Development of "real-world" *ex vivo* porcine skin biofilm models to assess the efficacy of a range of wound care products

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Introduction

Biofilms can cause chronic wound infections, as they can penetrate deep into tissue evading antimicrobial treatments. Given the preponderance of biofilms in wounds and the associated impact on public health, it is important to explore anti-biofilm therapeutics that can prevent formation and persistence of biofilms in acute wounds, consequently decreasing the incidence of chronic wounds and improving wound-healing recovery times. *Ex vivo* porcine skin models have been wildly used to assess the efficacy of antimicrobials and wound-healing therapies. *Ex vivo* models are more reflective of real wounds compared to current methods that test on hard surfaces, aiming to bridge the gap between *in vitro* and *in vivo* testing. The aim of this study is to utilize an *ex vivo* porcine skin model to develop "real-world" biofilm for assessing the efficacy of wound care products.

Method

Porcine skin explants were inoculated with *Pseudomonas aeruginosa*, incubated for 7-days at 37°C, to encourage mucoid biofilm formation. (Figure 4- A)

Treatment:

Type 1 (T1) - Mucoid biofilm skin samples were treated with each debridement tool (three debridement tools were used in this study) using circular clockwise and counter-clockwise motions for 2 minutes.

Type 2 (T2) - 10cm x 10cm wound dressing contain antimicrobial (WD) samples were placed onto mucoid biofilm skin samples and incubated for 24 hours at 37°C.

Biopsies were removed from treated/untreated skin. Biopsies were placed into Phosphate buffered saline (PBS) or neutralizer, for T1/T2, for viable bacteria quantification using plate counts. In addition, biopsies were placed into sterile water in T1/T2, for protein quantification using a BCA Protein Assay kit.

(T1)

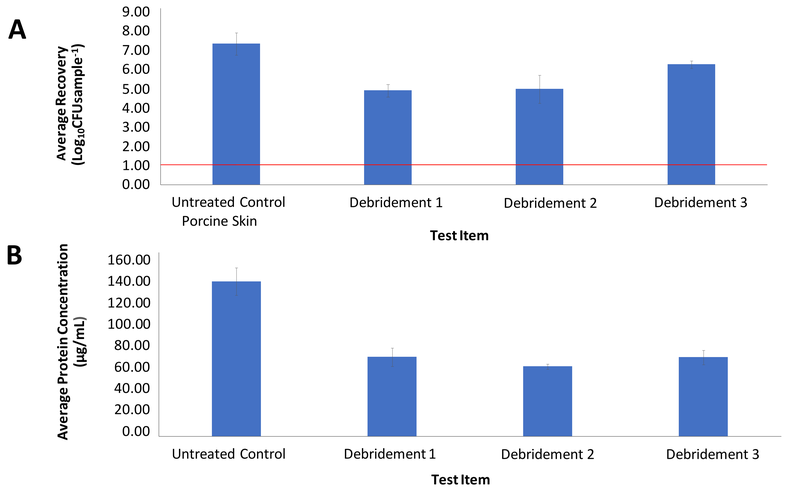


Figure 1. Average viable *P. aeruginosa* recovered from 5 x 5 cm treated porcine skin samples (A) and average protein recovery from 8 mm treated porcine skin samples (B) compared to untreated porcine skin samples. CFU = colony forming units. Error bars represent standard deviation. Red line represents limit of detection.

(T2)

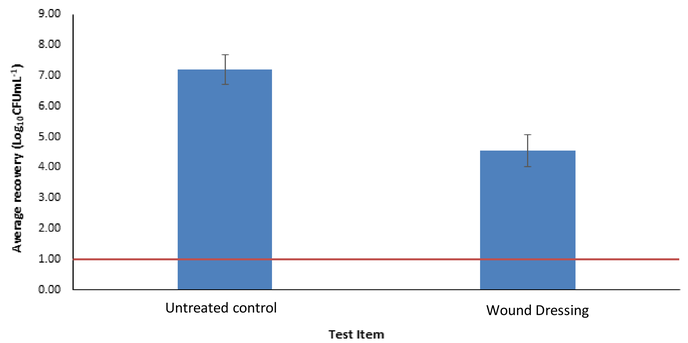


Figure 2. Average viable *P. aeruginosa* recovered from each 8 mm untreated and treated skin biopsies, following 7 days incubation with *P. aeruginosa* at 37 °C (untreated control) and 7 days incubation with *P. aeruginosa* at 37 °C followed by 24 hours treatment with dressing samples (treated skin). Error bars represent standard deviation. Red line represents limit of detection (1.00 Log). CFU = colony forming units.

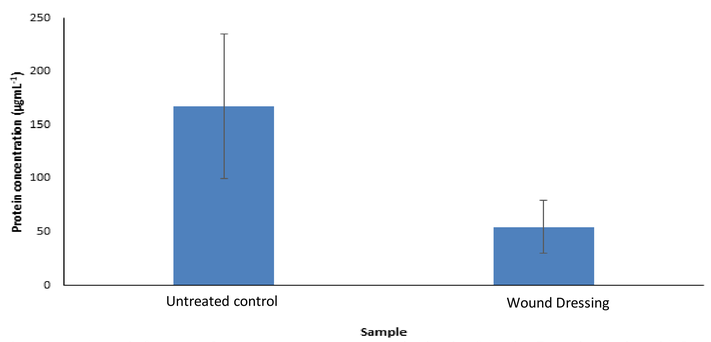
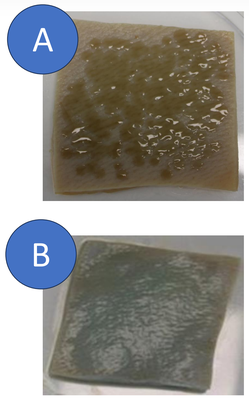


Figure 3. Average total protein from each 8 mm untreated and treated skin biopsies, following 7 days incubation with *P. aeruginosa* at 37 °C (untreated control) and 7 days incubation with *P. aeruginosa* at 37 °C followed by 24 hours treatment with dressing samples (treated skin). Error bars represent standard deviation.



(T1)

Figure 4.
(A) *Ex vivo* porcine skin explant following incubation with Pseudomonas aeruginosa at 37°C for 7 days

(B) *Ex vivo* porcine skin explant following 2 minutes debridement treatment

Results

T1 - Following treatment with debridement 1, an average reduction of 99.35% of viable *P. aeruginosa* was observed ($p < 0.05$). There was also a 48.77% reduction in protein ($p < 0.05$). Results from Debridement 2 and 3 (Two leading brands) were not significantly different to results demonstrated by Debridement 1 tested. (Figure 1 & 4)

T2 - A significant > 2.5 Log reduction (99.91 %) in viable *P. aeruginosa* was achieved on 8 mm biopsies when 7-day pre-formed biofilms cultured on porcine skin were treated for 24 hours with wound dressing samples. An average reduction of 112.82 ± 24.64 µgmL-1 (67.36%) in total protein following 24 hours treatment with test dressing samples, compared to the untreated skin samples at 7 days (Figure 2 & 3).

Conclusions

In vitro models which mimic the real-world scenario are extremely important in predicting clinical performance as they provide a complex yet quantitative assessment of medical devices.

Results demonstrates that both products reduce *P. aeruginosa* biofilm and protein quantities within a chronic wound. Within a clinical setting this would be beneficial to decrease the viable bacterial load within the mucoid exudate, associated with a chronic wound, and facilitate the progression towards typical healing pathways.

Future work could include ELISA and RT-qPCR for gene expression and SEM imaging, to support innovative product development.

This project was carried out by NAMSA, NAMSA, Sci-Tech Daresbury, Keckwick Lane, Cheshire, UK, WA4 4AD Tel +44 1925 864838, www.namsa.com