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Use of internally validated in vitro biofilm models to assess antibiofilm performance of silver-containing gelling fibre dressings

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Use of internally validated *in vitro* biofilm models to assess antibiofilm performance of silver-containing gelling fibre dressings

Objective: To assess the efficacy of five silver-containing gelling fibre wound dressings against single-species and multispecies biofilms using internally validated, UKAS-accredited *in vitro* test models.
Method: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* single- and multispecies biofilms were cultured using Centres for Disease Control (CDC) biofilm reactors and colony drip flow reactors (CDFR). Following a 72 hour incubation period, the substrates on which biofilms were grown were rinsed to remove planktonic microorganisms and then challenged with fully hydrated silver-containing gelling fibre wound dressings. Following dressing application for 24 or 72 hours, remaining viable organisms from the treated biofilms were quantified.
Results: In single-species *in vitro* models, all five antimicrobial dressings were effective in eradicating *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm bacteria. However, only one of the five dressings (Hydrofiber technology with combination antibiofilm/antimicrobial technology) was able to eradicate the more tolerant single-species *Candida albicans* biofilm. In a more complex and stringent CDFR biofilm model, the hydrofiber dressing with combined antibiofilm/antimicrobial technology was the only dressing that was able to eradicate multispecies biofilms such that no viable organisms were recovered.
Conclusion: Given the detrimental effects of biofilm on wound healing, stringent *in vitro* biofilm models are increasingly required to investigate the efficacy of antimicrobial dressings. Using accredited *in vitro* biofilm models of increasing complexity, differentiation in the performance of dressings with combined antibiofilm/antimicrobial technology against those with antimicrobial properties alone, was demonstrated.
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biofilms • *Candida albicans* • multispecies • *in vitro* biofilm model • *Pseudomonas aeruginosa* • *Staphylococcus aureus* • silver-containing gelling fiber wound dressings

Persistent infections have been attributed to biofilm, such as infections associated with cystic fibrosis,[1] catheter-associated urinary tract infections[2] and hard-to-heal wounds.[3] Studies have shown that managing biofilm has a beneficial impact on wound healing.[4–6] Wound bioburden is most effectively managed today with regular debridement, cleansing and antimicrobial wound dressings.[7,8] Selecting an effective topical antimicrobial agent can be challenging for clinicians when the data used to support antimicrobial claims is usually based on *in vitro* studies involving single-species planktonic bacteria. Malone et al.[9] reported the prevalence of biofilms in hard-to-heal wounds to be approximately 78% and concluded that their data supported clinical assumptions that biofilms are ubiquitous in human hard-to-heal wounds. The same expert panel also highlighted the importance of *in vitro* methods that better mimic the clinical situation. There is a current industry-wide lack of robust data that clinicians can refer to when selecting appropriate topical antimicrobials with additional antibiofilm performance.[10,11] The medical device sector, including advanced wound care, is starting to see a shift in terms of data that reflects the biofilm aetiology. There has been an emergence of antibiofilm claims relating to existing technologies, and novel technologies are being specifically designed to incorporate antibiofilm modes of action.[12]

Published studies describe the assessment of medical devices using a multitude of *in vitro* biofilm methods, in addition to collecting basic planktonic data via AATCC Test Method 100 and ASTM E2149 methodologies. Biofilm models include, but are not limited to, high-throughput adapted minimum biofilm eradication concentration (MBEC) methods,[12] filter disc supported biofilms,[12] and more specific *in vitro* biofilm models suitable for biofilm assessment using isothermal microcalorimetry.[13] Other well-characterised biofilm models include the Centers for Disease Control (CDC) reactor,[14] flow cell reactors and drip flow reactors.[15] In addition, there are numerous publications referencing custom-designed research.[16] With such a plethora of test models, it is challenging to compare product performance using standard platforms.

Key steps towards standardising biofilm testing came in 2018. The American Society for Testing and

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Materials (ASTM) published *in vitro* test methods that define biofilm growth and biofilm treatment. The Environmental Protection Agency (EPA) then adopted these methods and they were used to support label claims for a hard, non-porous surface cleaning agent. These methods culture and treat reproducible *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. They were designed specifically for assessing biocides, however, Perfectus Biomed Ltd. gained UKAS accreditation (ISO 17025, Accreditation number 9192) by adapting and validating these methods specifically for wound care product assessment. These validated biofilm test methods have been accredited for the culture of reproducible single-species biofilms on solid surfaces. They provide a reliable screen for assessing wound care products against tolerant biofilm microorganisms rather than susceptible planktonic microorganisms. However, they do not closely replicate the complexities of a chronic wound. These complexities include the multispecies nature of clinical biofilm, biofilm maturity, appropriate dressing exposure time, and a continuous fluid flow that mimics wound exudation. The adapted methods presented in this study mitigate some of these limitations by adding rigor and a level of clinical applicability to the externally validated methods.

This study uses both standard and adapted versions of accredited methods to create a series of models of increasing complexity and clinical relevance, with an ultimate objective being to better understand how antimicrobial wound dressings, with and without incorporated antibiofilm technologies, perform in a complex simulated clinical scenario. Specifically, we challenged five silver-containing gelling fibre wound dressings against single- and multispecies *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* biofilms using standard and adapted UKAS-accredited ISO 17025 CDC reactor models and (CDFR) models.

Methods

Test wound dressings

A range of commercially available silver-containing gelling fibre wound dressings were included in this study:

- Dressing A: carboxymethylcelluose (CMC) dressing containing ionic silver, ethylenediaminetetraacetic acid (EDTA) and benzethonium chloride (BEC). (AQUACEL Ag+ Extra, ConvaTec Ltd., UK; AQUACEL Ag Advantage in the US)
- Dressing B: lipid-colloid and polyabsorbent fibre dressing containing silver sulphate (UrgoClean Ag, Urgo Ltd., UK)
- Dressing C: a polyvinyl alcohol (PVA)) dressing coated with a hydroxypropylcellulose (HPC) gel containing silver sulphate (Exufiber Ag+, Molnlycke Health Care AB, Sweden)
- Dressing D: CMC/calcium alginate dressing containing a silver sodium zirconium phosphate (Maxorb Extra Ag+, Medline Industries Inc., US)
- Dressing E: CMC dressing containing silver oxysalts (KerraCel Ag, Crawford Healthcare Ltd., UK).

Microbial strains and culture conditions

Staphylococcus aureus NCTC 8325, *Pseudomonas aeruginosa* NCIMB 10434 and *Candida albicans* ATCC MYA-2876 SC5313 were maintained as stock cultures in cryovials at –79°C for long-term storage.

Single-species biofilm in the CDC biofilm reactor

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Candida albicans* cultures (24 hours) were harvested from Tryptone Soya Agar (TSA) (Acumedia, Trafalgar Scientific, UK) or Sabouraud Dextrose Agar (SDA) (Acumedia, Trafalgar Scientific, UK) and used to prepare 50ml of $1x10^8$ CFU/ml suspensions in Tryptone Soya Broth (TSB) or Sabouraud Dextrose Broth (SDB; *Candida albicans* only) using internal methods. The inocula were diluted further in TSB or SDB to give $1x10^7$ CFU/ml suspensions and the prepared inocula were transferred to separate CDC reactors (400ml per reactor).

Each CDC reactor (BioSurface Technologies, Bozeman, US), containing 24 polycarbonate coupons in 8 rods and 400ml of the inoculated TSB or SDB, was incubated in batch phase (no additional flow of nutrients through the CDC reactor throughout incubation) for 72 hours at 37±2°C, stirring at 50rpm, to encourage biofilm development on the polycarbonate coupons.

Following 72 hour incubation, CDC reactor coupons were aseptically removed from their rods and rinsed three times in sterile phosphate buffered saline (PBS) to remove planktonic cells. Rinsed coupons were transferred to a 24-well challenge plate and sandwiched between fully hydrated (2ml of PBS and 1% TSB) 2cm x 2cm sections of test wound dressings. Untreated controls consisted of rinsed coupons immersed in 2ml of PBS and 1% TSB. Positive controls consisted of rinsed coupons immersed in 2ml chlorine-based bleaching agent with 5% anionic surfactant (Flash with Bleach; P&G, UK). Tests and controls were performed simultaneously, in triplicate, incubating in a sealed 24-well plate for 24 hour at 37±2°C.

Following the 24 hour test period, the treated CDC coupons were transferred to 2ml of neutraliser Dey-Engley Broth, (Acumedia, Trafalgar Scientific, UK) for 5 minutes. Following neutralisation, the coupons were sonicated (VWR, UK) for 5 minutes to recover remaining attached microorganisms. Serial dilutions were carried out in the resultant neutralisation medium and viable organisms were plated onto TSA or SDA and incubated at 37±2°C for 24 hours. The number of colonies were counted, CFU/ml calculated and converted to Log_{10}CFU/ml recoveries and Log_{10}CFU/ml reductions, compared with an average of the negative controls. The limit of detection for this study was 0.70 Log_{10}CFU/ml in accordance with Currie.[17]

Table 1. Quantity of viable bacteria recovered, and average Log reductions compared with the untreated control, following 24 hour exposure to 72 hour single-species *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms with dressings A-E. Dressings and the untreated control n=3

Test dressing	*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
	Average recovery (Log_{10}CFU/ml)	Average reduction (Log_{10}CFU/ml)	Average recovery (Log_{10}CFU/ml)	Average reduction (Log_{10}CFU/ml)
Untreated control	5.00±0.08	N/A	7.06±0.28	N/A
Positive control	≤0.70±0.00	4.30±0.08	≤0.70±0.00	6.36±0.28
Dressing A	≤0.70±0.00	4.30±0.08	≤0.70±0.00	6.36±0.28
Dressing B	≤0.70±0.00	4.30±0.08	≤0.70±0.00	6.36±0.28
Dressing C	≤0.70±0.00	4.30±0.08	≤0.70±0.00	6.36±0.28
Dressing D	≤0.70±0.00	4.30±0.08	≤0.70±0.00	6.36±0.28
Dressing E	≤0.70±0.00	4.30±0.08	≤0.70±0.00	6.36±0.28

N/A—not applicable; Limit of detection 0.70 Log_{10}CFU/ml

Multispecies biofilm in the CDC biofilm reactor

The 24 hour cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were harvested from TSA or SDA and used to prepare individual suspensions of $1x10^8$ CFU/ml in 50ml, $1x10^6$ CFU/ml in 5ml and $1x10^7$ CFU/ml in 5ml of growth media (75% deionised water, 20% TSB, 5% SDB and 0.1% glucose) of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, respectively. The concentration of the initial inocula were confirmed by serial dilution and plate counting. The prepared mixed species microbial inocula were combined into 500ml of growth media (60ml mixed species inocula was added to 440ml of NB), and 400ml transferred to a single CDC reactor containing polycarbonate coupons. The CDC reactor was incubated for 72 hours at 37±2°C, stirring at 50rpm to encourage biofilm development.

Following 72 hours incubation, CDC reactor coupons were rinsed to remove planktonic organisms. Triplicate coupons were then transferred to a 24-well challenge plate and sandwiched between 2cm x 2cm sections of hydrated wound dressings. Triplicate coupons were immersed in 2ml untreated control (growth media) and 2ml of chlorine-based bleaching agent with 5% anionic surfactant (positive control). Solutions and dressings were tested concurrently. Test biofilms were incubated in challenge plates for 24 hours at 37±2°C.

Following the 24 hours test period, the CDC coupons were transferred to 2ml of neutraliser and the coupons were sonicated for 5 minutes to recover remaining biofilm microorganisms. Serial dilutions were carried out on the resultant neutralisation medium and total viable organisms were plated onto on Brain Heart Infusion Agar (BHIA) and incubated at 37±2°C for 24 hours. Mixed colonies were identified based on colony colour and size, which was confirmed using Gram staining. The counted colonies were calculated as CFU/ml and converted to Log_{10}CFU/ml recoveries and Log_{10}CFU/ml reductions, compared with the negative control. The limit of detection for this study was 0.70 Log_{10}CFU/ml in accordance with Currie.[17]

Multispecies biofilm in the colony drip flow reactor model

The CDFR method was adapted from Woods et al.[18] The 24 hour cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were harvested from TSA or SDA plates and used to prepare a $1x10^7$ CFU/ml *Staphylococcus aureus* suspension, a $1x10^4$ *Pseudomonas aeruginosa* CFU/ml suspension and a $1x10^5$ CFU/ml *Candida albicans* suspension in growth medium. The prepared suspension (10µl) was transferred to a 0.22µm pore-diameter porous polycarbonate membrane (Whatman, SLS, Nottingham, UK), which was placed on top of a 2.5cm-diameter absorbent pad (Millipore, SLS, UK). The membrane and absorbent pad were adhered to glass slides within a CDFR (Bio Surface Technologies, Bozeman, US) and left at room temperature (18–23°C) for 30 minutes. The flow system was attached such that the growth media flowed downward from the influent port to the effluent port at a rate of 5ml/hour per channel. The assembled system was then incubated at 37±2°C for 72 hour to allow biofilm to develop.

Following 72 hours incubation, biofilm membranes were removed from the reactor and rinsed in PBS to remove planktonic organisms. The rinsed membranes were transferred to a challenge plate and sandwiched between two growth media-saturated (5ml) 4cm x 4cm sections of test wound dressing (n=3 per test dressing). A set of membranes were immersed in 5ml growth media (untreated control) or a chlorine-based bleaching agent with 5% anionic surfactant (positive control). Controls and tests were performed concurrently for 72 hours at 37±2°C to reflect clinical practice. The limit of detection for this study was 0.70 Log_{10}CFU/ml.

Following the 72 hours test period, the biofilm membranes were transferred to 10ml of neutraliser (DE broth) and the membranes were sonicated for 5 minutes to recover remaining biofilm microorganisms. Serial dilutions were carried out on the resultant recovery medium and total viable organisms were plated onto BHIA and incubated at 37±2°C for 24 hours. Mixed colonies were identified based on colony colour and size, which was confirmed using Gram staining. The counted colonies were calculated as CFU/ml and converted to Log_{10}CFU/ml recoveries and Log_{10}CFU/ml reductions, compared with the negative control. The limit of detection for this study was 0.70 Log_{10}CFU/ml in accordance with Currie.[17]

Statistical analyses

To assess statistical differences between viable organisms recovered from the test and control coupons, Log_{10}CFU/ml data was analysed using a Student's t-test (two-tailed, unequal variance). Data means were considered significantly different where $p<0.05$.

Results

Single-species biofilm cultured in CDC reactor

No viable *Staphylococcus aureus* or *Pseudomonas aeruginosa* were recovered from 72 hours biofilm coupons following 24 hour exposure to any of the antimicrobial test dressings. This equated to reductions of greater than 4.30±0.08 Log_{10}CFU/ml ($p<0.001$) and 6.36±0.28 Log_{10}CFU/ml ($p<0.001$), respectively, compared with the average of the untreated controls (Table 1).

Viable *Candida albicans* were not recovered from 72 hour biofilm coupons following 24 hour exposure to dressing A. This equated to a reduction of 4.28±0.20 Log_{10}CFU/ml compared with an average of the untreated controls ($p<0.001$). However, viable *Candida albicans* were recovered from coupons treated for 24 hours with dressings B, C, D and E, which resulted in average reductions compared with the untreated control of 1.75±0.29, 2.45±0.74, 0.73±0.25 and 2.43±0.67 Log_{10}CFU/ml, respectively ($p<0.01$) (Fig 1). Significantly less *Candida albicans* was recovered following exposure to dressing A when compared with dressings B, C, D and E ($p<0.001$ in each comparison).

Multispecies biofilm cultured in CDC reactor

An average of 7.51±0.10 Log_{10}CFU/ml total viable microorganisms were recovered from 72 hour untreated control multispecies biofilm coupons. No viable organisms were recovered from 72 hour biofilms following 24 hour exposure to dressing A. This equated to a reduction of greater than 6.81±0.10Log_{10}CFU/ml compared with the untreated controls ($p<0.001$). Following exposure to dressings B, C, D and E, average Log reductions of 5.42±0.45, 6.05±1.86, 1.94±0.33 and 6.67±0.99 Log_{10}CFU/ml of total viable microorganisms were observed, respectively ($p<0.001$ versus control) (Fig 2). Significantly less viable organisms were recovered from dressing A compared with dressings B ($p<0.01$) and dressing D ($p<0.01$).

Fig 1. Quantity of total viable *Candida albicans* recovered from 72 hour single-species biofilm CDC coupons after 24 hour exposure to dressings A–E. Dressings and the untreated control were tested in triplicate (n=3). ***indicates Log_{10} reductions that were significantly different to the untreated control, $p<0.001$. Limit of detection is 0.70 Log_{10}CFU/ml

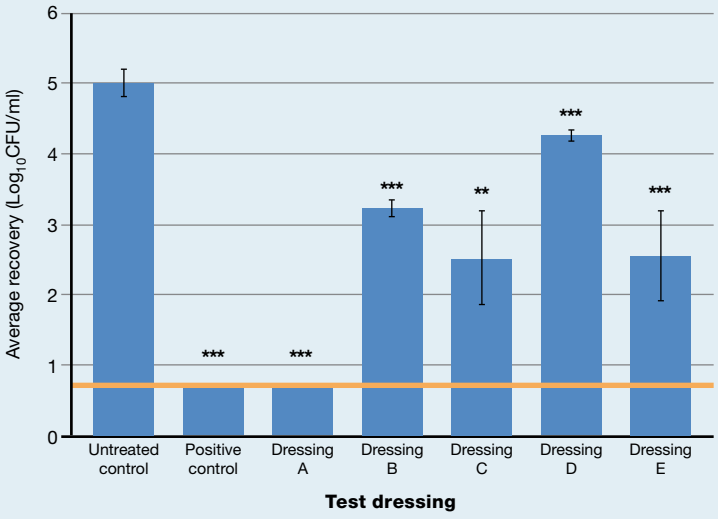
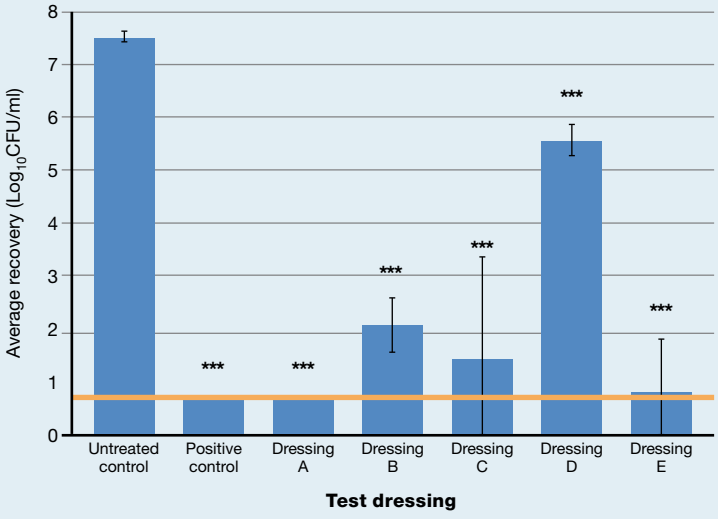


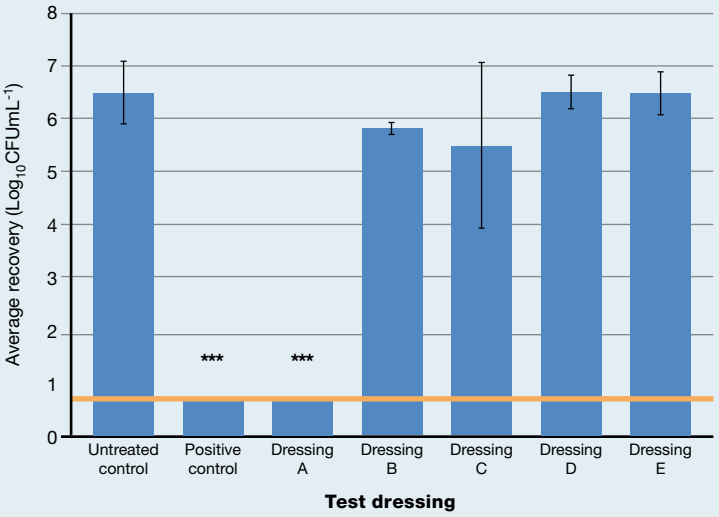
Fig 2. Quantity of total viable organisms recovered from 72 hour multispecies biofilm CDC coupons after 24 hour exposure to dressings A–E. Dressings and the untreated controls were tested in triplicate (n=3). ***indicates Log_{10} reductions that were significantly different to the untreated control, $p<0.001$. Limit of detection is 0.70 Log_{10}CFU/ml



Multispecies biofilm cultured within the colony drip flow reactor

An average of 6.46±0.59 Log_{10}CFU/ml total viable microorganisms were recovered from 72 hour biofilm untreated control membranes. No viable organisms were recovered from 72 hour biofilm membranes following 72 hour exposure to dressing A ($p<0.001$). This equated to a greater than 5.76±0.00 Log_{10}CFU/ml

Fig 3. Quantity of total viable organisms recovered from 72 hours multispecies biofilm CDFR membranes after 72 hours exposure to dressings A–E. Dressings and the untreated control were tested in triplicate (n=3). ***indicates Log_{10} reductions that were significantly different to the untreated control, p<0.001. Limit of detection is 0.70 Log_{10}CFU/ml

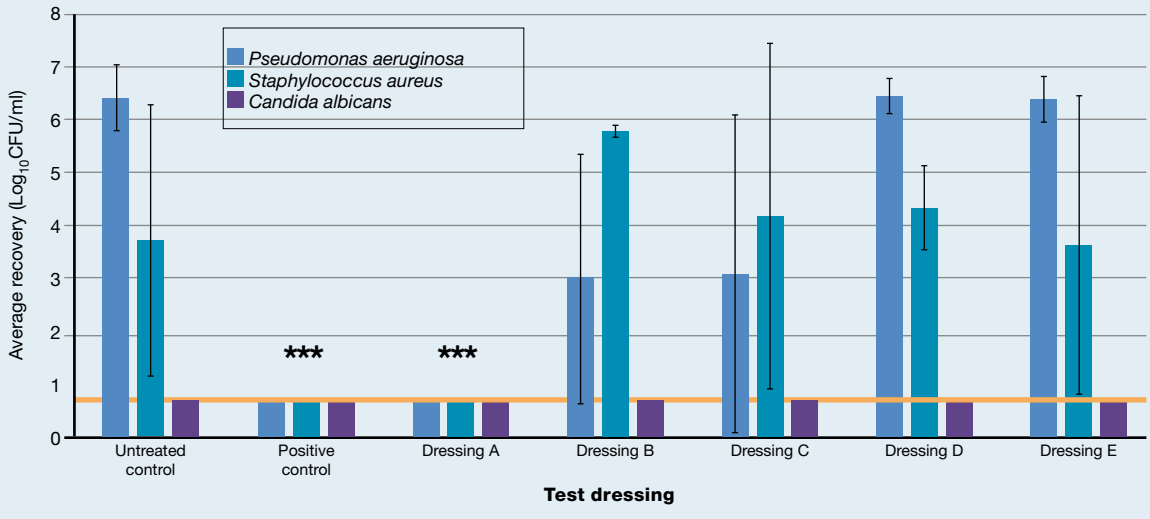


log reduction compared with the untreated controls. Following exposure to dressings B, C, D and E, average reductions of 0.66±0.10, 1.01±1.56, no reduction, and 0.01±0.41 Log_{10}CFU/ml of total viable microorganisms were observed, respectively (Fig 3). Reductions by dressings B, C, D and E compared with the untreated control were not statistically significant (p>0.05). *Pseudomonas aeruginosa* and *Staphylococcus aureus* were recovered from membranes treated with dressings B, C, D and E. No viable *Candida albicans* was recovered from the total viable microorganism samples (Fig 4). Significantly fewer viable organisms were recovered from biofilm membranes exposed to dressing A compared with dressings B, C, D and E (p<0.001 for each comparison).

Discussion

The CDC reactor biofilm model uses surface attachment as a measurement of biofilm formation (ASTM E2562-17).[19] The CDC reactor is an good model for the growth of reproducible biofilm. However, the biofilms are adhered to a smooth, non-porous, solid surface meaning there is no opportunity for the microorganisms to invade the surface. This in turn makes associated biofilm easier to treat than a more integrated, tissue-adhered biofilm. Within this CDC model, all five silver-containing gelling fibre wound dressings demonstrated *in vitro* efficacy against 72 hour single-species *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm microorganisms. However, the challenge of treating single-species *Candida albicans* biofilm resulted in product differentiation, whereby only dressing A reduced the fungal load below the limit of detection. Log_{10} reductions of between 0.73±0.25 and 2.45±0.74 were observed for Dressings B, C, D and E, demonstrating the differentiating impact of fungal biofilm models. *Candida albicans* infections, resulting from biofilms of a bilayer of yeast cells and hyphae,[20] are both clinically important and under-reported.[21] When compared with bacterial cells, yeast cells are larger, and the composition of the cell walls differ so that *Candida albicans* infections are typically less susceptible to antimicrobial treatment.[22] This is demonstrated commercially when antimicrobial products successfully gain antibacterial but not antifungal claims. *Candida albicans* resistance to

Fig 4. Quantity of viable *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* organisms recovered from 72 hours multi-species biofilm CDFR membranes after 72 hours exposure to dressings A-E. Dressings and the untreated control were tested in triplicate (n=3). ***indicates Log_{10} reductions that were significantly different to the untreated control, p<0.001. Limit of detection is 0.70 Log_{10}CFU/ml



antifungals is well documented, while the biofilm phenotype has been cited as a potential contributor to *Candida albicans* drug resistance.[23] Links between a decreased susceptibility to antifungal agents in biofilm-encased *Candida albicans* compared with planktonic *Candida albicans* has been related to the high levels of extracellular material produced by candida biofilms.

The inclusion of a fungal challenge enhanced dressing differentiation in terms of antibiofilm performance, but the single-species CDC model did not address complexities of multispecies cultures. The multispecies models proved to be effective dressing differentiators in that only dressing A consistently reduced the mixed species challenge below the levels of detection whereas in the single species *Staphylococcus aureus* and *Pseudomonas aeruginosa* models dressings A, B, C, D and E all demonstrated consistent biofilm reductions.

In order to further challenge the test dressings in a model that better mimicked a hard-to-heal wound, the dressings were ultimately assessed in a CDFR multispecies biofilm model that included the challenge of fluid flow. Unlike the CDC reactor, the CDFR biofilms are grown on a 0.2µm polycarbonate membrane and receive a slow fluid flow of nutrients via a cellulose support pad from underneath the membrane. Nutrients are therefore supplied to the establishing biofilm from the surface by which they are attached for 72 hour, simulating an exuding wound. The dressing treatment duration was increased to 72 hour to mimic a typical clinical dressing change interval. It is important to test products against mature, established biofilms that closely mimic the real-world environment. When multispecies biofilms were formed within the CDFR, *Candida albicans* was not recovered at the end of the 72 hour test period. However, dressings that had previously demonstrated efficacy against single-species biofilms (dressings B, C, D and E) had a reduction in efficacy against the multispecies biofilm. This suggests that the multispecies influence enhances the virulence of the remaining organisms. *Pseudomonas aeruginosa* and *Candida albicans* mixed-species populations are associated with an increase in multidrug resistance-associated proteins and an increase in mutability.[23] Within a mixed *Pseudomonas aeruginosa* and *Candida albicans* population, interspecies competition is thought to trigger iron-regulated virulence factors such as proteases and cytotoxic molecules,[24] both of which may degrade the wound bed and delay wound healing. This suggests that multispecies biofilm populations have the potential to be both less susceptible to antimicrobial treatments while being more damaging to the wound bed.

The antimicrobial test dressings used in the study are categorised as silver-containing gelling fibre wound dressings. The product differentiation demonstrated in this study could, in part, be attributed to the chemical form of silver used in the dressing, such as ionic silver bound to CMC, silver sulphate, silver sodium zirconium phosphate, silver oxysalt, or the availability of silver from the dressing. However, these cannot be the only contributing factors. All dressings contain silver salts that can dissociate to give antimicrobial silver ions but dressing A out-performed the other dressings in the more challenging CDFR multispecies biofilm tests. Other factors considered to impact performance include, the concentration of silver, the carrier dressing material, for example CMC, alginate, poly(vinyl alcohol), and the method of dressing manufacture.[25] Manufacturing variables such as temperature have been shown to affect the antimicrobial efficacy of the dressing; heat-treated nanocrystalline silver dressings ranged in efficacy from excellent to negligible as a result of varying temperature treatments.[26] However, in the absence of manufacturing insight for dressings A–E, it is not possible to comment on this factor. Dressing A supports healing by managing wound exudate, infection and biofilm.[27] These combinations of factors are collectively referred to as the dressing's 'combination antibiofilm/antimicrobial technology'.[12,13,28,29] This antibiofilm technology is implicated as the differentiator between dressing A and dressings B-E in the more challenging models presented in this study.

Clinical data further supports the antibiofilm impact of dressing A. Dressing A was investigated for safety and efficacy in two clinical studies. Biofilm was suspected in 54% and 74% of wounds initially, but in only 27% and 45%, respectively, by the end of the studies.[27,30] A concurrent increase in granulation tissue was also reported as the studies progressed.[30]

The differentiation in dressing performance between the single-species and multispecies biofilm models demonstrates the criticality of selecting more complex *in vitro* models to predict clinical performance. If the clinical need is to use effective antibiofilm therapy to improve wound healing outcomes, then the selected test methods should be able to differentiate products in terms of their ability to manage mature, multispecies biofilm communities in fluid-challenged models over clinically relevant durations. Health professionals should demand unbiased data that incorporates multispecies biofilm, the inclusion of challenging fungal pathogens, the growth substrate and fluid flow, and moreover, the use of validated and accredited test methods to support their choice of antimicrobial wound dressings.

Limitations

The single-species CDC reactor biofilm model does not attempt to address clinical complexities such as proteinaceous wound exudate, fluid flow, multispecies cultures or patient's comorbidities, which increase the challenge posed to antimicrobial dressings by binding with active agents, enhancing microbial attachment, giving rise to microbial synergies, and dampening the body's natural immune response, respectively. The

multispecies CDC model considered the mixed-species challenge, and the CDFR biofilm model additionally addressed fluid flow. However, these models did not address interactions of the wound dressings with biofilms formed on wounded tissue or host immune responses.

Conclusions

In vitro biofilm test methods cannot completely model the clinical scenario because every clinical case is unique, however it is possible to assess wound care products using reliable, reproducible models that mimic a range of clinically relevant complexities. These more complex models are a better differentiator of products, and therefore health professional should demand more compelling evidence to inform their choice of advanced wound dressings. Wound care clinicians should request data that has been generated using validated, independently accredited biofilm models that have been designed to replicate some of the challenges of hard-to-heal wound scenarios. In this study, the increasing complexities of the models used successfully differentiated silver-containing gelling fibre dressings in terms of their antimicrobial efficacy. This differentiation demonstrates the importance of considering proven antibiofilm technologies for the clinical management of biofilms in hard-to-heal wounds. **JWC**

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Reflective questions

- Why is *in vitro* test data important when selecting an antimicrobial wound dressing for clinical use?
- Why is biofilm testing important when evaluating the antimicrobial efficacy of wound care products?
- What are the advantages of mixed-species biofilm models compared with single-species biofilm models?