A NEW ANTI-BIOFILM HYDROFIBER® DRESSING: DEMONSTRATION OF ENHANCED SILVER PENETRATION & BIOFILM REMOVAL *IN VITRO*

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Introduction

There is increasing evidence that in the majority of chronic wounds there are bacteria present in their biofilm phenotype.[1] In this form bacteria produce a slime-like excretion (extracellular polymeric substance; EPS) that anchors to a surface and provides them with a protective environment which makes host defences, antibiotics and antiseptics much less effective.[2]

The majority of antimicrobial testing is performed using solutions of bacteria in their planktonic form, therefore the results may be misleading. Performing tests against biofilm is more difficult and quantification of results requires time-consuming skilled microbiological and/or microscopy work. An alternative and more rapid quantitative assay that could predict the effectiveness of antimicrobial products against biofilm in a representative environment would be a useful additional investigative tool.

Aim

To develop a model that uses chemical analysis to follow biofilm removal and predict antimicrobial effectiveness. To use this model to evaluate the effectiveness of a family of absorbent wound dressings.

Figure 1. 24-hour S. aureus biofilm supported on a 25 mm Anodisc in contact with growth medium in a deep 6-well culture plate (biofilm is stained pink for clarity in these photographs)

Method

Preparation. *Staphylococcus aureus* (NCIMB 9518) in log-phase growth was diluted in tryptone soy broth (TSB) to approximately 1x10^6 cfu/ml. Microbial counts were performed to determine an accurate concentration. 7 ml volumes of TSB were aseptically dispensed into each well of a deep 6-well plate (BD Biosciences). Anodisc filters (25 mm dia. membrane filters (0.2 µm), Whatman) were carefully placed onto the support ribs within each well such that the TSB was only in contact with the downward-facing face (Fig 1). 0.1 ml aliquots of the bacterial suspension were pipetted on to the centre of the upper face of each Anodisc. The plate lid was replaced and the plate incubated at 35 ± 3°C. After 24 hours Anodiscs were removed and rinsed for 1 minute with 30 ml volumes of 0.85% w/v sodium chloride solution (saline) to remove planktonic bacteria and unattached matter.

Dressing Challenge. Biofilm-covered Anodiscs were placed biofilm uppermost into individual 55 mm Petri dishes. 24 mm diameter circles of test dressings were applied (Fig 2) and immediately hydrated with 0.7 ml volumes of saline. The lids were replaced and dishes incubated for 24 hours at 35 ± 3°C. Three replicates were performed for each dressing type (Fig 3) (with three controls where no dressing was applied). An example of the hydrated dressing being removed is shown in Fig 2.

Figure 2. Test dressing application and removal

Figure 3. Test dressings

Generic Name	Coding	Commercial Name
Hydrofiber® dressing	**HF**	AQUACEL®
Silver Hydrofiber® dressing	**SHF**	AQUACEL® Ag
Anti-biofilm Hydrofiber® dressing	**ABHF**	AQUACEL® Ag+ (CE Marked 2013)

References:
1. James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds. Wound Rep Reg 2008; 16:37-44.
2. Metcalf DG, Bowler PG (2013). Biofilm delays wound healing: A review of the evidence. Burns Trauma 1:5-12.
3. Flemming H-C, Wingender J (2010). The biofilm matrix. Nature Rev Microbiol 8:623-633.

Method (cont.)

Analysis

After 24 hours incubation, Anodiscs and dressings were placed separately into individual plastic sample tubes containing 10 ml volumes of 1.2 M hydrochloric acid (aq). Tubes were agitated for 10 minutes or until all of the residual biofilm had dissolved (Fig 4). The resultant solutions were filtered through 0.45 µm membrane filters (Whatman) to remove any bacteria or dressing fibres and then assayed for potassium (K^+), magnesium (Mg^{++}), calcium (Ca^{++}) and silver (Ag^+) content by an inductively-coupled plasma mass spectrometer (ICP-MS, Agilent Technologies 7700 Series – Fig 4).

Figure 4. Dissolution of residual biofilm and the ICP-MS used to analyse samples

Results & Discussion

Group II divalent metal ions – magnesium (Mg^{++}) and calcium (Ca^{++}) play an important part in the stability of EPS acting as cross-links between polysaccharides.[3] Group I metal ions are ubiquitous in EPS but can be readily exchanged. Sodium is the most abundant Group I metal ion but is too concentrated for simultaneous analysis with the divalent ions. Potassium (K^+) is a less abundant and therefore usable alternative. All analytes are present at measurable and adequately reproducible levels. Silver (Ag^+) is virtually absent (Fig 5).

Figure 5. Analytes found to be present in biofilm

µg per biofilm sample (n=3)	Magnesium (Mg^{++})	Potassium (K^+)	Calcium (Ca^{++})	Silver (Ag^+)
Biofilm	**5.37** ± 0.81	**3.17** ± 0.75	**57.0** ± 7.0	**0.013** ± 0.006

Measuring the same metals in the test dressings shows levels of magnesium and calcium to be reproducible and relatively low. Potassium is present at a similar level to biofilm. Again results are quite reproducible (Fig 6). It is unlikely that any single treatment will fully remove biofilm. Therefore, in order to prevent its recovery and re-establishment, it is important also to reduce the bioburden as much as possible. Transfer of silver to the biofilm will be an indication of antimicrobial activity. Fig 7 shows the results of silver assay of the residual biofilm after a single dressing 24-hour application.

Figure 6. Analytes found to be present in test dressings

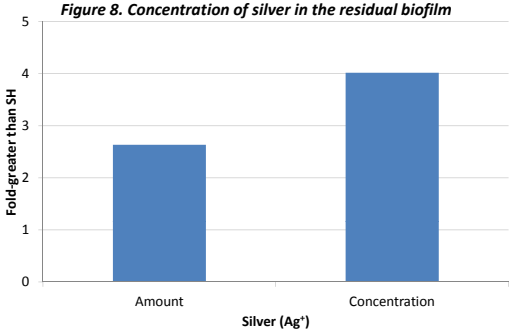
µg per dressing sample (n=3)	Magnesium (Mg^{++})	Potassium (K^+)	Calcium (Ca^{++})
HF	**0.077** ± 0.003	**2.2** ± 0.3	**2.03** ± 0.06
SHF	**0.047** ± 0.001	**3.4** ± 0.1	**2.03** ± 0.03
ABHF	**0.045** ± 0.001	**4.7** ± 0.1	**1.94** ± 0.02

Figure 7. Residual silver in biofilm after test dressing application

µg in biofilm sample (n=6)	Ag
Untreated Biofilm	**0.0**
HF	**0.0**
SHF	**7.3**
ABHF	**19.3**

However, it has already been established that the residual biofilm after application of ABHF is less than that left by SHF. Taking this into account the relative concentration of silver in the residual biofilm can be calculated (Fig 8).

Figure 8. Concentration of silver in the residual biofilm



Conclusion

This model requires further development and validation but it shows great potential in assisting with our understanding of how products interact with biofilm and to help in the development of better products. All Hydrofiber® dressings (HF, SHF and ABHF) removed approximately 70-80% of the calcium and magnesium from *S. aureus* biofilms. This will have lead to a reduction in biofilm EPS integrity. The rank order of effectiveness at removing biofilm (as indicated by potassium assay) was:

ABHF > SHF > HF (78% : 66% : 37%)

This order is consistent with visual observations. Biofilm treated with ABHF contained 2.6-times the amount of silver as biofilm treated with SHF. When the lower residual mass is taken into account, the concentration of silver in the biofilm is 4-times greater. These assays suggest that AQUACEL® Ag+ will be significantly better than the existing AQUACEL® and AQUACEL® Ag dressings in managing bacteria in the biofilm state.