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RESEARCH LETTER

Assessment of Silver-Containing Gelling Fiber Dressings Against Antibiotic-Resistant Pathogens Using an in vitro Biofilm Model

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Introduction

Dressings play a key role in wound care by providing the optimal conditions for healing through bioburden control and exudate management. Wound dressings containing a source of ionic silver—a broad-spectrum antiseptic—are commonly used to manage wound bioburden. However, in hard-to-heal wounds microorganisms largely exist in biofilm form, making them more difficult to manage than those in planktonic form.[1] Biofilm has long been implicated as a barrier to healing of hard-to-heal wounds. Microorganisms in biofilm are encased in a matrix of extracellular polymeric substances and have slower replication and metabolic rates, thus providing strong defense against the host immune system as well as antibiotics and antiseptics. There are several dressings available containing fibers that gel on absorption of wound fluid. Gelling of such dressings enables absorption of large amounts of exudate and micro-contouring to the wound bed, reducing dead spaces where microorganisms can grow. While maintaining a moist wound environment is considered best practice, establishing the right balance is crucial as excessive moisture can also result in damage (eg maceration of peri-wound skin).

There are a number silver-containing gelling fiber dressings available that provide both management of exudate and bioburden control. The gelling properties, as well as the silver content and form, can differ substantially in such dressings. Several in vitro studies have been performed to assess the antibiofilm activity of various wound dressings; however, the methods used vary in robustness and validity, making it difficult to differentiate between the dressings. Given the challenge of biofilm combined with the emergence of antibiotic-resistant pathogens and increasing concerns around antimicrobial stewardship, there is a need for stringent and more realistic models to differentiate the ability of antimicrobial dressings to reduce biofilm.

The aim of this in vitro study was to evaluate the effectiveness of a range of silver-containing gelling fiber wound dressings against antibiotic-resistant biofilm bacteria in a stringent, robust biofilm model.

Materials and Methods

Test Dressings

Four dressings were evaluated to compare their in vitro antibiofilm activity:

- Carboxymethylcellulose (CMC) dressing containing ionic silver, ethylenediaminetetraacetic acid (EDTA) and benzethonium chloride (BEC) ("CISEB"; Aquacel® Ag+ Extra™, Convatec Ltd., Deeside, UK)
- CMC dressing containing silver oxysalts (Ag^{3+}) ("CSO"; KerraCel® Ag, 3M, Bracknell, UK)
- Polyacrylate (polyabsorbent) fiber dressing with an acrylic core and silver sulphate ('PSS'; UrgoClean Ag, Urgo Medical Ltd., Loughborough, UK)
- Non-woven polyvinyl alcohol fiber dressing containing silver sulphate ('PVASS'; Exufiber® Ag+, Mölnlycke Health Care Ltd., Milton Keynes, UK)

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Preparation of Biofilm Model

Separate suspensions of each challenge organism, extended spectrum beta lactamase-producing antibiotic-resistant *Pseudomonas aeruginosa* (RPA; NCTC 13437, National Collection of Type Cultures, Salisbury, UK) and community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA; USA300; HPA Reference: H045260142, Health Protection Agency, Salisbury, UK), were prepared in maximum recovery diluent (MRD; Neogen Corporation, Lansing, MI, USA) and adjusted to yield a concentration of approximately 1×10^8 colony forming units (CFU)/mL. An inoculation medium was prepared by diluting 0.1 mL of the bacterial suspension in 9.9 mL of 50:50 v/v tryptic soy broth (Neogen Corporation, Lansing, MI, USA) and fetal bovine serum (FBS, Biowest, Nuaillé, France) in sterile 100 mL Duran bottles.

Samples of a sterile knitted viscose gauze (N-A® Gauze, 3M, Bracknell, UK) were aseptically prepared (40 mm diameter) and transferred into the Duran bottles containing the inoculation medium. The bottles were then incubated at 35°C (± 3°C) for 48 hours in a shaking incubator set at 150 rpm. Following incubation, the biofilm-gauze samples were washed in 0.85% saline (2×100 mL volumes) and cut to a uniform size (35 mm diameter) using a biopsy punch. A total viable count (TVC) was then performed on biofilm-gauzes to confirm the level of biofilm (T_{0hr} count).

A series of tryptic soy agar (TSA) contact plates were inserted into the center of separate simulated wound assemblies (Figure 1). Biofilm-gauzes were then individually transferred onto each TSA contact plate to simulate a biofilm-colonized wound bed (n=3 per dressing for each time point).

Evaluation of Test Dressings

Test dressings (10×10 cm samples) were applied to the simulated biofilm-colonized wound model, hydrated with 8 mL of simulated wound fluid (SWF; 50:50 v/v MRD and FBS) and covered with a transparent film dressing (Tegaderm™ Film, 3M, Bracknell, UK) (Figure 1A). A biofilm-gauze without a dressing was included as a control to monitor biofilm viability over the course of the challenge period (n=1 for each time point).

Following incubation at 35°C (± 3°C) for 6, 24, 48, 72, 96 or 120 hours, three simulated biofilm-colonized wounds for each test dressing and one control were tested for each time point. The test dressings were removed (Figure 1B) and the biofilm-gauzes transferred into separate stomacher bags containing Dey-Engley neutralizing broth (Neogen Corporation, Lansing, MI, USA) to neutralize residual antimicrobial activity. Each bag was then homogenized using a stomacher to release biofilm bacteria from the gauze such that TVCs could be performed on the resultant solutions.

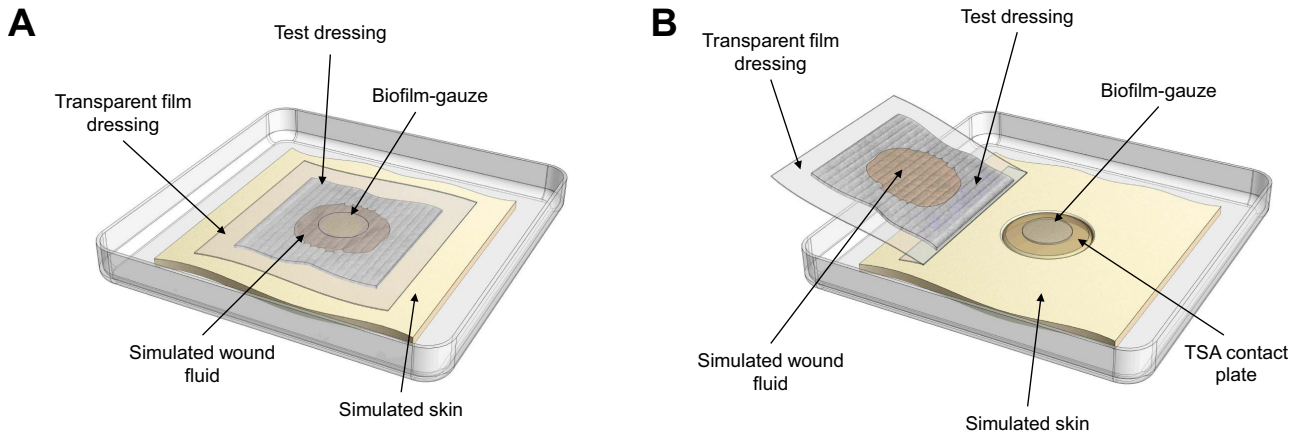

Figure 1 Simulated wound biofilm model with CISEB and secondary transparent film dressing application within the wound assembly (**A**) and following removal of dressing for enumeration of surviving biofilm on the gauze (**B**).
Abbreviations: CISEB, carboxymethylcellulose dressing containing ionic silver, ethylenediaminetetraacetic acid and benzethonium chloride.

Results

The initial bacterial challenge was approximately 1×10^{10} CFU/gauze for RPA biofilm and 4×10^{9} CFU/gauze for CA-MRSA biofilm. Levels of both RPA and MRSA remained high throughout the test period for the no-dressing biofilm-gauzes (biofilm controls). All test dressings reduced biofilm bacteria levels for both species within 6 hours of application, however, the extent of kill varied markedly between dressings (Figure 2).

The greatest reduction in biofilm was observed for CISEB, which reduced the RPA biofilm population by ~6 $\log_{10}$ (million-fold reduction) within 48 hours (Figure 2A). This kill rate was sustained for the duration of the challenge period, with CISEB reducing the RPA biofilm population to non-detectable levels (<30 CFU/gauze) by 96 hours (>8.8 $\log_{10}$ reduction in RPA biofilm bacteria). Similar antibiofilm activity was observed against CA-MRSA biofilm (Figure 2B), where CISEB reduced the viable CA-MRSA biofilm population by >5 $\log_{10}$ within 48 hours. This kill rate was sustained with a steady decrease in the CA-MRSA biofilm population to non-detectable levels (<30 CFU per test) by 96 hours, an >8.4 $\log_{10}$ reduction, which was maintained until the end of the test period (120 hours).

Smaller reductions in biofilm bacteria were observed with the other three test dressings compared with CISEB, with biofilm cell survival remaining comparatively high throughout the test for both species (Figure 2). The greatest reductions were observed for RPA biofilm, with maximum reductions of 3.2 $\log_{10}$ (at 24 hours), 3.5 $\log_{10}$ (at 96 hours) and 1.28 $\log_{10}$ (at 72 hours) for CSO, PSS and PVASS, respectively. Even more modest reductions were observed for CA-MRSA biofilm, with maximum reductions of 1.1 $\log_{10}$ (at 96 hours), 0.9 $\log_{10}$ (at 24 hours), 0.6 $\log_{10}$ (at 6 hours) for CSO, PSS and PVASS, respectively.

Discussion

There are several silver-containing gelling fiber dressings available that claim to have antibiofilm activity. Our findings show that there were marked differences in the antibiofilm activity of the tested dressings in this challenging in vitro biofilm model, with CISEB showing far greater activity than CSO, PSS and PVASS. This work follows previous studies which evaluated the antibiofilm activity of silver-containing dressings, utilizing stringent biofilm models that consider key aspects of the wound environment (eg, the use of SWF, provision of moisture from below, a realistic simulated wound topography, use of a secondary dressing, and relevant testing timepoints linked to dressing wear times).[2,3] The current study also continues previous work which evaluated the antibiofilm activity of silver-containing gelling fiber wound dressings using adapted and internally validated standard in vitro biofilm test methods.[3]

The greater antibiofilm activity of CISEB compared with the other three silver-containing gelling fiber dressings observed in this study may be attributed to the additional antibiofilm agents within the dressing. EDTA is a metal chelating agent and BEC is a surfactant, which together act to disrupt biofilm. When silver was applied in combination with these antibiofilm agents, a synergistic effect was observed compared with their administration alone.[4] Our findings are inconsistent with a previous study which reported comparable antibiofilm activity between CSO and CISEB against non-antibiotic-resistant strains of *P. aeruginosa* and *S. aureus* using in vitro and ex vivo models.[5] Similarly, an in vitro evaluation of PSS demonstrated a reduction in MRSA biofilm of 4.6 $\log_{10}$ after 24 hours and up to 4 $\log_{10}$ after 7 days, which is far greater than the maximum reduction of 0.5 $\log_{10}$ observed in the current study.[6] Dressing performance is more readily differentiated in biofilm models that are realistic and stringent, and the inconsistent findings with the previous studies are likely attributed to the different testing methods used.

It should be noted that the dressing treatment times were based on previous testing using this biofilm model[2] in which a longer incubation time (120 hours) was required to reduce CA-MRSA to the detection limit by CISBE, whereas RPA only required 96 hour treatment. The observation that CA-MRSA was reduced to the limit of detection limit 24 hours earlier in this study may be attributed to variations in the cover dressing used and the adjustment in SWF hydration of the CISEB dressings due to the change in cover dressing (the film dressing is not absorbent).

There are several limitations to the current study. In vitro models cannot necessarily replicate how real wound biofilm may form (ie, in surface-attached patches or colonies, and/or in aggregates within wound tissues) and it is unclear how the findings of this study translate to real-life clinical practice. Our work attempts to simulate the clinical situation by using gauze-attached biofilm on a moist simulated wound bed with a "skin" surrounding. Similar in vitro models utilizing permeable membranes over

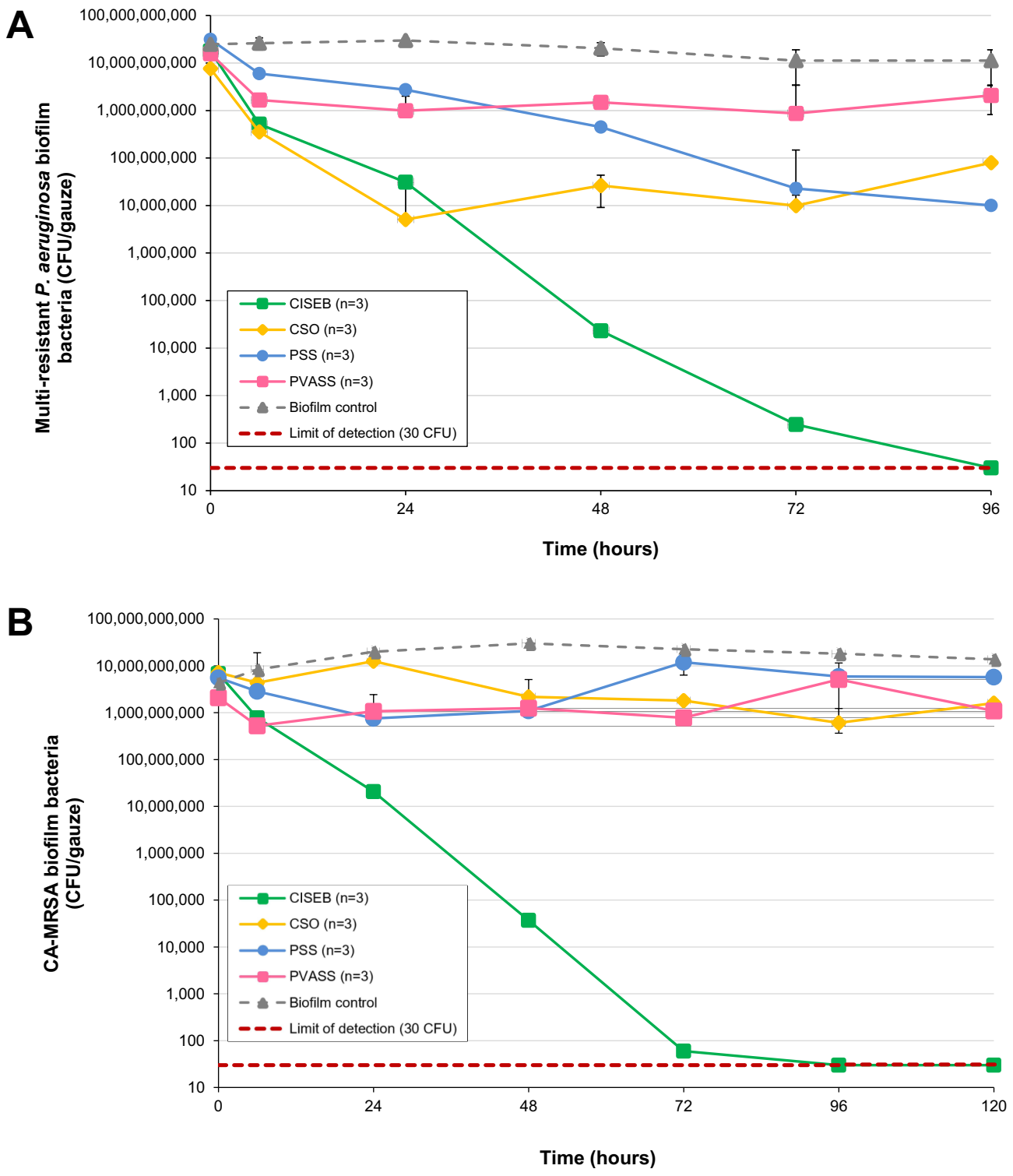

Figure 2 Antimicrobial activity of test dressings against RPA biofilm over 96 hours (**A**) and CA-MRSA biofilm over 120 hours (**B**).
Abbreviations: CFU, colony-forming units; CISEB, carboxymethylcellulose dressing containing ionic silver, ethylenediaminetetraacetic acid and benzethonium chloride; CSO, carboxymethylcellulose dressing containing silver oxysalts; PSS, polyacrylate (polyabsorbent) fiber dressing with an acrylic core and silver sulphate; PVASS, non-woven polyvinyl alcohol gelling fiber dressing containing silver sulphate.

moist agar have been reported.[7] However, thick biofilm in three-dimensions, such as that cultured on gauze, represents a worst-case, challenging scenario. Moreover, the test model included single-species aerobic biofilm, whereas clinical wound biofilm may be polymicrobial and may include anaerobic bacteria and fungi. Other factors, such as pH of the system and dressing

attributes such as the ability to manage exudate or the ability to conform to simulated wound surfaces of the dressing, were also not evaluated. However, it is known that the CMC in CISEB dressing is buffered to a low pH,[8] and previous studies have also demonstrated marked differences in the ability of various gelling fibers to absorb exudate.[9]

In conclusion, this robust and stringent in vitro biofilm model has demonstrated marked differences in the ability of various silver-containing gelling fiber dressings to kill two antibiotic-resistant pathogens in their biofilm form. Further work is warranted to assess the ability of silver-containing gelling dressings to reduce wound biofilm bioburden in clinical practice.

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Disclosure

All authors are employees and/or shareholders of Convatec Ltd.

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