The antimicrobial efficacy of a silver alginate dressing against a broad spectrum of clinically relevant wound isolates

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ABSTRACT

Wound dressings impregnated with silver have a role to play in aiding to reduce both the dressing and wound microbial bioburden. It is therefore imperative that antimicrobial wound dressings have efficacy on a broad range of clinical significant microorganisms. Accordingly, this study aimed to determine the antimicrobial efficacy of a silver alginate dressing against 115 wound isolates that had been isolated routinely from patients at West Virginia University Hospital. Standardised corrected zones of inhibition (CZOIs) were performed on all clinical isolates. It was found that the silver alginate dressing was able to inhibit the growth of all microorganisms tested. In particular, the silver alginate dressing inhibited the growth of *Candida albicans* and yeasts with CZOI of 3–11·5 mm. All meticillin-resistant *Staphylococcus aureus* (MRSA) strains were found to be sensitive to the silver alginate dressing with a CZOI range calculated at 3–7·8 mm. Sensitivity to the silver alginate dressing was also evident for *S. aureus* and vancomycin-resistant Enterococci. CZOIs of 4·25 mm were calculated for *Enterococcus faecium* and 9·8 mm for *viridans streptococcus*. The bacteria which demonstrated the highest tolerance to ionic silver included *Enterobacter cloacae* and *Acinetobacter baumannii*. Contrary to this the most responsive microorganisms to ionic silver included strains of staphylococci, *viridans streptococcus* and *Candida albicans*. No antibiotic-resistant isolates, as identified by Kirby Bauer Clinical Laboratory Standards Institute classification system, were found to be resistant to ionic silver. When a selected number of microorganisms were grown in the biofilm phenotypic state enhanced tolerance to silver was observed, compared to their non biofilm counterparts. Overall, this study has demonstrated the broad antimicrobial activity of a silver alginate dressing on wound isolates grown in the non biofilm and biofilm state. This finding is clinically relevant as both the non biofilm and biofilm phenotypic states of microorganisms are evident in wounds and therefore significant to delayed healing. Consequently, it is imperative that antimicrobial wound dressings demonstrate antimicrobial activity against microorganisms in both phenotypic states.

Key words: Alginate • Biofilm • Infection • Silver • Wound

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Key Points

- the efficacy of antimicrobials on wound biofilms is of paramount importance because biofilms are now considered to delay wound healing
- therefore, disruption and control of biofilms in chronic wounds represent a fundamental requirement of a 'wound management strategy'
- silver impregnated wound dressings have been shown to be efficacious on antibiotic-resistant and silver-resistant bacteria
- additionally, their usage has been associated with enhanced wound healing rates
- in this study, the aim was to evaluate the antimicrobial efficacy of a silver alginate dressing against clinical isolates using preliminary screening methods [corrected zone of inhibition (CZOI)]
- although we recognised the limitations of the CZOI for antimicrobial efficacy screening, in particular the lack of a true cidal activity as indicated in other studies, it was for this reason we also employed the use of the biofilm poloxamer CZOI assay
- our aim was to enhance our knowledge of the antimicrobial activity of a silver alginate dressing further by investigating its broad spectrum of activity on both planktonic and sessile microorganisms
- it was our hypothesis that a silver alginate dressing would remain efficacious independent of selected cell wall composition (Gram positive and Gram negative), antibiotic-resistance category and biofilm phenotype on microorganisms commonly recovered from wounds
- in total, 115 microbial strains were evaluated in this study that had been routinely isolated from 108 intensive care unit patients with infected wounds

INTRODUCTION

Chronic wounds are a global problem, independent of socioeconomic and geographic boundaries. In the USA alone, chronic wounds involve 5·7 million patients per year at a cost in excess of 20 billion dollars annually. The incidence and prevalence of chronic wounds are set to increase given the disease pathophysiology and targeted ageing populations (1).

The repair of a wound involves the coordination of an array of different biological processes, including inflammation, angiogenesis, the development of granulation tissue and cellular remodeling culminating in the formation of a healed wound. However, progression of a wound to healing is impeded by a number of factors, in particular colonisation of the wound by microorganisms. An increasing wound microbial bioburden, and an increase in bacterial virulence and pathogenicity, has a significant effect in increasing the likelihood of the wound becoming infected (2). This is because many microorganisms in a wound produce an array of factors detrimental to healing, including toxins, enzymes and pro-inflammatory cytokines (3).

Common microorganisms that are routinely isolated from wounds have included *Staphylococcus aureus*, *Corynebacterium* sp, *Candida albicans* and *Pseudomonas aeruginosa* (4). However, chronic wounds are colonised with a diverse polymicrobial microflora known to affect healing rates and also to increase the risk of an infection developing. The microorganisms found within a wound exist both within a planktonic and biofilm phenotypic state (2). Consequently, antimicrobials used for the management of chronic wounds infections or the prevention of an infection must therefore demonstrate activity against microorganisms in both phentotypic states, as tolerance to antimicrobials is significantly different (5). In particular, the efficacy of antimicrobials on wound biofilms is of paramount importance because biofilms are now considered to delay wound healing (6–9). Therefore, disruption and control of biofilms in chronic wounds represent a fundamental requirement of a 'wound management strategy' (10).

Antimicrobial agents, including ionic silver, have a proven ability to kill and inhibit the growth of microorganisms when present within and on the outside of a wound dressing (6,11,12). A number of recent in vitro studies have confirmed that some silver wound dressings have efficacy on biofilms (13–17). In addition, silver impregnated wound dressings have been shown to be efficacious on antibiotic-resistant and silver-resistant bacteria (18). Additionally, their usage has been associated with enhanced wound healing rates (19).

In this study, the aim was to evaluate the antimicrobial efficacy of a silver alginate dressing against clinical isolates using preliminary screening methods [corrected zone of inhibition (CZOI)]. The disc diffusion antimicrobial sensitivity testing method was employed because it was considered as an easy, inexpensive and reliable method for rapid screening of a large number of microorganisms (20). Although we recognised the limitations of the CZOI for antimicrobial efficacy screening (21), in particular the lack of a true cidal activity as indicated in other studies (22), it was for this reason we also employed the use of the biofilm poloxamer CZOI assay (15). Poloxamer is a diblock copolymer of polyoxyethylene and polyoxypropylene and is used to induce a biofilm phenotypic state in microorganisms (23,24). Gilbert and colleagues have suggested that poloxamer hydrogels provide a reliable and reproducible method for testing the antimicrobial efficacy of antimicrobials on biofilms (23). Bacteria that are grown on poloxamer mimic many of the properties of biofilm-grown bacteria, including resistance to antimicrobials (15,23–28).

In conjunction with previous studies (12,29, 30), our aim was to enhance our knowledge of the antimicrobial activity of a silver alginate dressing further by investigating its broad spectrum of activity on both planktonic and sessile microorganisms. It was our hypothesis that a silver alginate dressing would remain efficacious independent of selected cell wall composition (Gram positive and Gram negative), antibiotic-resistance category and biofilm phenotype on microorganisms commonly recovered from wounds.

METHODS AND MATERIALS

Test microorganism

The microbial strains used in this study were clinical isolates kindly donated by West Virginia University Hospital, West Virginia, USA. In total, 115 microbial strains were

Table 1 Clinically isolated wound isolates included in this study

Microorganism	Number tested
Gram-positive bacteria	
MRSA	26
Staphylococcus aureus	11
VRE	16
Vancomycin-resistant *Enterococcus faecium*	2
Enterococcus faecium	2
Streptococcus pneumoniae	1
Group C *Streptococcus* sp	1
β-haemolytic Group A *Streptococcus* sp	2
β-haemolytic Group B *Streptococcus* sp	1
β-haemolytic Group C *Streptococcus* sp	1
Listeria sp	1
Staphylococcus lugdenensis	1
Coagulase-negative Staphylococci	3
Viridans Streptococcus	1
Gram-negative bacteria	
Pseudomonas aeruginosa	13
Gram-negative rods	4
Enterobacter cloacae	1
Acinetobacter baumanii	1
Acinetobacter sp	2
Aeromonas sp	1
Citrobacter freundii	2
Escherichia coli	3
Klebsiella pneumoniae	1
Salmonella sp	1
Serratia marcescens	2
Stenotrophomonas maltophilia	1
Yeast	
Candida albicans	9
Other yeasts	5
Total	115

MRSA, meticillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant Enterococci.

evaluated in this study that had been routinely isolated from 108 intensive care unit patients with infected wounds. The diversity and numbers of clinical isolates included in this study can be found in Table 1. All microorganisms were maintained as frozen cultures in a −80°C freezer. The cultures were revived in tryptic soy broth (TSB) and evaluated for purity by streaking on Mueller Hinton agar (MHA) prior to being used in the study.

Test dressings

Wound dressings that were evaluated in this study included a silver alginate (AMS, Winsford, UK) and a non silver gauze, and a non silver hydrofiber (ConvaTec, Flintshire, UK) dressing.

Test methods

Plating procedure (agar method)

Each purified microorganism, grown overnight on MHA plates, was added to 70 µl of TSB, vortexed (30 seconds) and then inoculated into 5 ml of blood bank saline (0·85%). Inoculated saline (containing 1×10^6 CFUs/ml of each microorganism) was then swabbed (using a sterile cotton swab) onto MHA plates, using Clinical Laboratory Standards Institute (CLSI) techniques.

Biofilm test method and procedure (poloxamer method)

The biofilm test model can be located elsewhere (13,15). In brief, poloxamer was incorporated into Mueller Hinton broth (MHB) at a concentration of upto 30% and refrigerated overnight (4°C). The dissolved poloxamer was then autoclaved and returned to the fridge. Liquefied poloxamer was then poured into petri dishes. Each petri dish was incubated overnight at 37°C before inoculation. Following the incubation the poloxamer gel plates were inoculated with a $1{\cdot}0 \times 10^6$ CFU/ml (colony forming unit) microbial suspension. The suspension was spread over the surface of the poloxamer gel plates to ensure complete coverage.

Measurement of the CZOI

All dressings were cut into 1 × 1 cm squares, pre-soaked (in saline for 30 seconds) and placed upon lawns of inoculated MHA or poloxamer plates. All plates were incubated for 24 hours and the CZOI was then determined. From both the MHA and poloxamer plates, the CZOI was determined by measuring the zone of clearing vertically and horizontally in millimetre for the test and control wound dressings. Hydration of wound dressing samples can cause them to contract inwards while increasing in height; therefore, a CZOI was calculated by subtracting the dimensions of the dressing, vertically and horizontally, from the zone of clearing around the dressing thus obtaining a CZOI value. All testing was done in duplicate.

Statistical analysis

A Student's *t*-test was used to compare the CZOIs. All data were analysed using Microsoft Excel software.

Table 2 Mean CZOIs to demonstrate the efficacy of silver alginate on wound solates

Organism	Number (clinical)	Mean CZOI (mm)	Range (mm)	SD
Yeast				
Candida albicans	9	8·7	3·0–11·5	±1·3
Yeasts	5	8·2	6·3–9·8	±2·1
Gram-positive bacteria				
MRSA	26	5·3	3·0–7·8	±0·0
Staphylococcus aureus	11	7·1	4·5–10·8	±0·6
Coagulase-negative *Staphylococcus* sp	3	9·0	8·2–10·7	±1·8
VRE	16	3·1	2·5–6·0	±0·18
Vancomycin-resistant *Enterococcus faecium*	2	6·0	5·0–7·0	±1·4
Enterococcus faecium	2	4·9	4·3–5·5	±0·9
β-haemolytic Group A *Streptococcus* sp	2	8·5	7·8–9·3	±2·1
β -haemolytic Group B *Streptococcus* sp	1	5·5	5·5	±0·0
β-haemolytic Group C *Streptococcus* sp	1	2·8	0–5·5	±3·9
Group C *Streptococcus* sp	1	4·0	0–8	±5·7
Streptococcus pneumonia	1	7·0	7·0	±0·0
Staphylococcus lugdenensis	1	7·5	7·5	±0·0
Listeria sp	1	6·0	6·0	±0·0
Viridans Streptococcus	1	10·0	8·0–12·0	±0·7
Gram-negative bacteria				
Pseudomonas aeruginosa	13	7·2	4·5–9·3	±1·3
Acinetobacter baumannii	1	1·8	1·5–2·0	±0·4
Acinetobacter sp	2	3·7	3·0–4·8	±0·2
Aeromonas sp	1	3·3	3·0–3·5	±0·4
Citrobacter freundii	2	3·9	2·0–5·75	±2·7
Escherichia coli	3	3·8	0·5–1·0	±3·0
Serratia marcescens	2	4	3·5–4·3	±0·6
Salmonella sp	1	1·5	0–3·0	±2·1
Stenotrophomonas maltophilia	1	4·3	4·0–4·5	±0·4
Gram-negative rod	4	2·7	2·3–3·5	±0·8
Klebsiella pneumoniae	1	3·3	2·0–4·5	±1·8
Enterobacter cloacae	1	1·5	0–3·0	±2·1

CZOI, corrected zone of inhibition; MRSA, meticillin-resistant *Staphylococcus aureus*; SD, standard deviation; VRE, vancomycin-resistant Enterococci.

Key Points

- the use of the CZOI method established that the silver alginate dressing has antimicrobial activity on a broad range of wound isolates
- the results indicated that the silver alginate dressing was able to inhibit the growth of all microorganisms tested
- bacteria which demonstrated a high tolerance to ionic silver included *Enterobacter cloacae* and *Salmonella* sp, with mean CZOIs comparable at 1·5 mm
- contrary to this, the most responsive microorganisms to ionic silver included strains of Staphylococci *viridans streptococcui* and *C. albicans*
- no antibiotic resistant isolates, as identified using the by Kirby Bauer CLSI classification system, were found to be resistant to ionic silver

RESULTS

The use of the CZOI method established that the silver alginate dressing has antimicrobial activity on a broad range of wound isolates. Table 2 provides a summary of the CZOI of all microorganisms growing in the non biofilm phenotypic state following exposure to the silver alginate dressing. The results indicated that the silver alginate dressing was able to inhibit the growth of all microorganisms tested. In particular, the activity of the silver alginate dressing was demonstrated against *C. albicans* and yeasts with a CZOI range of 3–11·5 mm calculated. All meticillin-resistant *S. aureus* (MRSA) strains were found to be sensitive to the silver alginate dressing; the CZOI range was calculated at 3–7·8 mm. Sensitivity to the silver alginate dressing was also demonstrated for *S. aureus* and vancomycin-resistant Enterococci (VRE). A mean CZOI of 4·9 mm were calculated for *Enterococcus faecium* and 10·0 mm for *viridans streptococcus*.

Bacteria which demonstrated a high tolerance to ionic silver included *Enterobacter cloacae* and *Salmonella* sp, with mean CZOIs comparable at 1·5 mm. Contrary to this, the most responsive microorganisms to ionic silver included strains of coagulase-negative Staphylococci (CNS), *Streptococcus* sp, and *C. albicans*. No antibiotic-resistant isolates, as identified by the Kirby Bauer CLSI classification system, were found to be resistant to ionic silver.

When a selected number of microorganisms were grown in the biofilm phenotypic state enhanced tolerance to silver was observed, when a comparison was made between

Table 3 Mean CZOI comparisons for a selected number of planktonic and biofilm-grown bacteria and yeast

	Mean CZOI (mm)			
Microorganism	Non biofilm state (agar)	SD	Biofilm state (poloxamer)	SD
Yeast				
Candida albicans	11·0	±0·0	3·5	±1·4
C. albicans	6·8	±0·4	3·5	±0·0
Yeast	6·3	±1·1	0·0	±0·0
Yeast	7·3	±0·4	3·8	±1·1
Gram positive				
MRSA	7·8	±1·1	1·8	±1·1
MRSA	4·3	±0·4	1·3	±1·1
MRSA	3·5	±0·0	0·5	±0·0
MRSA	5·5	±0·7	0·5	±0·0
MRSA	3·0	±1·4	0·5	±0·0
Staphylococcus aureus	4·3	±0·4	1·3	±0·4
S. aureus	6·8	±0·4	2·0	±0·0
Staphylococcus lugdenensis	7·5	±0·0	3·5	±0·0
VRE	4·3	±0·4	5·5	±2·1
VRE	4·3	±0·0	3·8	±1·1
VRE	4·5	±0·7	0·0	±0·0
VRE	4·5	±0·0	4·0	±0·7
VRE	3·0	±0·7	4·3	±0·4
Gram negative				
Pseudomonas aeruginosa	6·5	±0·7	5·0	±0·0
P. aeruginosa	5·5	±0·7	3·5	±0·0
Acinetobacter sp	3·3	±0·4	3·5	±0·7
Cirobacter freundii	2·0	±0·0	0·5	±0·0
Escherichia coli	0·8	±0·4	1·3	±0·4
Salmonella sp	1·5	±2·1	1·3	±0.4

CZOI, corrected zone of inhibition; MRSA, meticillin-resistant *Staphylococcus aureus*; SD, standard deviation; VRE, vancomycin-resistant Enterococci.

their non biofilm counterparts. However, this difference was found not to be statistically significant ($P = 0{\cdot}05$) (Table 3). In addition to this a number of bacterial strains, namely VRE, *Acinetobacter* sp and *Escherichia coli* demonstrated an enhanced tolerance to silver in the non biofilm state when compared to the biofilm phenotypic state. The reasons for this are being investigated further. Regardless of these 'erroneous' results overall, it has been demonstrated that microbial tolerance to silver is enhanced, in both yeasts and bacteria, when they are grown in their biofilm phentotypic state compared to their non biofilm state.

All control gauze and non silver hydrofiber dressing demonstrated no antimicrobial activity as indicated by the lack of zones of inhibition around the dressing.

DISCUSSION

Wounds are known to be colonised with a vast array of microorganisms including those that can be readily cultured and those that cannot be recovered by traditionally used culturable-based techniques (6,31). Both culturable and 'viable but non culturable microorganisms' are known to reside in a wound and together represent the wound microbial bioburden. The presence of viable microorganisms at high levels and their detrimental by-products delay wound healing (6,31,11). Consequently, wound dressings exploited for the management of infected wounds need to demonstrate antimicrobial activity on a varied population of microorganisms which are known to be present in chronic wounds.

Within chronic wounds the residing microbial population exists both in the planktonic and biofilm phenotypic states (32,33). Those microorganisms growing as a biofilm are considered to delay wound healing. However, eradiation of biofilms is difficult as they exhibit enhanced tolerance to antimicrobials. Consequently, as advances are being made in our

Key Points

- it has been demonstrated that microbial tolerance to silver is enhanced, in both yeasts and bacteria, when they are grown in their biofilm phentotypic state compared to their non biofilm state
- wound dressings exploited for the management of infected wounds need to demonstrate the antimicrobial activity on a varied population of microorganisms which are known to be present in chronic wounds
- as advances are being made in our understanding of wound pathophysiological, microbiology and healing, it has become more significant today that antimicrobial wound dressings demonstrate a broad antimicrobial effect, not only on different genera and species of microorganisms, but also on microorganisms in the non biofilm or planktonic phenotypic state and the biofilm phenotypic state

Key Points

- it was our hypothesis that the silver alginate dressing would remain efficacious independent of selected cell wall composition (Gram positive and Gram negative), antibiotic resistance categories and biofilm phenotype
- the silver alginate dressing was found to be effective at inhibiting the growth of both Gram-positive and Gram-negative bacteria and yeasts which had been isolated and cultured from wounds
- it was found that the antimicrobial efficacy of ionic silver was dependent on bacterial cell wall composition, that is, Gram positive or negative, and species of bacteria
- in this study, the silver alginate dressing was found to be more effective on Gram-positive than Gram-negative bacteria
- in addition to demonstrating a broad spectrum of activity on microorganisms in the non biofilm state, the silver alginate dressing also confirmed universal antimicrobial efficacy against microorganisms within the biofilm phenotypic state
- the silver alginate dressing helps to significantly reduce both the wound and dressing bioburden, reduce the risk of infection and therefore enhance conditions for improved wound healing
- the study confirmed the broad antimicrobial efficacy of a silver alginate dressing on non biofilm and biofilm phenotypes states of clinically derived microorganisms
- however, it has been demonstrated in this study that the efficacy of ionic silver is significantly reduced when microorganisms were grown in the poloxamer biofilm model
- as with all in vitro studies it is important that interpretation and extrapolation of the results generated are used in conjunction with clinical results

understanding of wound pathophysiological, microbiology and healing, it has become more significant today that antimicrobial wound dressings demonstrate a broad antimicrobial effect, not only on different genera and species of microorganisms, but also on microorganisms in the non biofilm or planktonic phenotypic state and the biofilm phenotypic state.

Silver is used widely in wound care, often impregnated into wound dressings, for the treatment of microbial infections. Silver alginate wound dressings have demonstrated beneficial effects on wound healing, based principally on the physical properties of the dressing (29). In particular, silver alginate wound dressings have been shown to have the ability to 'modulate' wound exudates levels and maintain a 'therapeutic' level of antimicrobial activity at the wound dressing interface and within the wound dressing itself (30). In addition, silver alginate wound dressings have clinically been shown to prevent wound infections (12).

In conjunction with previous studies this study aimed to help enhance our knowledge regarding the antimicrobial activity of a silver alginate dressing by investigating its broad spectrum of activity and effectiveness on both planktonic and sessile microorganisms utilising traditionally used CZOI in vitro models (15). It was our hypothesis that the silver alginate dressing would remain efficacious independent of selected cell wall composition (Gram positive and Gram negative), antibiotic-resistance categories and biofilm phenotype.

The silver alginate dressing was found to be effective at inhibiting the growth of both Gram-positive and Gram-negative bacteria and yeasts which had been isolated and cultured from wounds. The dressing demonstrated antimicrobial efficacy against MRSA, VRE, *S. aureus*, vancomycin-resistant *E. faecium*, *E. faecium*, *Streptococcus pneumonia*, Group C *Streptococcus* sp, α-haemolytic *Streptococcus* sp (α-Strep), β-haemolytic Group A *Streptococcus* sp, β-haemolytic Group B *Streptococcus* sp, β-haemolytic Group C *Streptococcus* sp, *Listeria* sp, *Staphylococcus lugdenensis*, *P. aeruginosa*, *Acinetobacter* sp, *Acinetobacter baumannii*, Gram-negative rods, *Aeromonas* sp, *Citrobacter freundii*, *E. coli*, *Klebsiella pneumonia*, *Salmonella* sp, *Serratia marcescens* and *Stenotrophomonas maltophilia*. However, the antimicrobial efficacy of the silver alginate dressing was found to differ based on the types of microorganisms being tested. In particular, it was found that the antimicrobial efficacy of ionic silver was dependent on bacterial cell wall composition, that is, Gram positive or negative, and species of bacteria. In this study, the silver alginate dressing was found to be more effective on Gram-positive than Gram-negative bacteria.

In addition to demonstrating a broad spectrum of activity on microorganisms in the non biofilm state, the silver alginate dressing also confirmed universal antimicrobial efficacy against microorganisms within the biofilm phenotypic state. Such a finding is clinically significant as microorganisms residing within a wound are known to exist within both the planktonic and biofilm phenotypic states. Although both phenotypic states are significant to wound healing (6,8), microorganisms that reside in the biofilm phenotypic state are known to be more recalcitrant to antimicrobials. In particular, the silver alginate dressing will help to significantly reduce both the wound and dressing bioburden, reduce the risk of infection and therefore enhance conditions for improved wound healing.

Overall, the study confirmed the broad antimicrobial efficacy of a silver alginate dressing on non biofilm and biofilm phenotypic states of clinically derived microorganisms. However, it was also demonstrated that the efficacy of ionic silver is significantly reduced when microorganisms were grown in the poloxamer biofilm model. Nevertheless, as with all in vitro studies it is important that interpretation and extrapolation of the results generated are used in conjunction with clinical results.

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