

Antibacterial Efficacy of Antibiotic Loaded Calcium Sulfate Beads Against *Staphylococcus aureus* Biofilms on Different Orthopaedic Implant Surfaces

Tripti Thapa Gupta¹, Phillip A. Laycock², Sean S. Aiken², Daniel Wozniak¹

1. Department of Microbial Infection and Immunity, The Ohio State University, Columbus, Ohio, USA
2. Biocomposites Ltd., Keele Science Park, Keele, Staffordshire, UK

Introduction

Bacterial biofilm formation on implant surfaces has been a major cause of orthopedic infections. Once biofilm has developed on the surface, it is challenging to eradicate with the conventional clinical microbiological methods, and the lack of definitive diagnosis may result in an underestimation of infection rates [1]. Because these underlying infections are difficult to diagnose and treat, debridement and prosthesis removal are the only viable treatment options [1].

To address these limitations, local antibiotic delivery using calcium sulfate beads has emerged as a promising approach. This approach was developed to achieve high, sustained antibiotic concentrations directly at the site of infection. Antibiotic loaded calcium sulfate beads represent a biodegradable local antibiotic delivery strategy that enables sustained antibiotic release and does not require surgical removal after implantation [1,2].

This study therefore aims to evaluate the effectiveness of antibiotic loaded calcium sulfate beads in decontaminating biofilm formed on clinically relevant orthopedic implant surfaces. Antibiotic efficacy was assessed through viable plate count on coupons representative of three implant materials, and confocal microscopy to compare treated versus untreated biofilms.

Materials & Methods

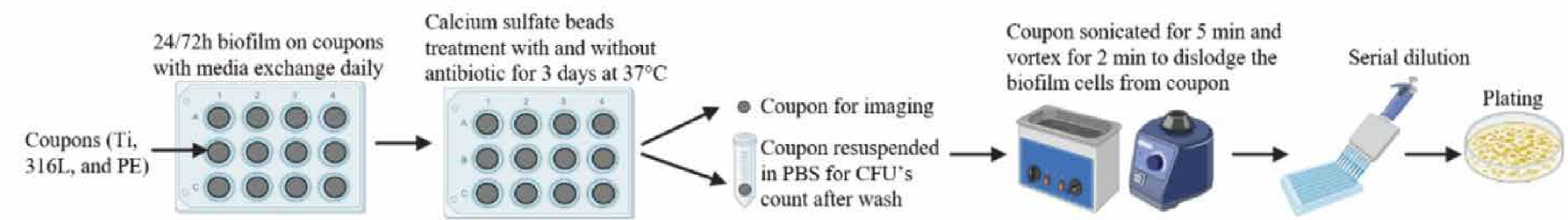


Fig 1. Schematic showing the experimental method used in this study

- Green Fluorescent Protein (GFP) expressing strain of *Staphylococcus aureus* (AH1726) was diluted to 10⁶ CFU/mL for biofilm formation for 1 and 3 days
- Titanium (Ti), stainless steel (316L), and polyethylene (PE) coupons were placed in 12 well plates and inoculated with bacterial culture
- The culture media made in 10% synovial fluid (SF) was exchanged every 24 hours for 3 days to allow biofilm maturation
- Mature biofilms were then exposed to either 4 and 8 antibiotic loaded calcium sulfate beads. 10 cc STIMULAN® Rapid Cure (Biocomposites Ltd) mixing kit was used to prepare beads
- Two antibiotic combinations were tested: Vancomycin (1g)+Gentamicin (0.24g): V+G and Vancomycin (1g)+Tobramycin (0.24g): V+T. Coupons with mature biofilms were treated with V+G and V+T with 4 and 8 beads for 3 days at 37°C on a rocker
- Following treatment, surviving bacteria were dislodged from the coupons via 5 min of sonication followed by 2 min of vortexing and quantified using viable plate counts
- Log reduction values were calculated, and statistical significance was determined using an unpaired two-tailed t-test assuming equal variance and a threshold of a P value of <0.05
- Representative images of GFP *S. aureus* biofilms were obtained by confocal microscopy to qualitatively compare treated and untreated biofilms

Results

Complete eradication of 1-day *S. aureus* biofilms by vancomycin+gentamicin (V+G) and vancomycin+tobramycin (V+T) with 4 and 8 beads

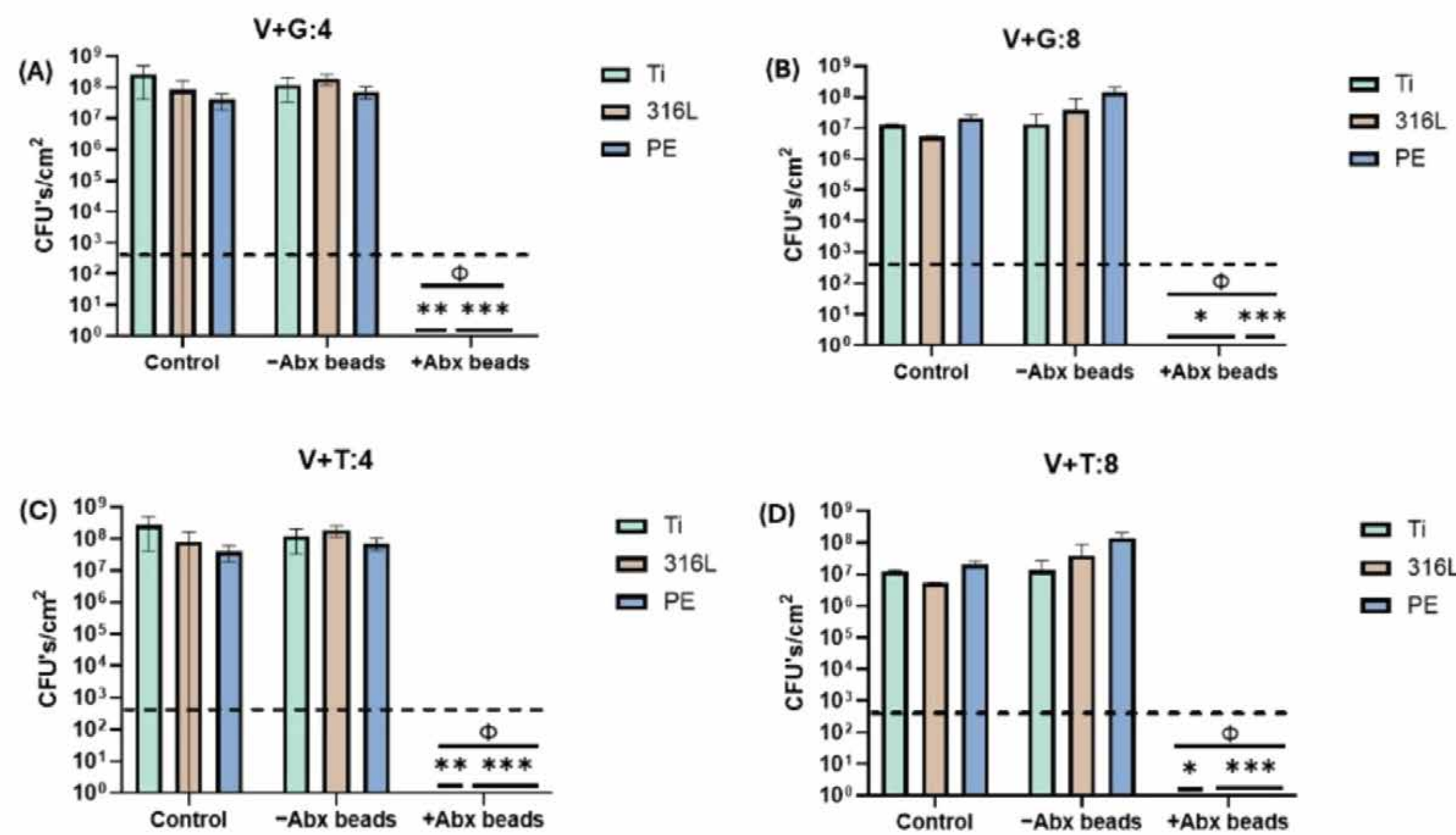


Fig 2. Quantification of viable *S. aureus* following treatment of 1-day biofilms with vancomycin + gentamicin (V+G) or vancomycin + tobramycin (V+T) delivered via 4 or 8 antibiotic-loaded calcium sulfate beads, ϕ indicates no detectable CFU.

Significant log reduction of 3-day *S. aureus* biofilms by vancomycin+gentamicin (V+G) and vancomycin+tobramycin (V+T) with 4 and 8 beads

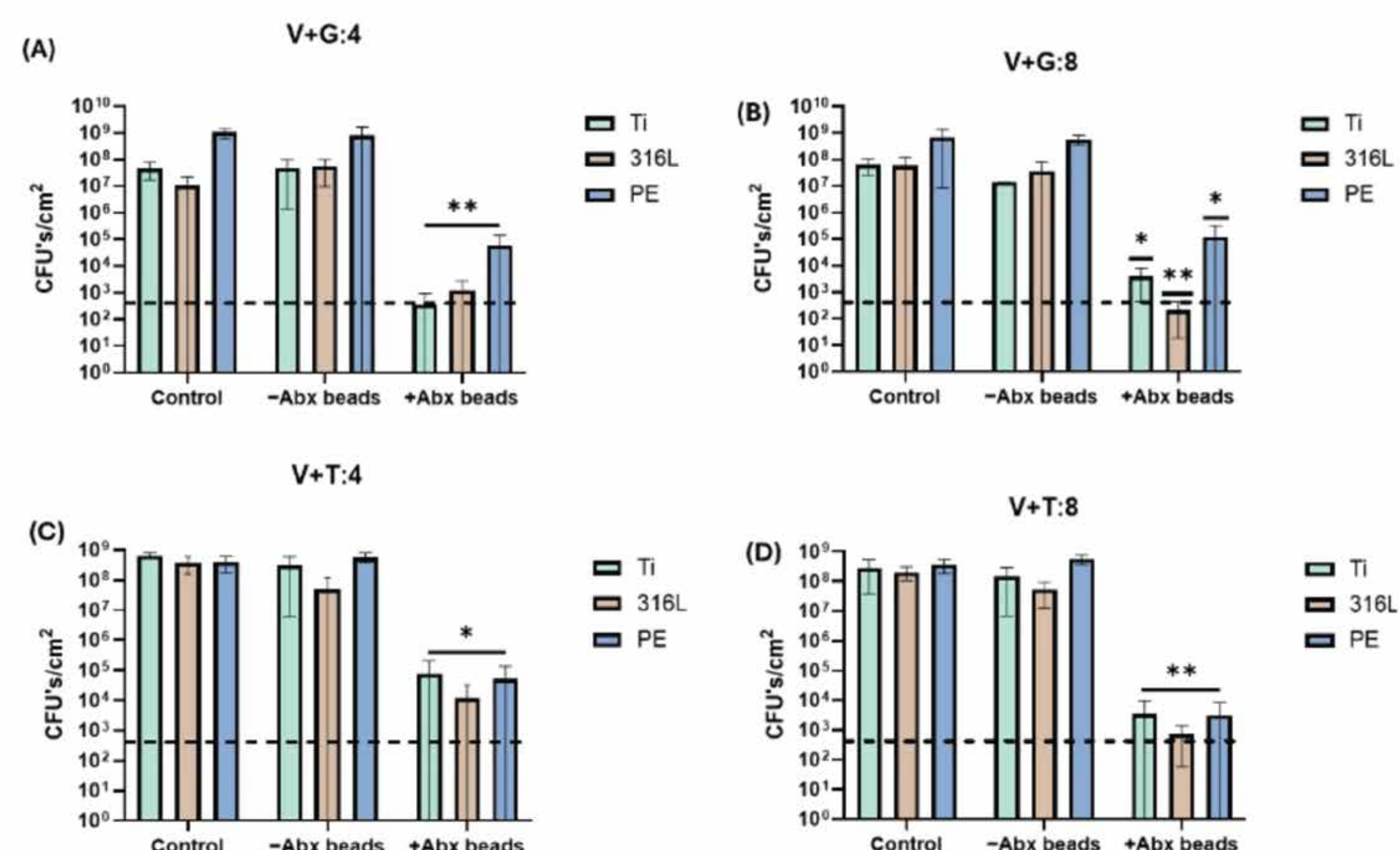
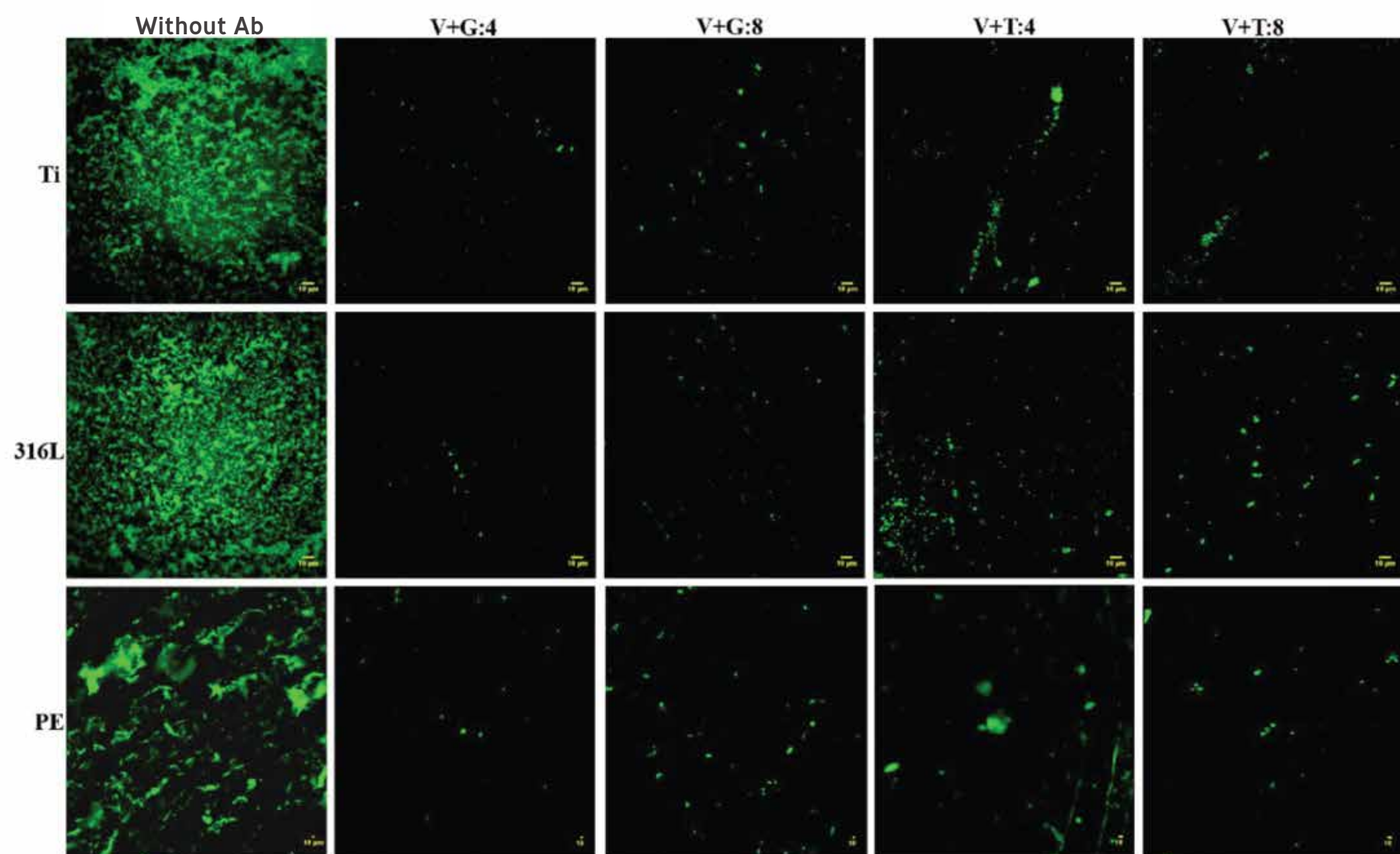
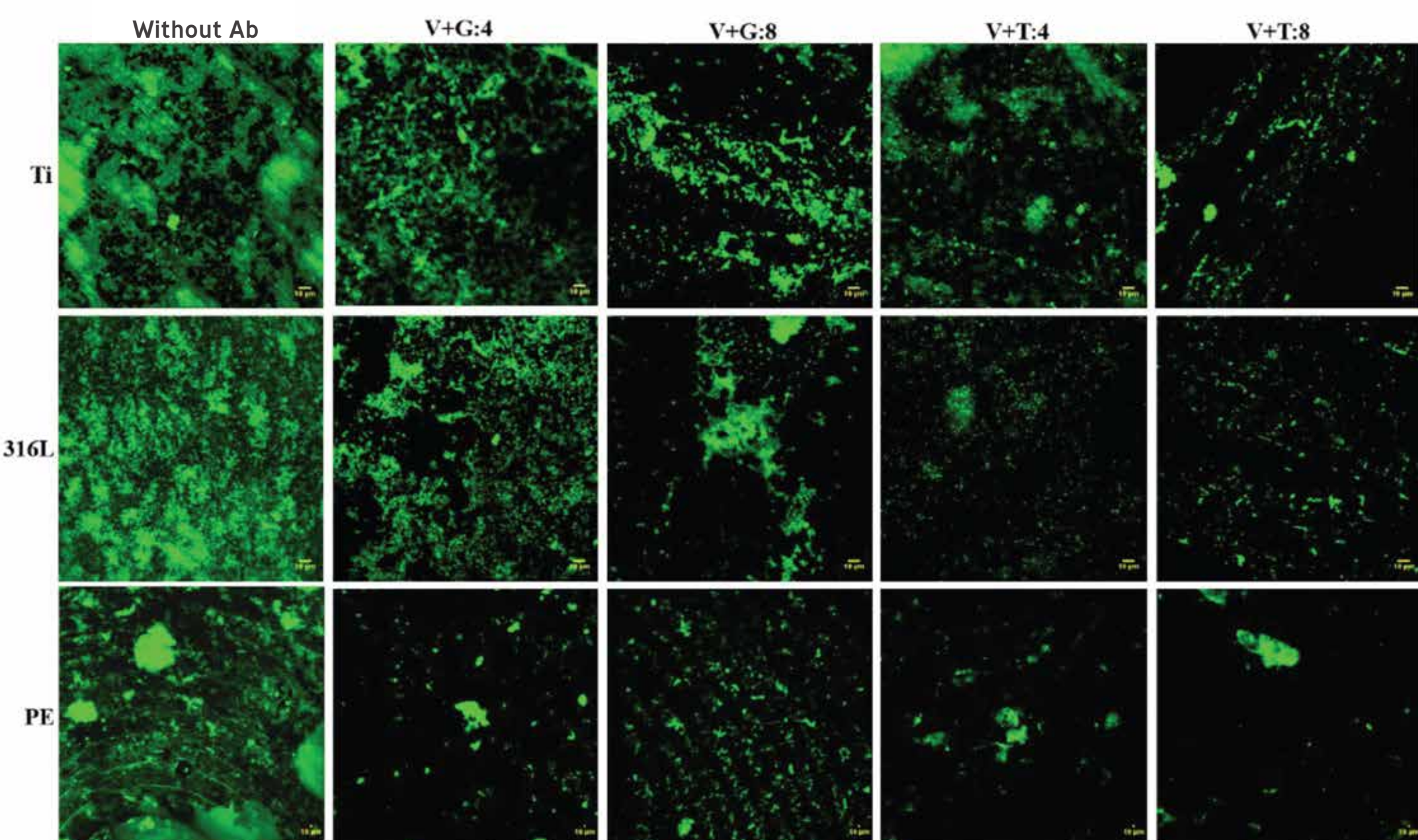


Fig 3. Quantification of viable *S. aureus* following treatment of 3-day biofilms with vancomycin+gentamicin (V+G) or vancomycin+ tobramycin (V+T) delivered via 4 or 8 antibiotic-loaded calcium sulfate beads.

Confocal imaging showed minimal residual bacteria following treatment of 1-day biofilms with antibiotic-loaded calcium sulfate beads



Confocal imaging showed major biofilm reduction following treatment of 3-day biofilms with antibiotic-loaded calcium sulfate beads



Conclusion

Antibiotic-loaded calcium sulfate beads containing vancomycin+gentamicin or vancomycin+tobramycin completely eradicated 1-day *S. aureus* biofilms and significantly reduced 3-day biofilms (3-5 log reduction). CFU analysis and confocal microscopy consistently demonstrated substantial biofilm reduction, highlighting the potential of combination antibiotic-loaded beads for treating biofilm-associated implant infections.

This research was funded by Biocomposites Ltd. (Keele, Staffordshire, UK)

References

1. Howlin, R., et al., Antibiotic-loaded synthetic calcium sulfate beads for prevention of bacterial colonization and biofilm formation in periprosthetic infections. *Antimicrobial agents and chemotherapy*, 2015. 59(1): p. III-120.
2. McConoughey, S.J., et al., Comparing PMMA and calcium sulfate as carriers for the local delivery of antibiotics to infected surgical sites. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 2015. 103(4): p. 870-877.