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ORIGINAL ARTICLE

# Prevention of *Propionibacterium acnes* biofilm formation in prosthetic infections *in vitro*

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**Background:** The role of *Propionibacterium acnes* in shoulder arthroplasty and broadly in orthopedic prosthetic infections has historically been underestimated, with biofilm formation identified as a key virulence factor attributed to invasive isolates. With an often indolent clinical course, *P acnes* infection can be difficult to detect and treat. This study investigates absorbable cements loaded with a broad-spectrum antibiotic combination as an effective preventive strategy to combat *P acnes* biofilms.
**Methods:** *P acnes* biofilm formation on an unloaded synthetic calcium sulfate ($CaSO_4$) bone void filler cement bead was evaluated by scanning electron microscopy over a period of 14 days. Beads loaded with tobramycin alone or vancomycin alone (as comparative controls) and beads loaded with a vancomycin-tobramycin dual treatment were assessed for their ability to eradicate planktonic *P acnes*, prevent biofilm formation, and eradicate preformed biofilms using a combination of viable-cell counts, confocal microscopy, and scanning electron microscopy.
**Results:** *P acnes* surface colonization and biofilm formation on unloaded $CaSO_4$ beads was slow. Beads loaded with antibiotics were able to kill planktonic cultures of $10^6$ colony-forming units/mL, prevent bacterial colonization, and significantly reduce biofilm formation over periods of weeks. Complete eradication of established biofilms was achieved with a contact time of 1 week.

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**Conclusions:** This study demonstrates that antibiotic-loaded $CaSO_4$ beads may represent an effective antibacterial and antibiofilm strategy to combat prosthetic infections in which *P acnes* is involved.
**Level of evidence:** Basic Science Study; Microbiology

**Keywords:** *Propionibacterium acnes*; biofilm; shoulder arthroplasty; prosthetic infection; calcium sulfate; antibiotics

The number of prosthetic infections (PIs) continues to rise because of the ever-increasing number of joint arthroplasties carried out worldwide.[10] Joint remodeling or replacement has the capacity to improve function and mobility for the patient, but conversely, the risk of infection has the potential to result in significant morbidity and cost to the health care service.[27,34]

PIs are strongly linked to the formation of bacterial biofilms, seeded initially during the intraoperative process or later through hematogenous spread and intrusion through the scar.[42,53,54] Infiltrating bacteria are able to attach to and form a biofilm on a heterogeneous range of host and manufactured surfaces, such as prosthetic components, surrounding tissue, and bone architecture.[27] Although physically distinct, these act as communicating niches where reseeding can occur from one area despite successful bacterial clearance from another, thus making complete eradication challenging. Moreover, the biofilm phenotype itself confers bacterial cells with an increased resistance to clearance by the host immune system and substantial tolerance to antimicrobials.[14] Consequently, the difficulty in clearing biofilm-mediated chronic infection means reinfection rates can rise up to 20% after revision compared with 1% to 4% for all primary procedures.[7,24,25]

*Staphylococcus aureus* and coagulase-negative staphylococcus are considered the predominant causative pathogens in PI, based largely on culture-dependent methods.[47] However, gaining increasing prominence is the skin commensal and facultative anaerobe *Propionibacterium acnes*, whose role in PI pathogenesis has historically been underestimated but whose impact in shoulder arthroplasty has been well documented.[38] A slow-growing, aerotolerant anaerobe, *P acnes* can be difficult to isolate using conventional methods, with incubation times of up to 3 weeks leading to false-negative cultures.[31] In addition, its relatively low virulence and abundance as part of the normal skin microbiota, coupled with an often indolent clinical course, make its clinical significance on identification in PIs difficult to determine.[8,13,52] However, neurosurgical and orthopedic procedures have been shown to be serious risk factors involved in *P acnes* infection.[45] In particular, its common association with hair follicles and sebaceous glands, whose numbers are elevated around the face, scalp, chest, and back, means *P acnes* is particularly numerous around the shoulder.[17] Consequently, it is the most predominant microorganism associated with shoulder infections and following revision shoulder arthroplasties.[39,41,46] Moreover, its location deep within sebaceous glands means that instruments used around superficial tissues may be contaminated with *P acnes* below the skin, making wound incision-site sterilization difficult and increasing the possibility of introduction into deep joint tissues.[22,26,36]

One approach in combating PIs involves the use of antibiotic-loaded cement spacers or beads to provide high localized antibiotic concentrations at the surgical site, with the aim of device protection and dead space management.[18] Dissolvable cements, such as calcium sulfate, have the added benefit of sustained elution kinetics and complete reabsorption, therefore not requiring a further surgical procedure to remove.[16] This study investigates the ability of sustained antibiotic elution of a broad-spectrum combination from cement beads to prevent bacterial colonization and biofilm formation, as well as their impact on established biofilms of *P acnes*.

## Materials and methods

### Planktonic and biofilm minimum inhibitory concentration and minimum bactericidal concentration antibiotic assays

*P acnes* ATCC 11827 cultures (ATCC, LGC Standards, Teddington, UK) were grown in brain-heart infusion (Sigma-Aldrich, Gillingham, UK) anaerobically (AnaeroGen; Oxoid, Basingstoke, UK) for 72 hours at 37°C. The strain was selected as it is one of the most commonly used *P acnes* strains for biofilm studies.[1,11] Holmberg et al[15] in 2009 demonstrated the importance of biofilm formation as a characteristic of invasive *P acnes* clinical isolates; consequently, the propensity for the strain to form biofilms was of key importance in this study to be able to evaluate the antibiofilm efficacy of pharmaceutical-grade calcium sulfate alpha-hemihydrate (PG-CSH) beads. The cultures were diluted in fresh brain-heart infusion to an optical density ($OD_{620nm}$) corresponding to $10^6$ colony-forming units (CFU)/mL. As a result, this constitutes a modified minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay, performed so that starting cultures more closely matched the CFUs recovered from biofilm MBCs that cannot be readily controlled (planktonic control for MBC after 72 hours' growth, $2.1 \times 10^7$ CFU/mL; biofilm control for MBC after 7 days growth, $2.29 \times 10^7$ CFU/mL). Using the adjusted bacterial culture, a dilution series from 0.5 to 1024 μg/mL of vancomycin (Hospira UK Ltd, Maidenhead, UK) and tobramycin (Sigma-Aldrich) was performed, and the cultures were again incubated anaerobically for 72 hours at 37°C. Beyond 1024 μg/mL, vancomycin was noted to be incompletely soluble in the media; as a result, this was the maximum concentration of antibiotics evaluated. The antibiotic MIC was measured using a microplate reader (BMG Omega; BMG Labtech Ltd, Aylesbury, UK) at 680 nm ($OD_{680nm}$).