Efficacy of a Novel Ultrasonically Activated Water Stream for Removal of Antimicrobial Resistant Biofilms

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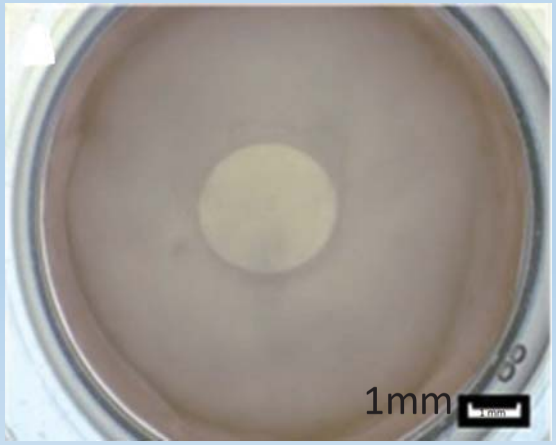
Aims & Objectives

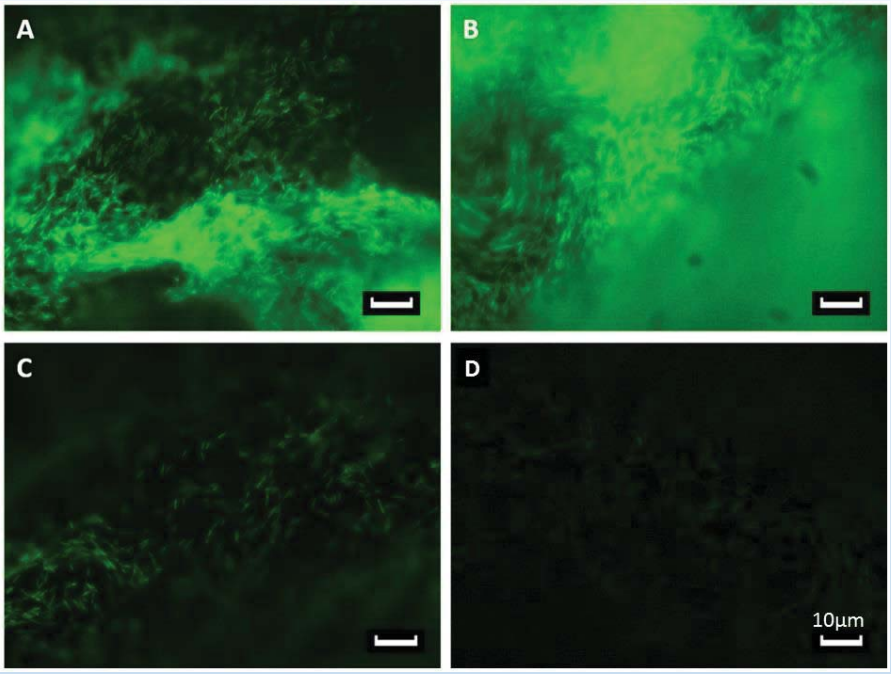
Bacterial biofilms are a significant problem in the management of chronic wounds, such as venous leg ulcers. In this phenotype, bacteria are protected from both antibiotics and physical removal. This study aimed to test the effectiveness of a novel ultrasonically activated fluid stream (UAS) in removing biofilm from biological, soft surfaces in an *in vitro* wound model.

Methods

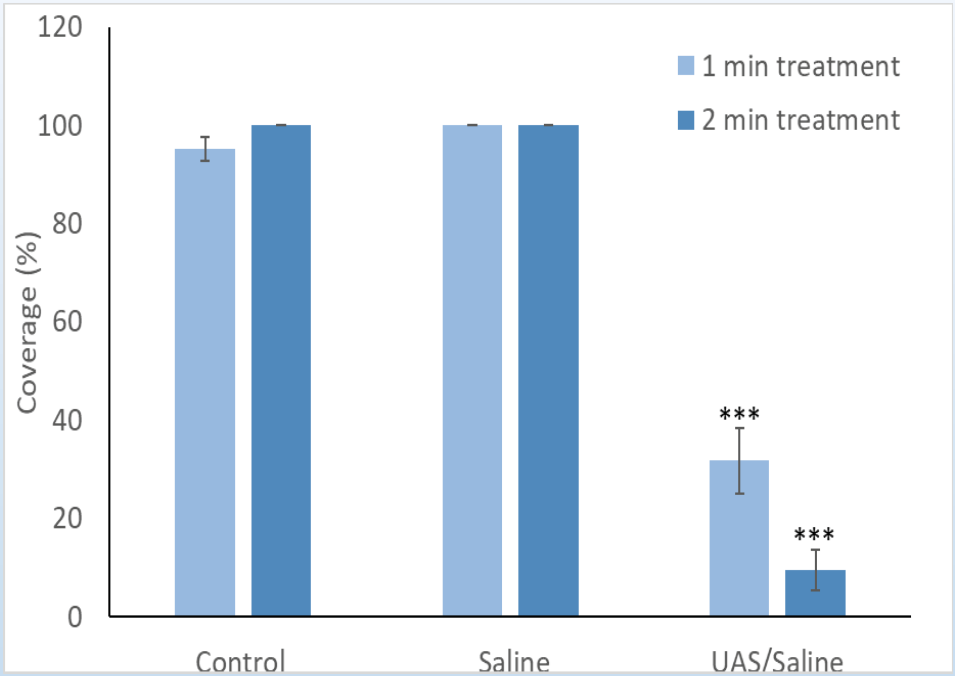
Two *in vitro* wound models were used: pig trotters; and a pre-wounded human skin model (EpiDerm™ FT, Mattek Inc, USA). Early stage biofilms were cultured within the wounds using fluorescent-tagged *Pseudomonas aeruginosa pMF230* and SYTO-9 pre-stained *E-MRSA-16*. Wound models were cleaned with either 0.9% saline or the UAS at a flow rate of 2 L/min, and residual bacteria visualised by direct *in situ* epifluorescence microscopy.



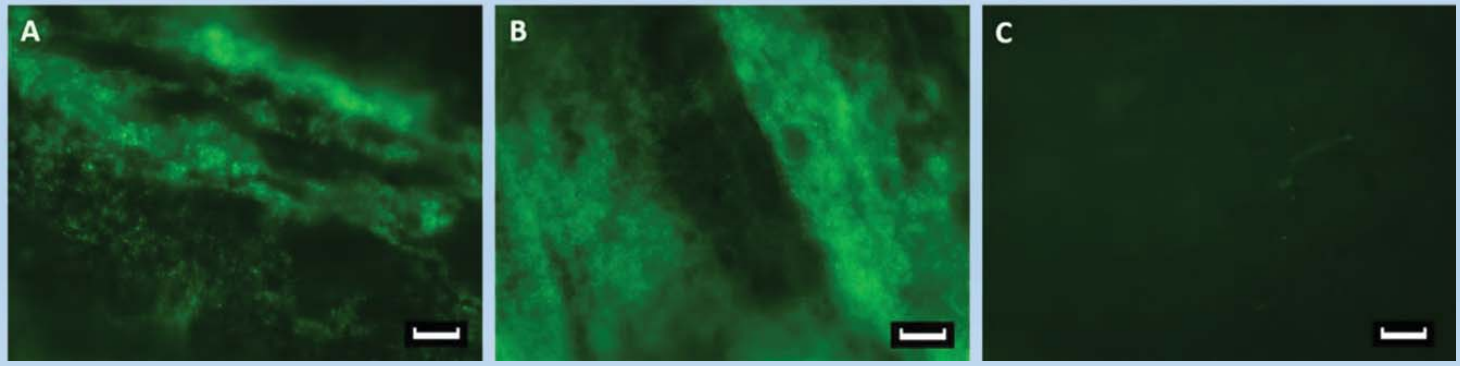
Results: Biofilm removal from pig trotter model



Direct EDIC/EF micrographs of GFP tagged *Pseudomonas aeruginosa pMF230*. A = control, B = 1 min saline wash, C = 1 min Starstream, D = 2 min Starstream. Scale bars represent 10µm.

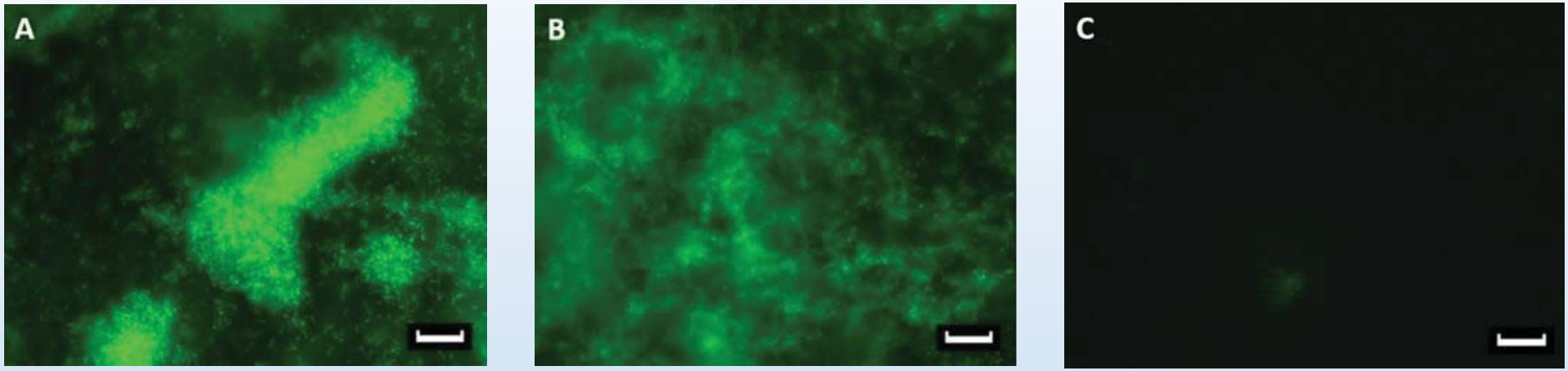


Percentage coverage of GFP tagged *Pseudomonas aeruginosa pMF230* biofilm. Error bars represent the SEM (N=3), One way ANOVA/Tukey post hoc test demonstrated *** = $p \leq 0.001$ when compared to the non-treated controls.

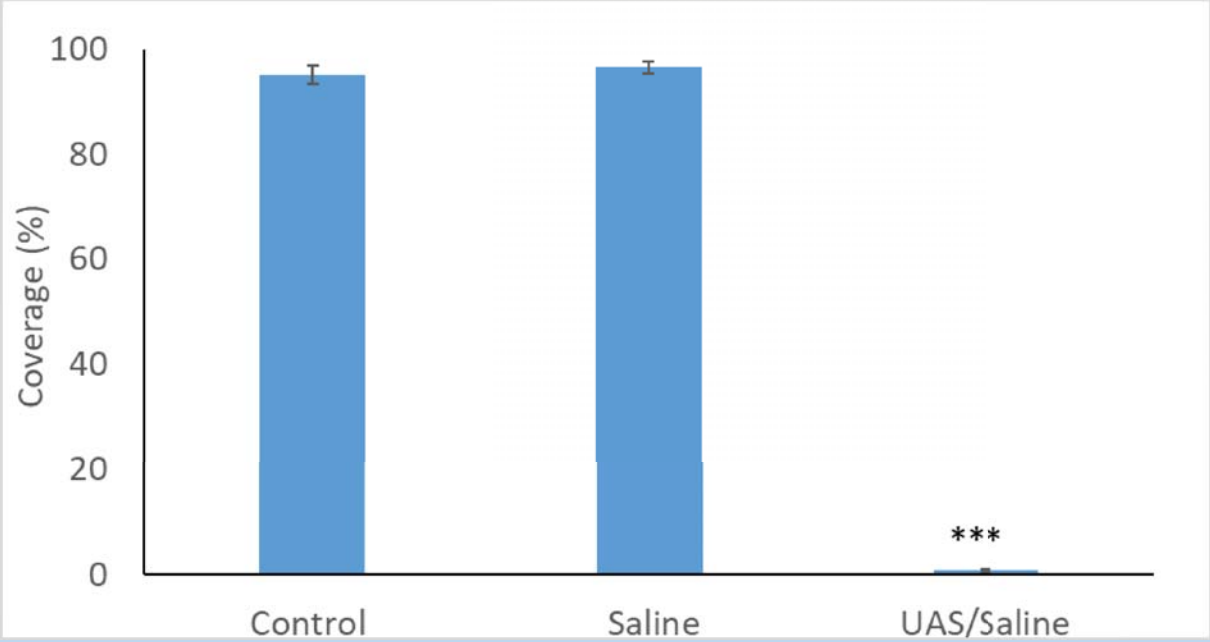


Direct EDIC/EF micrographs of SYTO-9 pre-stained *E-MRSA-16* early biofilm within pig trotter wounds. A = control, B = 1 min saline wash at a flow rate of 2 L/min, C = 1 min UAS treatment at a flow rate of 2 L/min. Scale bars represent 10µm.

Results: Biofilm removal from Epiderm™ FT model



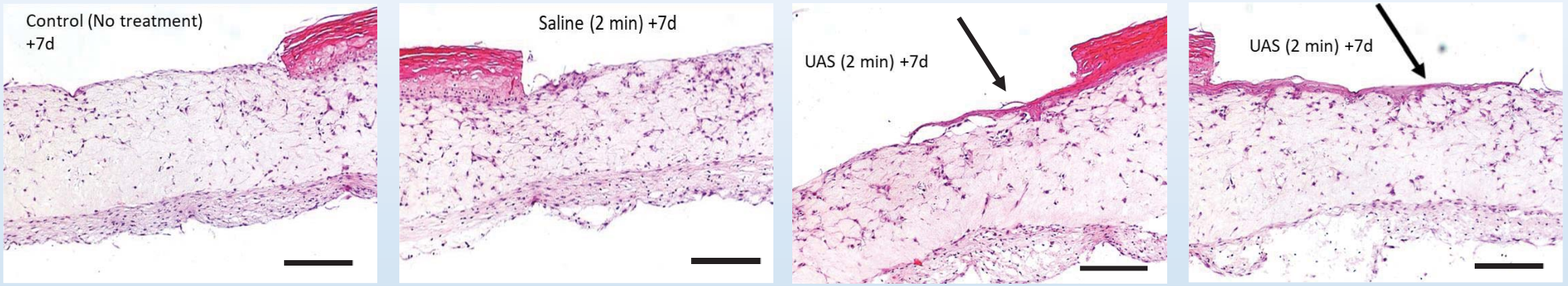
Direct EDIC/EF micrographs of GFP tagged *Pseudomonas aeruginosa pMF230*. A = control, B = 2 min saline wash, C = 2 min UAS. Scale bars represent 10µm.



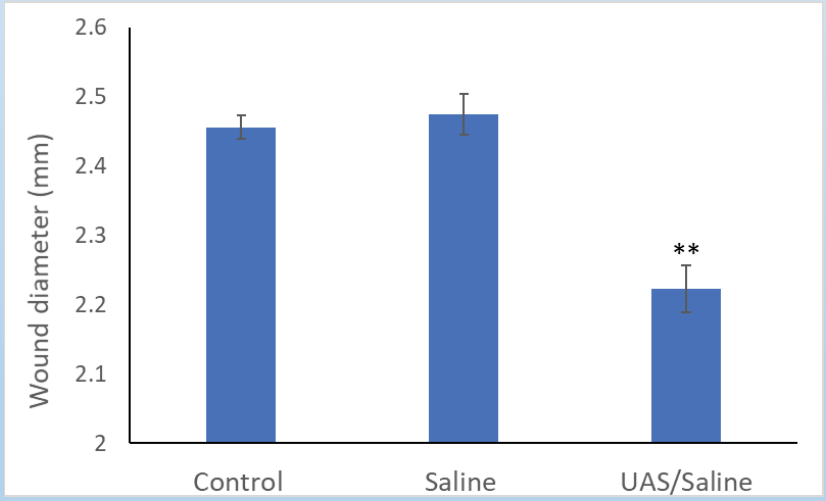
Percentage coverage of GFP tagged *Pseudomonas aeruginosa pMF230* biofilm following treatments showing significant biofilm removal by UAS. Error bars represent the SEM (N=3), One way ANOVA/Tukey post hoc test demonstrated *** = $p \leq 0.001$ when compared to the non-treated controls.

Results: Effect of ultrasonically activated stream on Epiderm™ FT human wound model

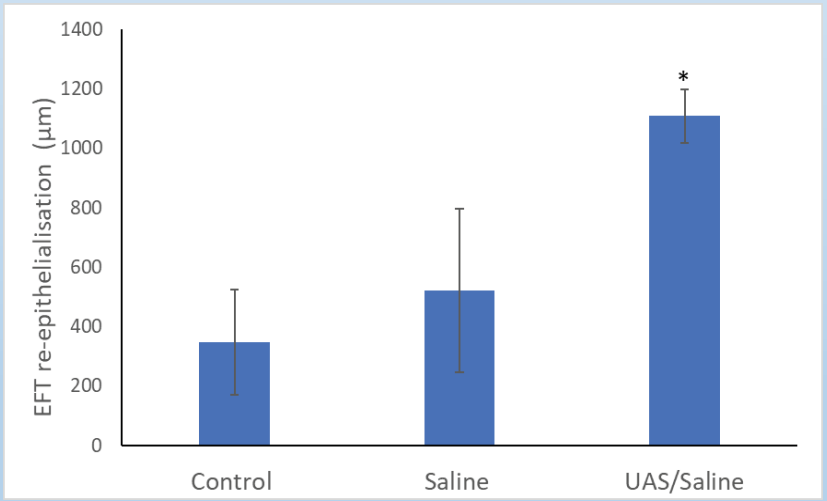
Histological examination showed no disruption to the delicate EpiDerm™ architecture, and a significant reduction in wound diameter and increase in re-epithelialisation ($p \leq 0.001$) in models treated with the UAS.



Example H&E stained sections (4µm) taken from the EFT wounds 7 days post cleaning. Arrows highlight the re-epithelialization across the wound bed. Scale bars represent 500 µm.



Mean ± SEM Reduction in wound diameter in Epiderm full thickness wound models. Image analysis results demonstrating wound diameter 7 days post cleaning. * = $p \leq 0.05$ when compared to non-treated controls.



Mean ± SEM Re-epithelialisation in Epiderm full thickness wound models. Image analysis results demonstrating amount of re-epithelialisation from wound edge 7 days post cleaning. * = $p \leq 0.05$ when compared to non-treated controls.

Conclusions

The combination of EpiDerm™ FT and sensitive in situ microscopic analysis is a viable model to investigate biofilms in wounds.
We have demonstrated that UAS is effective in both removing biofilm from biological, soft surfaces and reducing re-growth.
Additionally, our data suggest that treatment with the UAS improves wound healing independent of biofilm removal.
Work is ongoing to explore the potential mechanisms involved.

Acknowledgements

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