In vitro assessment of silver-containing gelling fiber dressings against wound surface-associated antibiotic-resistant pathogens

Kate Meredith, PhD [1], Matilda Coleborn, MSc[1], Daniel Metcalf, PhD[1]

[1]*Convatec Ltd, Deeside, UK*

Introduction

- Increasing awareness of the implications of wound surface-attached microbial communities is supported by a growing evidence base, and this microbial phenotype is now considered to be one of the key local barriers to wound healing[1]
- Current clinical practice around the management of surface-attached microbial communities focuses primarily on good wound bed preparation techniques and the use of antimicrobial dressings[1]
- There are several silver containing gelling fibers dressings available, in this study we use antibiotic-resistant bacteria within a stringent, robust model to compare the evaluate their antimicrobial efficacy against surface-attached microbial phenotype

STUDY OBJECTIVE

In this *in vitro* investigation silver-containing gelling fiber dressings in their ability to kill surface attached communities of extended-spectrum beta lactamase (ESBL)-producing antibiotic-resistant *Pseudomonas aeruginosa* or community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA)

Methods

Microbial challenge preparation

- Separate suspensions of each challenge organism, ESBL *P. aeruginosa* and CA-MRSA, were inoculated into Tryptone Soy Broth/Fetal Bovine Serum (50/50 v/v) to give a final concentration of approximately 1×10^6 CFU/mL
- N-A gauze samples 44 mm in diameter (the substrate for the surface attached bacteria), were added to the above suspensions, and incubated at 35±3°C for 48 hours in a shaking incubator. Following incubation, samples were washed in 0.85% saline, to remove planktonic bacteria
- A total viable count (TVC) was performed on the samples to confirm the levels of biofilm

Table 1. Test primary dressings

Test primary dressing	Challenge organisms & timepoints
CISEB: carboxymethylcellulose dressing containing ionic silver, ethylenediaminetetraacetic acid (EDTA) and benzethonium chloride (BEC)	ESBL *P. aeruginosa* (NCTC 13437): 6, 24, 48, 72 & 96 hrs
PSS: polyacrylate (polyabsorbent) fiber dressing with an acrylic core and silver sulphate	
CSO: carboxymethylcellulose dressing containing silver oxysalts	CA-MRSA (USA300): 6, 24, 48, 72, 96 & 120 hrs
PVASS: non-woven polyvinyl alcohol gelling fiber dressing containing silver sulphate	

Simulated wound assembly (SWA) setup

- SWA consists of a porcine leather-covered Perspex plate (simulating peri-wound skin), surrounding a central insert of a 55 mm-diameter Tryptone Soy Agar (TSA) contact plate (simulating a moist wound bed with a reservoir of isotonic nutrients), which supported the surface-attached bacteria (Figure 1)
- The wound area was covered with the test primary dressing and a transparent film dressing (secondary dressing) (n=3 for each time point) and incubated at 35±3°C (Table 1)
- A no-dressing control was also performed to monitor bioburden viability over the experiment course (n=1 for each time point)

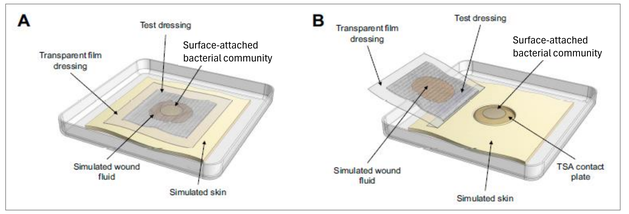

Figure 1. SWA with CISEB and secondary transparent film dressing application within the wound assembly (A) and following removal of dressing for enumeration of surviving surface-attached bacterial community on the gauze (B)

TVCs

- Following incubation, the bacterial surface-attached communities for all tests and controls were separately homogenized (to release the bacteria) in Dey-Engley Neutralizing Broth (to neutralize residual antimicrobial activity), and TVCs were performed

Confocal Laser Scanning Microscopy (CLSM)

- The bacterial surface-attached communities at T_0hr were examined by CLSM to establish the presence of biofilm before test dressing application
- BacLight® (Live/Dead stain™) was used to stain bacteria using the procedure as described by the manufacturer (Figure 2)

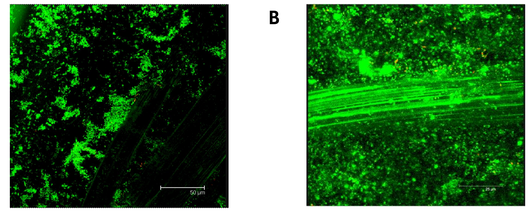

Figure 2. T_0 hour CSLM image of CA-MRSA (A) and ESBL *P. aeruginosa* (B) surface attached on N-A gauze before application of test dressings. Bacteria have been stained with BacLight™ Live/Dead® stain, which stains live bacteria green and dead

Results

Antimicrobial activity against ESBL *P. aeruginosa* surface-attached community

- Following dressing application, CISEB dressing was the only dressing to achieve a decrease in bacterial numbers to below detectable levels over the full test period (Figure 3), i.e., an approximate 9 $\log_{10}$ reduction by 96 hours
- Although some of the other dressings showed some reduction in numbers, none achieved greater than 4 $\log_{10}$ reduction over the testing period
- The viable bacteria numbers for PSS did show some decrease over time, but CSO and PVASS dressings showed an initial decrease followed by a slow increase in bacteria numbers
- This indicates that the CISEB dressing was most effective in reducing ESBL *P. aeruginosa* numbers compared with the other dressings tested

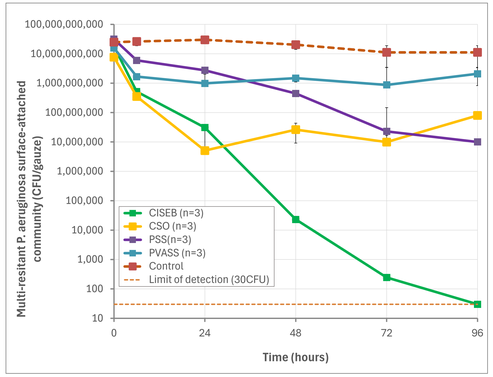

Figure 3. ESBL-producing *P. aeruginosa* surface-attached community results over 96 hours for test dressings and control

Antimicrobial activity against CA-MRSA surface-attached community

- Following dressing application, CISEB dressing was the only dressing to achieve a decrease in bacterial numbers to below detectable levels over the full test period (Figure 4), i.e. an approximate 10 $\log_{10}$ reduction by 96 hours
- CA-MRSA reduction in the presence of the other silver dressings was minimal, all showing a <3 $\log_{10}$ reduction over the testing period, and following initial decreases, viable bacteria numbers for these dressings remained consistent and did not further decrease, or in some cases increase
- This indicates that the CISEB dressing was most effective at reducing CA-MRSA surface-attached communities compared with the other dressings tested

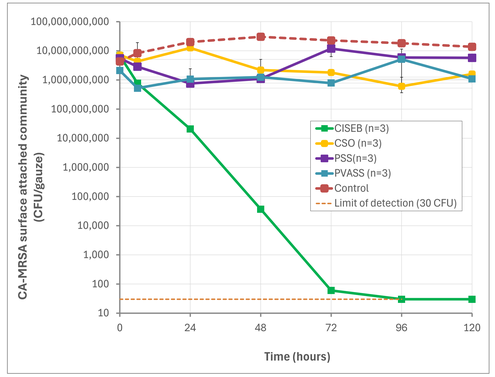

Figure 4. CA-MRSA surface-attached community results over 120 hours for silver test dressings and control

Discussion

The dressings used in this *in vitro* study are all reported to have antimicrobial activity against surface-attached bacterial communities in various laboratory test methods. Data generated in this challenging *in vitro* wound model, which simulates many of the physical features of a heavily-colonized wound, illustrates:

- The CISEB dressing was the most effective in reducing ESBL *P. aeruginosa* and CA-MRSA surface-attached communities over the testing period compared with CSO, PVASS, and PPS dressings
- This may be attributed to the additional components (EDTA and BEC) that aid in the breakdown of these communities along with the bacterial killing of the ionic silver within gelling dressing

CONCLUSION

This robust and stringent in vitro bacterial surface-attached wound model has demonstrated marked differences in the ability of various silver-containing gelling fiber dressings to kill two antibiotic-resistant pathogens

References & Footnotes

1. Metcalf DG & Bowler PG. *Burns Trauma* 2013;1:5–12.

Victoria Rowlands and Nicola Burke (both formerly Convatec Ltd.) for assistance with the laboratory testing. Editorial support was provided by Kenny Tran (Convatec Ltd.). Manjunath Penagondla providing artwork support for Figure 1 (Convatec Ltd.).

CISEB: Aquacel® Ag+ Extra™ (Convatec, UK); **PSS:** UrgoClean Ag (Urgo Medical Ltd); **CSO:** KerraCel ®Ag (3M); **PVASS:** Exufiber® Ag+ (Molnlycke Health Care Ltd).

Presented at Symposium on Advanced Wound Care (SAWC), 14-18th May 2024, Orlando, FL