In vitro assessment of a methylene blue and gentian violet-containing foam dressing and an advanced silver-containing gelling fiber dressing against surface-associated antibiotic-resistant bacteria

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Introduction

- There is increasing evidence that the presence of surface-associated or aggregated microbial communities (i.e., biofilm) is a key local barrier to wound healing[1]
- Current clinical practice around the management of surface-associated microbial communities focuses primarily on good wound bed preparation techniques and the use of antimicrobial dressings[1]
- There are several antimicrobial dressings with differing mechanisms available[2]
- In this study, we evaluated the antimicrobial activity of two dressings with distinct mechanisms against surface-associated antibiotic-resistant bacteria using a stringent, robust model

Objective

To evaluate two antimicrobial dressings with distinct mechanisms against surface-associated antibiotic-resistant bacteria using a stringent, challenging *in vitro* model

Methods

Microbial challenge preparation

- Separate suspensions of each challenge organism, extended-spectrum beta lactamase (ESBL) *Pseudomonas aeruginosa* (RPA) and community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), were prepared in Maximum Recovery Diluent to yield a concentration of approximately 1×10^8 colony-forming units (CFU)/mL
- A 0.1 mL volume of each bacterial suspension was then diluted in 9.9 mL volumes of Tryptone Soy Broth/Fetal Bovine Serum (50/50 v/v) in sterile 100 mL Duran bottles to provide an inoculation medium (1×10^6 CFU/mL)
- N-A gauze samples, 44 mm in diameter (the substrate for the surface-associated 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 or loosely attached bacteria
- A total viable count (TVC) was performed to confirm initial bacterial populations

Table 1. Dressings

Test primary dressing	Secondary dressing
CISEB*: Carboxymethylcellulose dressing containing ionic silver, ethylenediaminetetraacetic acid (EDTA), and benzethonium chloride (BEC)	Transparent film dressing
PVA-MBGV†: Polyvinyl alcohol foam dressing containing methylene blue and gentian violet	

Simulated wound assembly (SWA) setup

- The 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 contact plate (simulating a moist wound bed with a reservoir of isotonic nutrients), which supported the surface-associated bacteria (**Figure 1**)
- The wound area was covered with the test primary dressing (CISEB or PVA-MBGV), then a transparent film secondary dressing (n=3 for each time point), and incubated at 35±3°C (**Table 1**)
- As per product IFU, the PVA-MBGV dressing was moistened with 0.85% saline and any excess solution removed by squeezing
- A no-dressing control was also performed to monitor bacterial viability over the experiment course (n=1 for each time point)

TVCs

- Following incubation, the surface-associated bacterial 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 (**Table 2**)

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-associated bacterial community on the gauze (B)

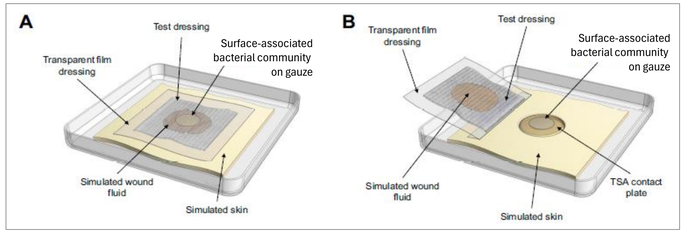


Table 2. Timepoints tested for each challenge organism

Challenge organisms	Time points tested (hr)					
	6	24	48	72	96	120
ESBL *P. aeruginosa* (NCTC 13437)						
CA-MRSA (USA300)						

Results

- PVA-MBGV produced an initial ~0.5 $\log_{10}$ reduction in **RPA** population at 6 hours, which was sustained throughout the 96-hour challenge period (**Figure 2**)
- CISEB reduced the RPA population by ~1.5 $\log_{10}$ at 6 hours and by ~6 $\log_{10}$ at 48 hours (million-fold reduction from initial challenge of ~1×10^{10} CFU/gauze) (**Figure 2**):
  - The RPA kill rate was sustained with the population reaching non-detectable levels (<30 CFU/gauze) by 96 hours (~8.8 $\log_{10}$ reduction)
- PVA-MBGV did not reduce **CA-MRSA** and population levels remained high throughout the 120-hour challenge period; the initial MRSA challenge (~3×10^9 CFU/gauze) was sustained at 48 hours with levels comparable to the no-dressing control at the remaining timepoints (**Figure 3**)
- CISEB reduced the CA-MRSA population by 1 $\log_{10}$ at 6 hours and >5 $\log_{10}$ at 48 hours:
  - The CA-MRSA kill rate was sustained and the population reached non-detectable levels by 96 hours (~8.4 $\log_{10}$ reduction) and 120 hours
- The no-dressing controls demonstrated challenge organism viability throughout the test periods

Figure 2. Surface-associated ESBL *P. aeruginosa* reduction over 96 hours for test dressings and control

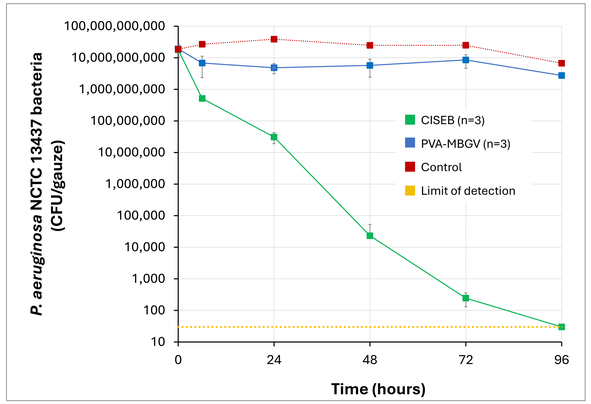
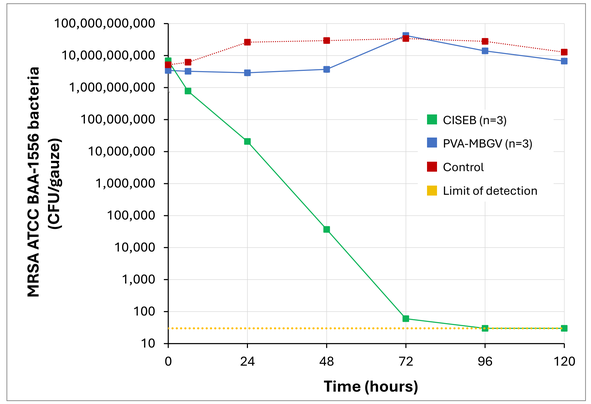


Figure 3. Surface-associated CA-MRSA reduction over 120 hours for test dressings and control



Discussion

- Within a stringent *in vitro* model of surface-associated antibiotic-resistant bacteria, PVA-MBGV demonstrated marginal activity against RPA and negligible efficacy against MRSA
- In comparison, within the same test method against the same challenge organisms, CISEB dressing reduced numbers of both challenge organisms to the limit of detection (<30 CFU/gauze), a ~8 $\log_{10}$ kill against both RPA and MRSA
- This may be attributed to the additional components (EDTA and BEC) that aid in the breakdown of these surface-associated communities along with optimized bacterial killing by ionic silver within gelling dressing

Conclusion

CISEB demonstrated superior antimicrobial activity against surface-associated RPA and CA-MRSA compared with PVA-MBGV, reducing populations to non-detectable levels

1. Metcalf DG, Bowler PG. Burns Trauma 2013; 1: 5-12.
2. Shi C, et al. Front Bioeng Biotechnol 2020; 8:182.

*Aquacel® Ag Advantage
†Hydrofera Blue® Classic

Presented at the Symposium on Advanced Wound Care (SAWC) Spring, April 30th–May 4th 2025, Grapevine, TX, USA