A novel treatment method for the removal of biofilm material

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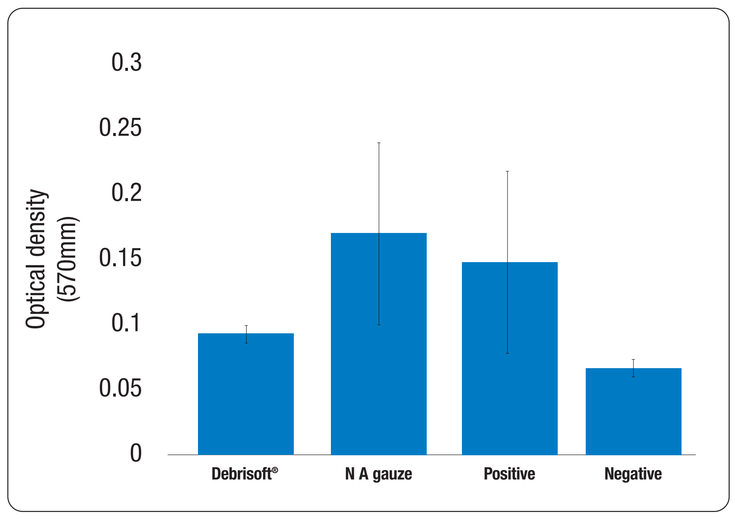


Figure 1. The optical density of crystal violet stained microtitre plate wells following incubation with a *Pseudomonas aeruginosa* inoculum (n=3). Positive wells did not receive treatment, negative wells did not contain a bacterial inoculum or undergo treatment. Bars represent standard deviations.

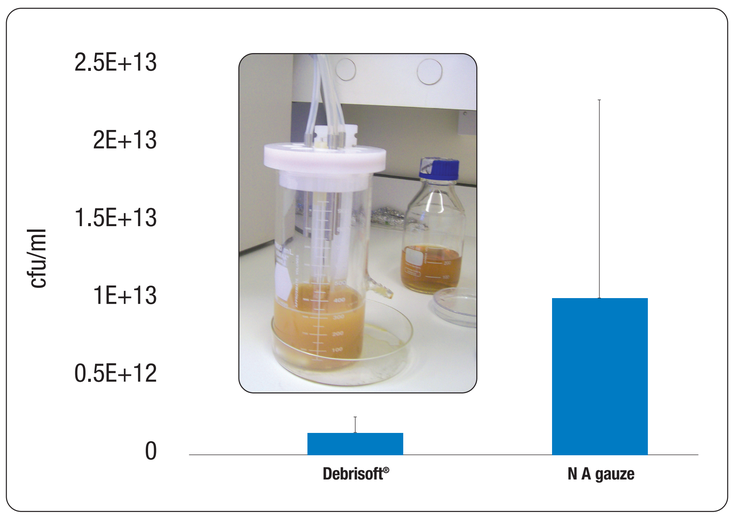
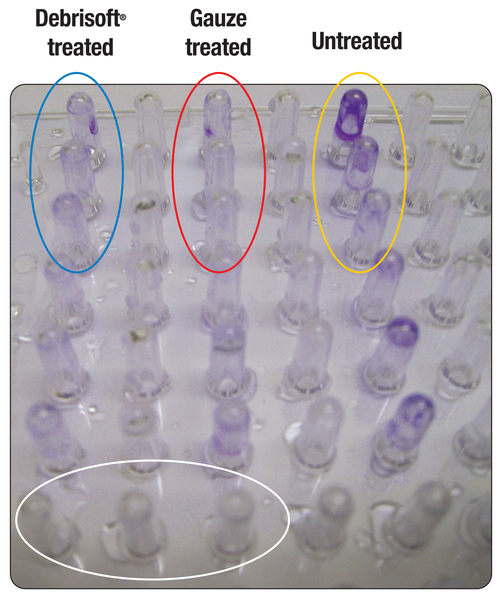


Figure 2. Bacterial counts recovered from polystyrene coupons



White oval = Negative control

The microtitre plate pin lid after staining with CV.

Aim

Assessing the removal of single and multi-species bacteria, that have attached to a solid surface, using a debridement product*.

Methods

Single species bacterial biofilms were formed using *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and Methicillin resistant *Staphylococcus aureus* (MRSA) on microtitre plates and pin lids. Following incubation, wells and pins were washed 3 times with sterile PBS. Six surfaces were treated with the debridement product, 6 were treated with NA Gauze, 6 were untreated (positive control) and 6 were incubated in sterile TSB only (negative control). Three surfaces from each treatment group were stained with 2% crystal violet (CV) dye and enumerated for bacterial attachments the remaining 3 surfaces were visualised using SEM microscopy.

Equal quantities of *S. aureus*, *P. aeruginosa* and *E. coli* were used to inoculate TSB in a CDC reactor containing polystyrene coupons. The reactor was incubated at 37°C, shaking at 50 rpm to encourage biofilm growth. Coupons were treated with the novel debridement product, NA Gauze or remained untreated (N=6). Following aseptic removal and rinsing, 3 coupons from each treatment were transferred into 10ml sterile TSB and placed in a sonic water bath for 5 minutes to remove attached bacteria. TSB was incubated overnight at 37°C and the detached bacteria were quantified.

Results

Mechanical disruption of attached bacteria decreased the bacterial load on a solid surface. The effectiveness of the mechanical removal varied with species reflecting variations in bacterial surface attachment appendices.

Crystal violet staining

A significant difference was seen between the optical density (OD) of the negative control wells and the wells that contained a biofilm. The well treated with the debridement tool and NA gauze both demonstrated less staining than the positive control (Figure 1).

Biofilms on CDC coupons

Treating the coupons with the debridement product resulted in a significant decrease in the bacterial loads recovered from the CDC coupons ($P<0.01$) (Figure 2).

Conclusions

Mechanical disruption of a biofilm was achieved using the novel debridement product. Biofilm breakdown is a critical factor in the removal of biofilm material from chronic, non-healing wounds and a vital component in encouraging healing in wounds that were previously stalled.

Discussion

There are limitations to *in vitro* work. For example a flat, hard surface cannot compare with a soft, contoured wound bed.

Pain and trauma associated with debris removal are not considered in the laboratory. Clinical evidence also demonstrates that the new debridement product traps debris and bacteria within the monofilament fibres (Bahr et al, 2011) and we are not aware of any clinical evidence to demonstrate that gauze can do this.

References

Bahr S et al (2011) Clinical efficacy of a new monofilament fibre-containing wound debridement product. Journal of Wound Care. 20(5)

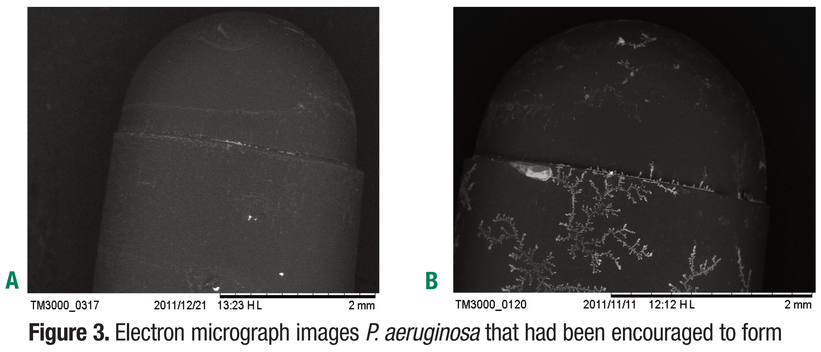


Figure 3. Electron micrograph images *P. aeruginosa* that had been encouraged to form a biofilm. Treated pin (A) and untreated pin (B).

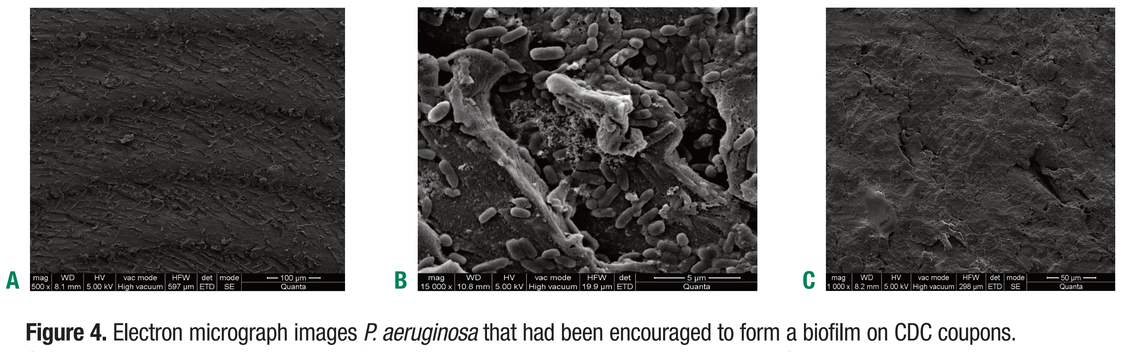


Figure 4. Electron micrograph images *P. aeruginosa* that had been encouraged to form a biofilm on CDC coupons. Coupons were negative (A) untreated (B) and treated with the novel treatment method (C).

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