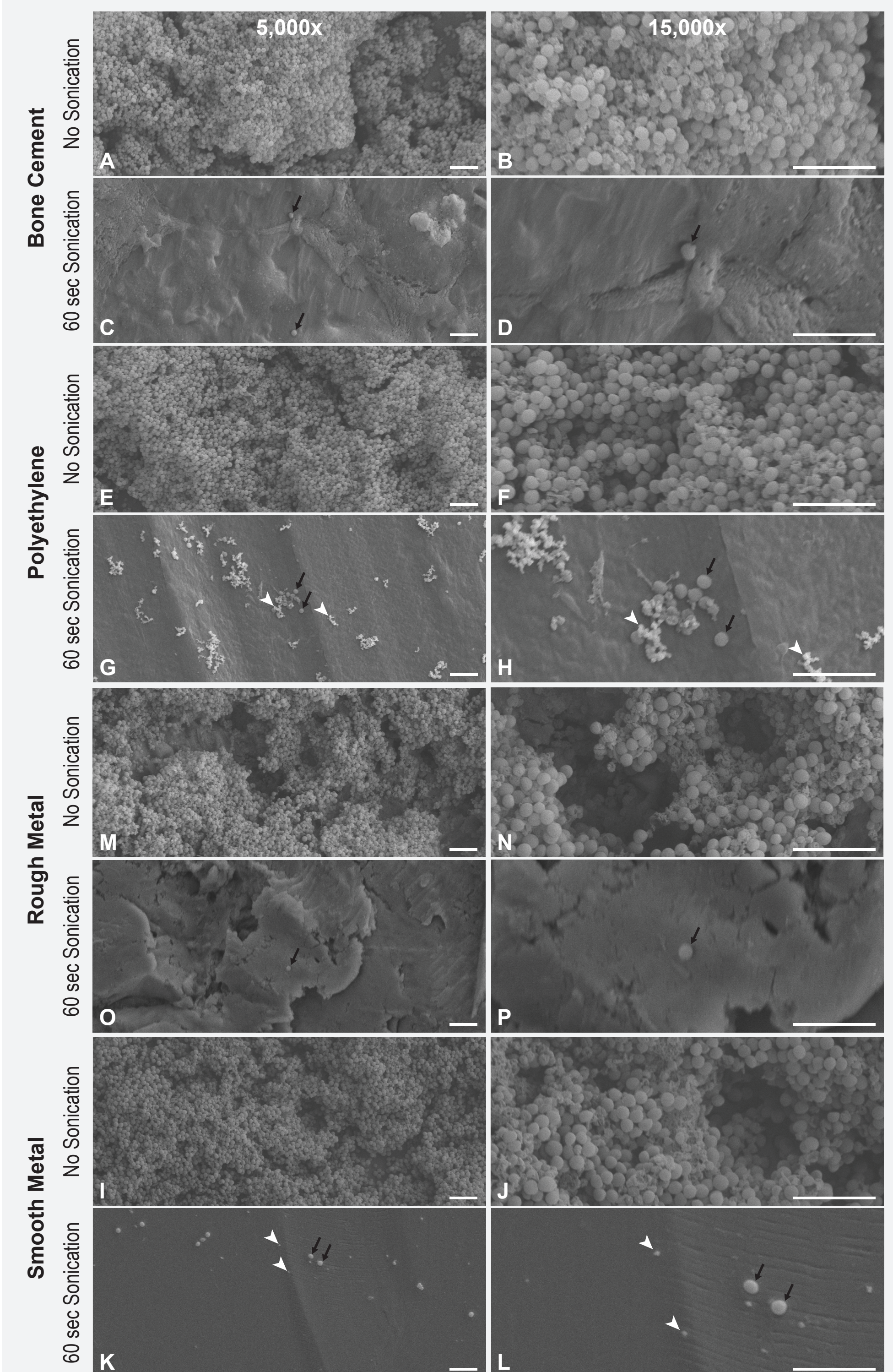


Figure 3. Scanning electron microscopy of orthopaedic material discs with biofilm and post-sonication

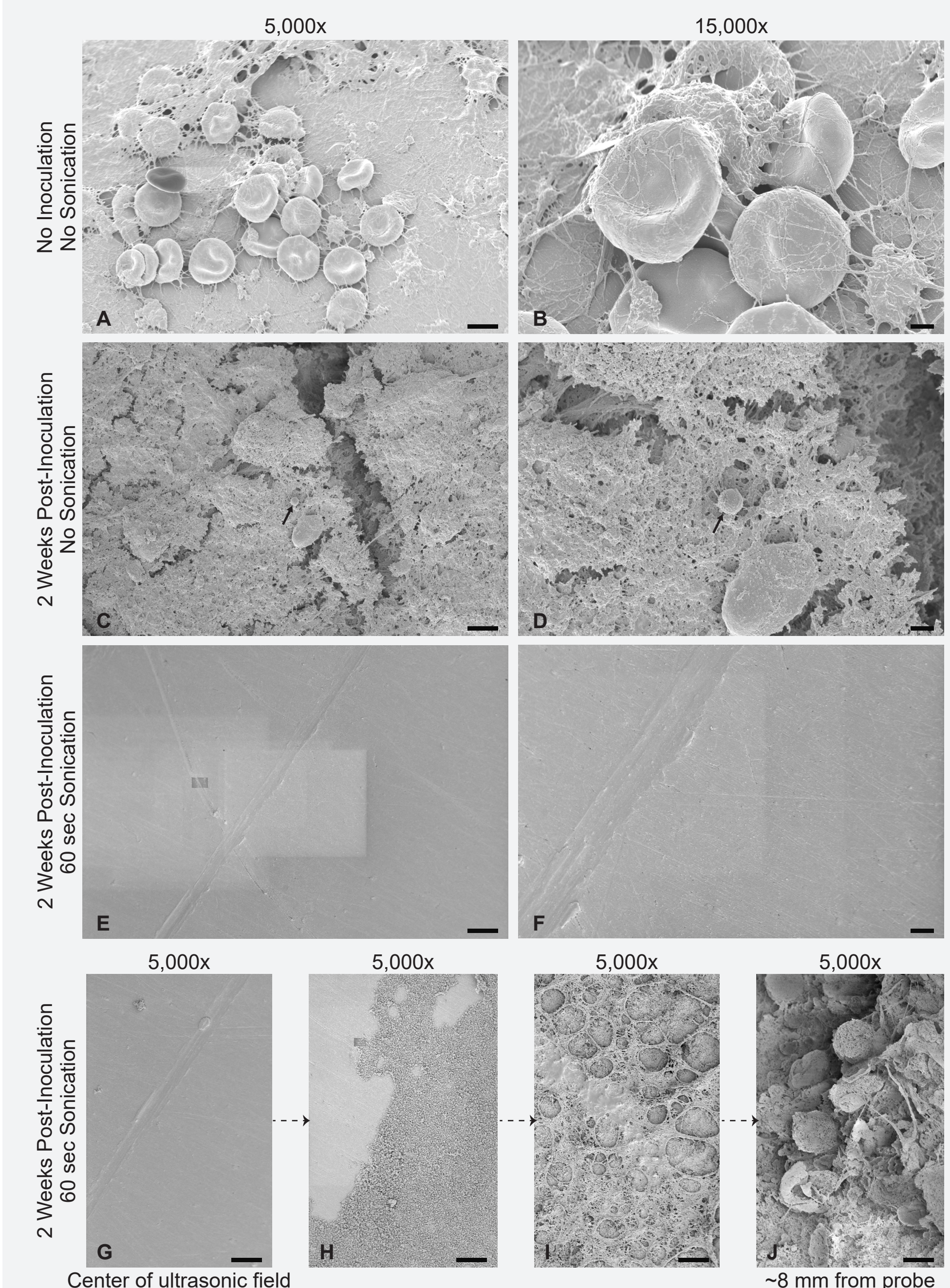


Figure 4. Scanning electron microscopy of PJI model implant with and without sonication

CONCLUSIONS

Submerged probe ultrasonication effectively disrupted *S. aureus* biofilms *in vitro* and *ex vivo*.

ACKNOWLEDGEMENTS

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