Biofilm prevention and killing of Gram-positive bacterial pathogens involved PJI by antibiotic-loaded calcium sulfate beads (ALCSB) *in-vitro*

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Aims:

Periprosthetic Joint Infection (PJI) causes significant morbidity and mortality in fixation and joint arthroplasty and has been extensively linked to the formation of biofilms. A common approach in PJI management is the adjunctive use of local and systemic antibiotics. In this study we evaluated the *in-vitro* efficacy of ALCSB* to inhibit biofilm formation and kill pre-existing biofilms of a number of key Gram-positive pathogens including Epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-16) and *Staphylococcus epidermidis.*

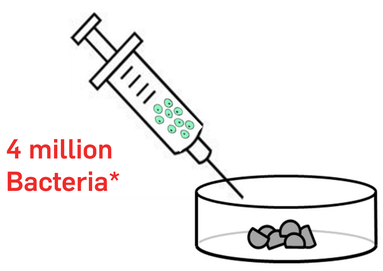
Method:

To assess biofilm prevention, microorganisms were treated with ALCSB containing vancomycin (1000mg/10cc pack), gentamicin (240mg/10cc pack) or combinations of both antibiotics. Media was removed and challenged with fresh bacteria for 14 daily challenges. CFU counts were taken after 1,2,3,7 and 14-days. For killing of pre-existing biofilms, ALCSB were added to 3-day biofilms. CFU counts were recorded at 1,3 and 7-days.

Results:

ALCSB are capable of preventing surface colonisation and biofilm formation in the presence of repeat bacterial challenges

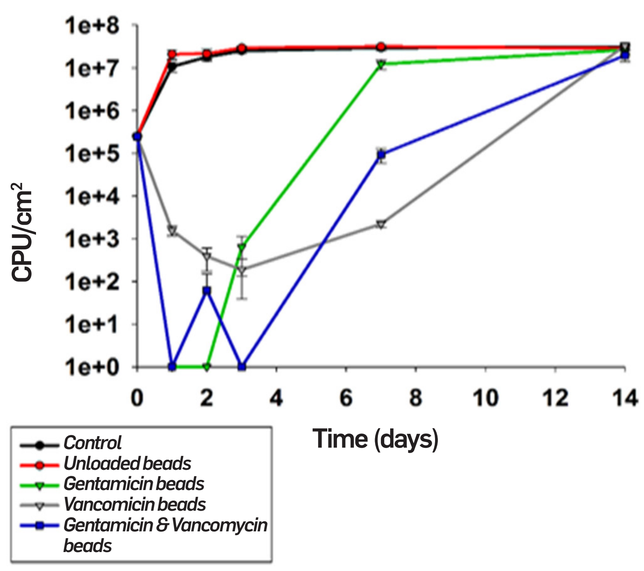
A **Ability of the beads to prevent biofilm formation after multiple bacterial challenges**



Repeat Challenge Model-

Every 24 hours:
- **Complete media exchanges**
- **Released Antibiotics removed**
- **Fresh inoculum**

B EMRSA-16



C S. epidermidis

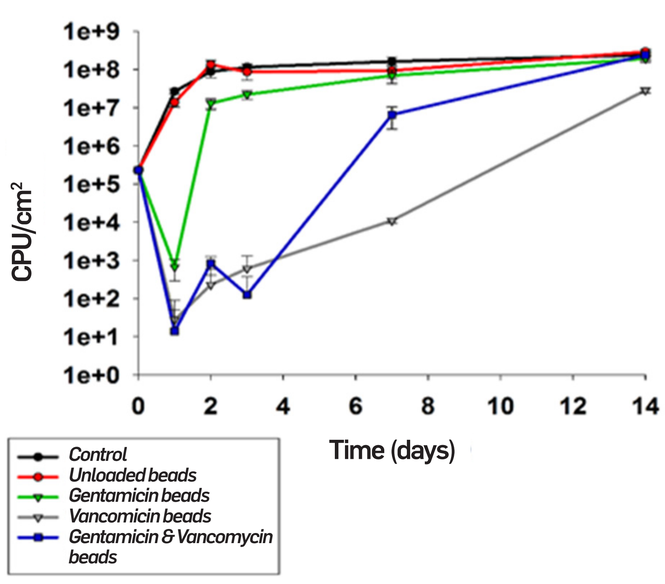
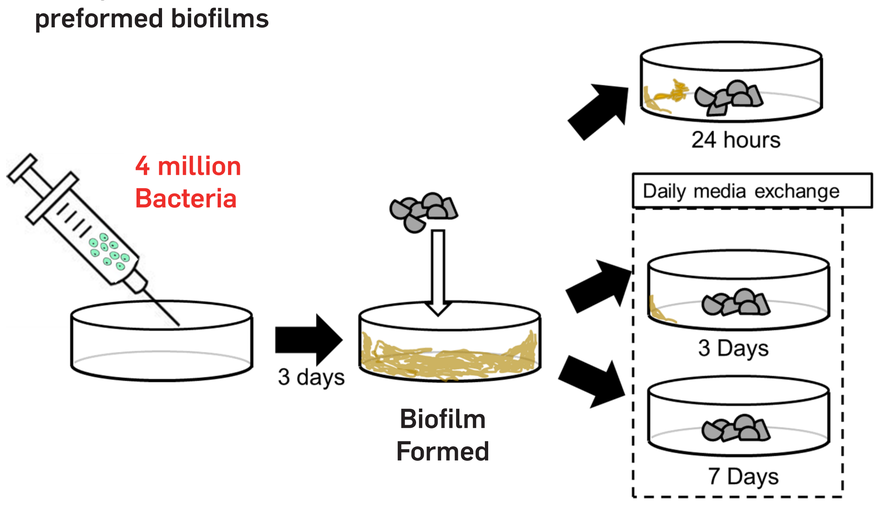


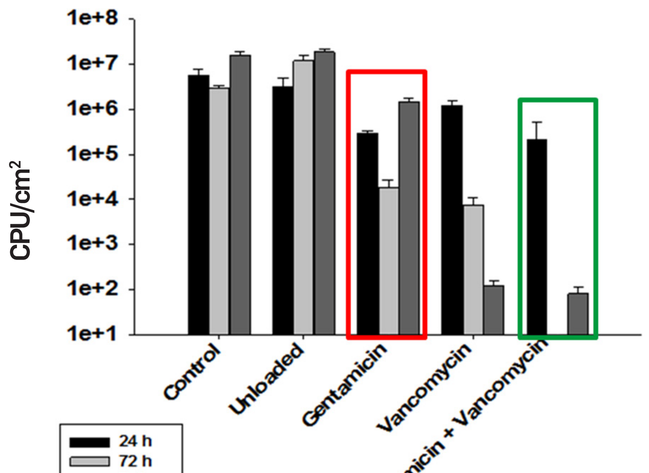
Figure 1A-C. Bacterial colonisation and biofilm formation on substratum in the presence of Vancomycin, Gentamicin or Vancomycin and Gentamicin loaded calcium sulfate beads and repeat bacterial challenges of 4x106 CFU/ml (challenge carried out every 24h for all bacterial species. CFU counts taken after 1,2,3,7 and 14 days. B) ALCSB with vancomycin & gentamicin achieved a complete kill of EMRSA-16 after challenge 1 and 3 and by challenge 14, was comparable with control groups. C) *S.epidermidis* biofilm prevention assays showed ALCSB achieved a 6-log reduction in CFU/cm2 after 2 challenges.

Pre-existing biofilms are harder to eradicate, although possible given longer incubation times with some of the bacterial species evaluated

A **Ability of the beads to eradicate preformed biofilms**



B EMRSA-16



C S. epidermidis

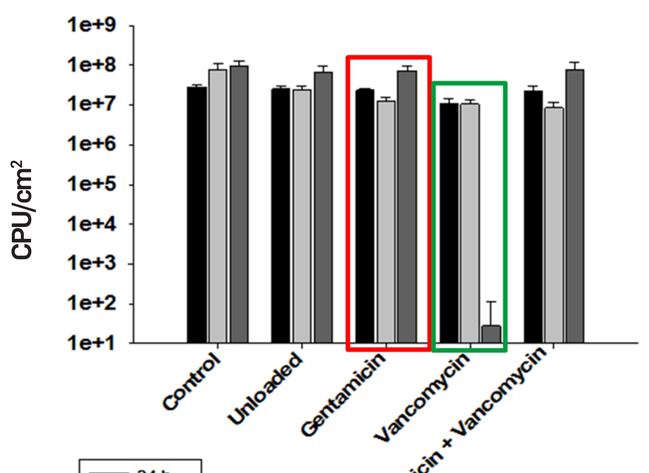


Figure 2A-C. The effect of antibiotic-loaded calcium sulfate beads with increasing contact times on pre-existing biofilms of *S.Aureus* EMRSA-16 and *S.Epidermidis*, grown for 72hr prior to incubation with the vancomycin and tobramycin in combination loaded beads. CFU counts taken after 1,3 and 7 days. B) ALCSB with vancomycin & gentamicin achieved complete kill of EMRS-16 biofilms after 72h. C) ALCSB had limited effect on pre-existing *S.epidermidis* biofilms at 24h (P=0.519) or 7-days (P=0.425) relative to unloaded beads.

Conclusions:

These *in-vitro* studies highlight the potential *in vivo* benefit of antibiotic-loaded calcium sulfate beads in the prevention of bacterial colonisation and biofilm formation in prosthetic infection management.

*Stimulan Rapid Cure, Biocomposites

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