

Are silver-containing dressings effective against bacteria in biofilms?

Lorna McInroy PhD, Breda Cullen PhD, Rachael Clark PhD,
Systagenix Wound Management, Gargrave, Skipton, North Yorkshire BD23 3RX

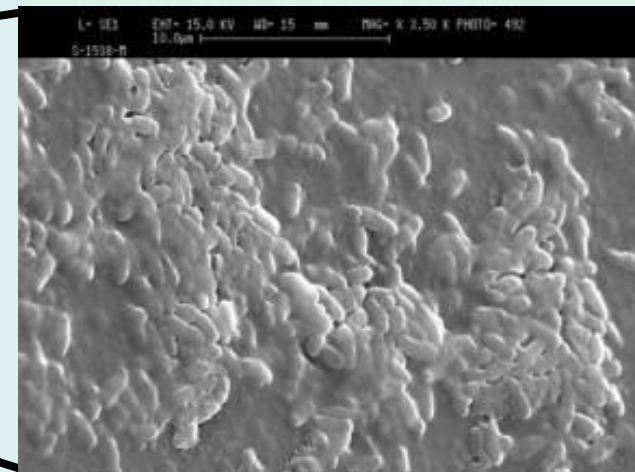
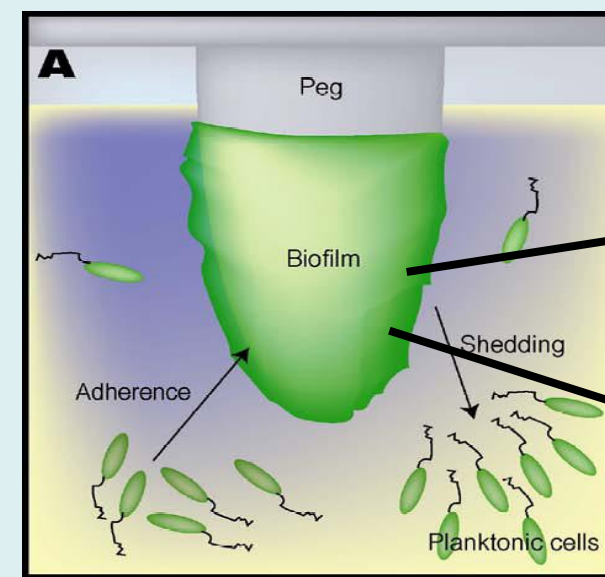
Abstract

Most wounds are populated by diverse floras, which have the potential to lead to infection, cause inflammation and delay healing. Recently, evidence for the presence of complex biofilms in wounds has been gaining recognition. Biofilms are defined as the coordinated multi-cellular behaviour of microorganisms encased in an extracellular polysaccharide matrix of both host and microbial origins. The ability of biofilms to form these complex communities is believed to impede host defences and antimicrobial treatments.

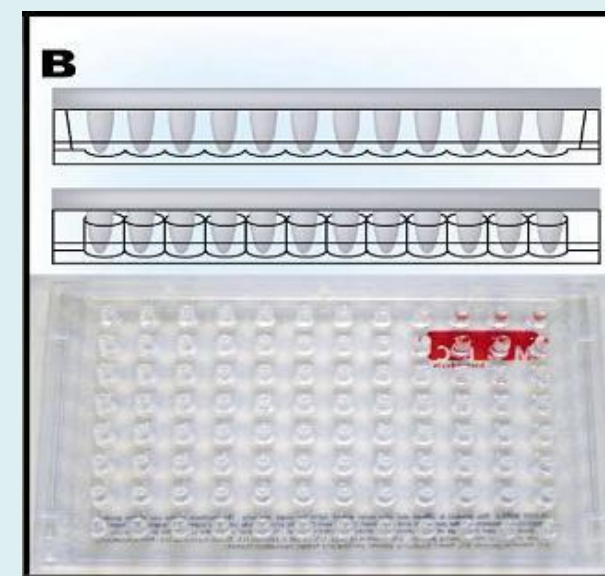
Silver dressings have been widely used in the treatment of infected chronic wounds, but there is a lack of evidence regarding their effect on biofilm disruption, in a relevant model. In this study we used a high throughput, *in vitro*, biofilm model to assess the effectiveness of a non-adherent silver alginate dressing to combat common wound pathogens including; *Pseudomonas aeruginosa*, *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* (VRE), in this environment. The organisms were grown in a substrate that closely reflects the host environment, in order to simulate a wound biofilm. We have also evaluated the ability of this dressing to disrupt and prevent biofilm formation *in vitro*.

Results demonstrate that the non-adherent silver alginate dressing was able to reduce bacterial bioburden in 24 hr pre-formed biofilms in all three organisms, with total kill achieved in two strains (*Staphylococcus aureus* MRSA 399 and *Enterococcus faecalis* (VRE) 06-0147 3122277). The presence of extracellular polysaccharides and disruption of biofilms was also verified using scanning electron microscopy techniques. In addition we demonstrated the ability of the non-adherent silver alginate dressing to prevent biofilm formation, with inhibition of *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* strains observed.

In light of emerging evidence on the presence of biofilms in chronic wounds, this study describes a new, more clinically relevant, model for measuring the efficacy of antimicrobials in the treatment in wounds.



High Throughput *in vitro* pre-formed biofilm killing model

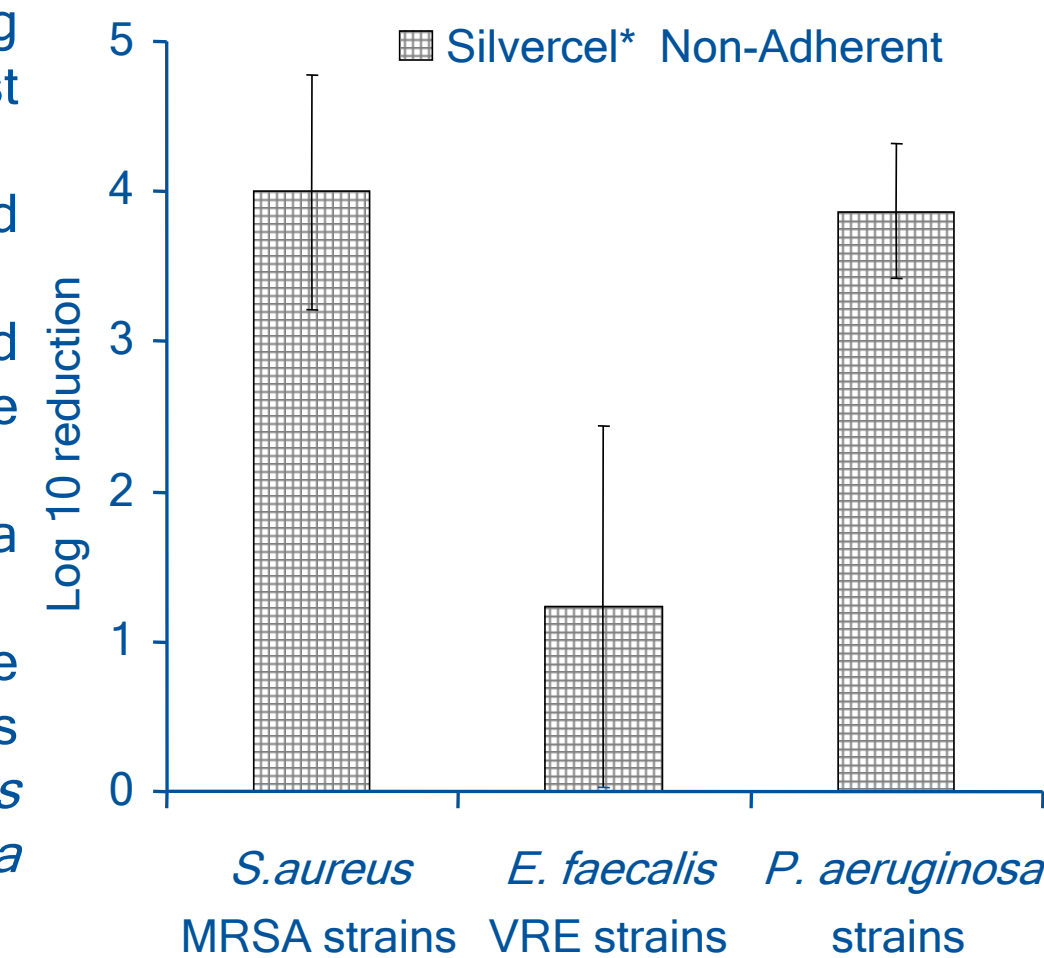


Images courtesy of Innovotech

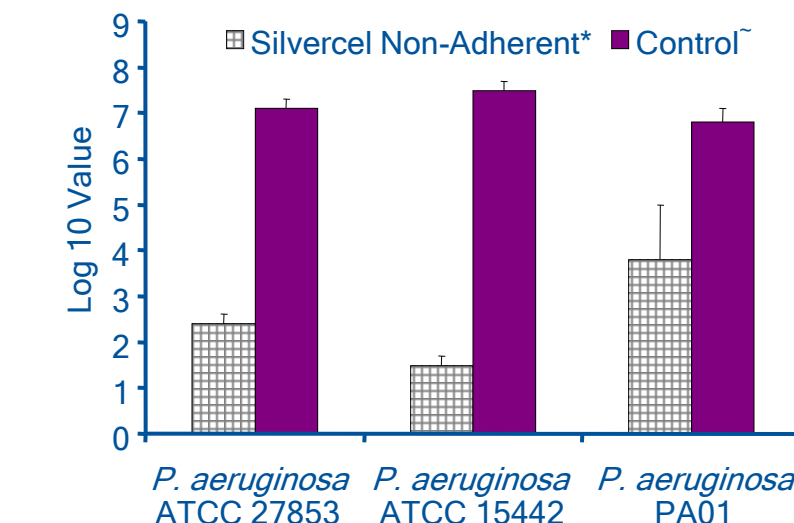
- 96 well format giving reproducible biofilms.
- Biofilms grown for 24 hours pre-antimicrobial challenge in human serum.
- Human serum used as challenge medium to reflect physiological conditions.
- Planktonic cells removed by washing leaving adherent cells attached to pegs to be challenged by antimicrobial.
- Dressings are placed firmly in the bottom of each well, with human serum media, and preformed biofilms are incubated for desired time.
- Endpoint measured by removing adherent cells via sonification and plating to give colony forming units (CFU)/peg.

Biofilm prevention *in vitro* Model

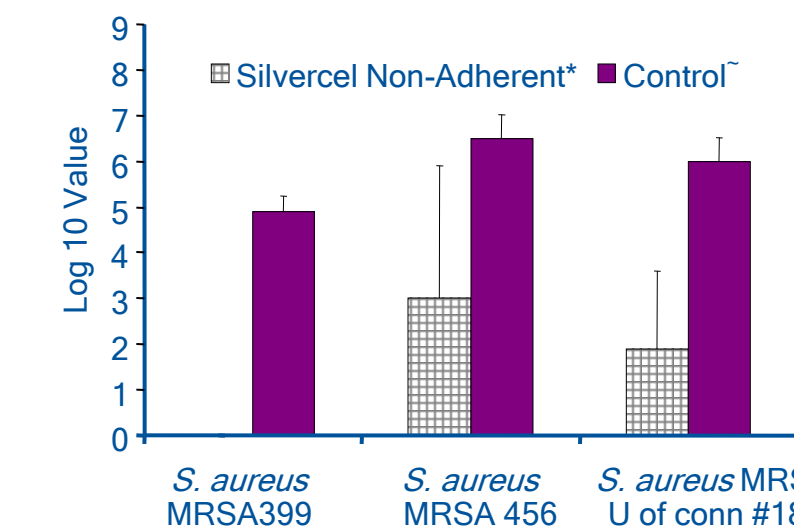
- 12 well quantitative method to measure adherence killing and biofilm formation of viable organisms attached to test materials.
- Samples were attached to a pegged lid and incubated with inoculum (10^5 cells) with overnight shaking.
- After 24hr contact time, samples were rinsed 3x, adhered bacteria were removed by sonification and plated to give CFU recovered/sample.
- Results are presented as \log_{10} reduction compared to a non-silver containing absorbent dressing control.
- This assay demonstrated the ability of silver to reduce microbial adherence to the dressing. Silver was particularly effective on inhibition of *Staphylococcus aureus* (MRSA) strains and *Pseudomonas aeruginosa* strains, given ≥ 3 log reduction criteria for efficacy.



Results of pre-formed biofilm killing

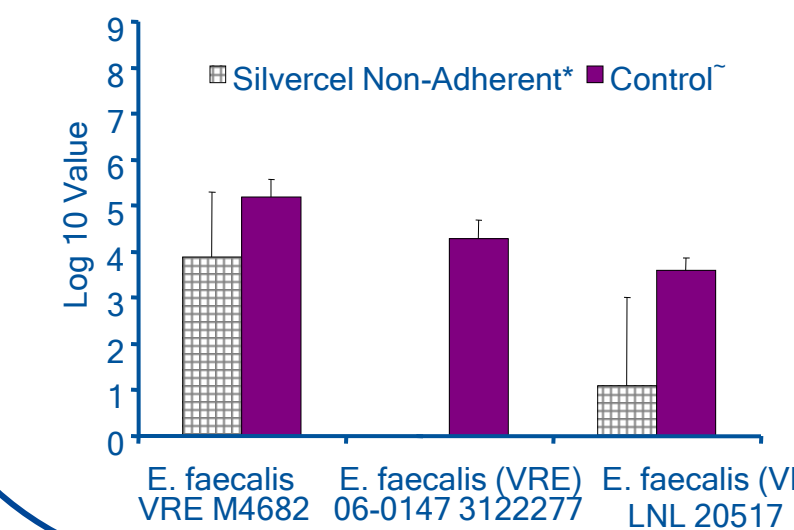


P. aeruginosa
ATCC 15442

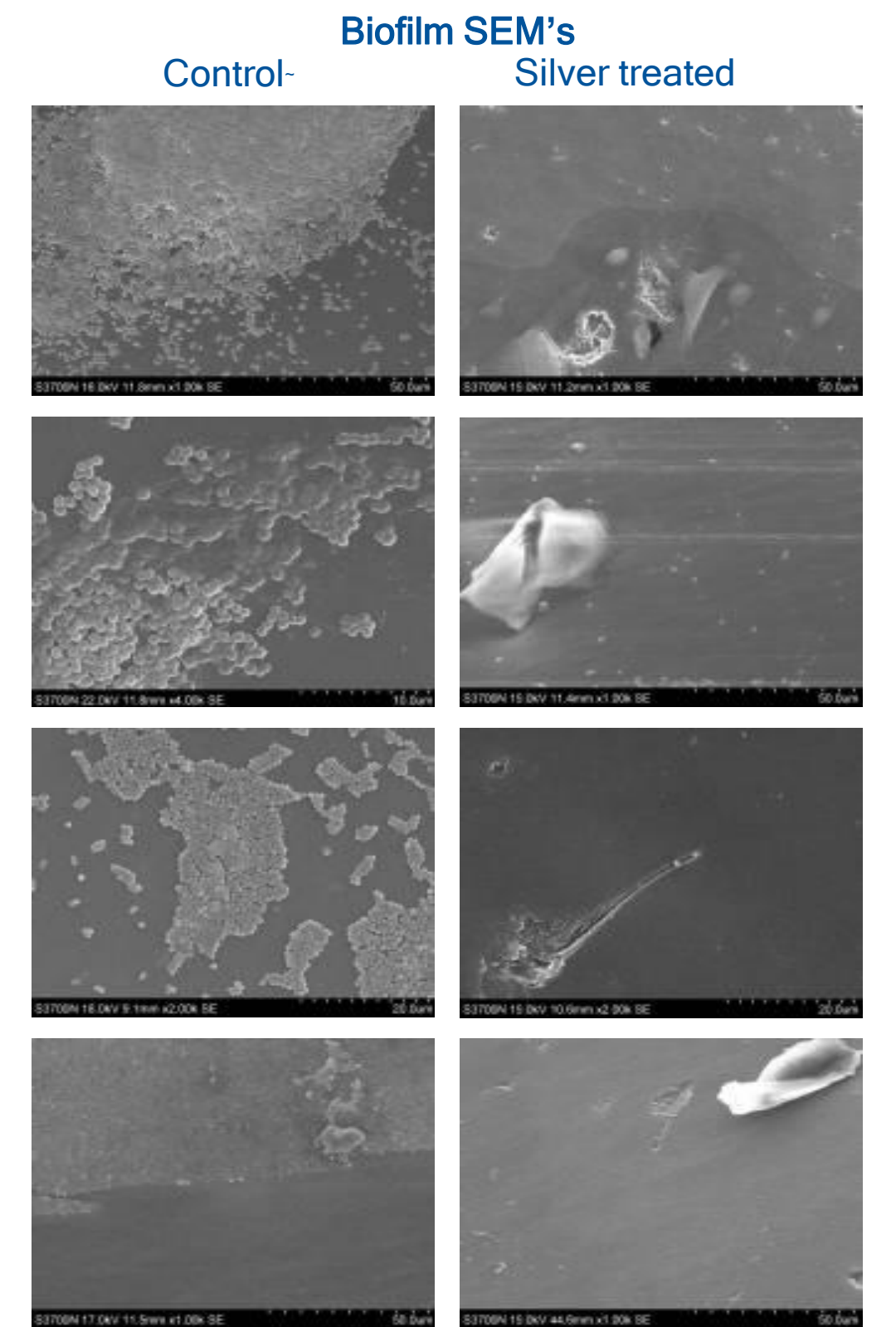


S. aureus
MRSA 399

S. aureus
MRSA 456



E. faecalis
VRE M4682



- Control refers to biofilm formed peg that was untreated after 24hrs

Conclusion

The data presented shows SILVERCEL* Non-Adherent is effective at reducing the bioburden of *S. aureus* (MRSA), *P. aeruginosa* and *E. faecalis* (VRE) in pre-formed biofilms within 24 hours. Notably, no viable bacteria were recovered from the biofilms of MRSA 399 and VRE 06-0147 strains when treated with Silver.

The representative SEM demonstrate the removal of bacteria from the pegs when treated with silver dressing compared to the untreated control showing large amounts of bacteria adhered to the surface with an exopolysaccharide matrix apparent in the micrographs. Silvercel* Non-Adherent was able to inhibit the formation of biofilms over 24hrs in *S. aureus* (MRSA) and *P. aeruginosa* strains giving > 3 log reduction. Future work will concentrate on using a multi-species biofilm model to evaluate antimicrobial efficacy.

The study gives insight into the differences in bacterial killing efficiency from the standard \log_{10} and zone of inhibition assays (See complementary SILVERCEL* Non-Adherent poster for data) compared to performance in biofilm models, this suggests the need to evaluate the efficacy of microbial agents in a more relevant *in vitro* assay for wounds.