The visualisation and speed of kill of wound isolates on a silver alginate dressing

Samuel J Hooper, Steven L Percival, Katja E Hill, David W Thomas, A J Hayes, David W Williams

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ABSTRACT

In chronic wound management, alginate dressings are used to absorb exudate and reduce the microbial burden. Silver alginate offers the added benefit of an additional antimicrobial pressure on contaminating microorganisms. This present study compares the antimicrobial activity of a RESTORE silver alginate dressing with a silver-free control dressing using a combination of *in vitro* culture and imaging techniques. The wound pathogens examined included *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, β-haemolytic *Streptococcus*, and strictly anaerobic bacteria. The antimicrobial efficacy of the dressings was assessed using $\log_{10}$ reduction and 13-day corrected zone of inhibition (CZOI) time-course assays. Confocal laser scanning microscopy (CLSM) was used to visualise the relative proportions of live/dead microorganisms sequestered into the dressings over 24 hours and estimate the comparative speed of kill. The RESTORE silver alginate dressing showed significantly greater $\log_{10}$ reductions and CZOIs for all microorganisms compared with the control, indicating the antimicrobial effect of ionic silver. Antimicrobial activity was evident against all test organisms for up to 5 days and, in some cases, up to 12 days following an on-going microbial challenge. Imaging bacteria sequestered in the silver-free dressing showed that each microbial species aggregated in the dressing and remained viable for more than 20 hours. Growth was not observed inside of the dressing, indicating a possible microbiostatic effect of the alginate fibres. In comparison, organisms in the RESTORE silver alginate dressing were seen to lose viability at a considerably greater rate. After 16 hours of contact with the RESTORE silver alginate dressing, >90% of cells of all bacteria and yeast were no longer viable. In conclusion, collectively, the data highlights the rapid speed of kill and antimicrobial suitability of this RESTORE silver alginate dressing on wound isolates and highlights its overwhelming ability to manage a microbial wound bioburden in the management of infected wounds.

Key words: Alginate • Bacteria • Confocal • Silver

INTRODUCTION

Microorganisms are present in all chronic wounds, including those that do not exhibit obvious signs of clinical infection (1). Contamination of a wound can involve the presence of a low level of microorganisms that are able to persist in the wound environment. In contrast, colonisation of wounds involves the presence of multiplying bacteria, although an associated host reaction may not be observed (2). On occasion, the presence of microorganisms in wounds, such as in the case of infection, is considered to be a contributing factor to delayed wound healing (3). Such chronic wounds are a major problem in developed countries, with

Authors: SJ Hooper, PhD, BSc, Tissue Engineering and Reparative Dentistry, Cardiff University, Cardiff, UK; SL Percival, PhD, BSc, Department of Pathology, West Virginia University; and, Director of Research and Innovation, Advanced Medical Solutions, Winsford, UK; KE Hill, PhD, BSc, Tissue Engineering and Reparative Dentistry, Cardiff University, Cardiff, UK; DW Thomas, BDS, FDSRCS, MScD, PhD, Tissue Engineering and Reparative Dentistry, Cardiff University, Cardiff, UK; AJ Hayes, PhD, MSc, BSc, School of Biosciences, Cardiff University, Cardiff, UK; DW Williams, PhD, BSc, Tissue Engineering and Reparative Dentistry, Cardiff University, Cardiff, UK

Address for correspondence: D Williams, Tissue Engineering and Reparative Dentistry, Cardiff University, Cardiff, UK

E-mail: WilliamsDD@cardiff.ac.uk

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Key Points

- the causes of chronic wounds are multiple, often involving underlying peripheral vascular diseases, uncontrolled diabetes, or prolonged pressure ulceration
- during wound infection, the number and virulence of infecting organisms are sufficient to overcome host defence mechanisms, thereby causing tissue damage and delayed healing
- it has been shown that biofilms exhibit a much higher tolerance to both host immune defences, as well as to any administered antimicrobial agents
- systemic antibiotic therapy is more frequently used for treating chronic wounds, although limited evidence on the effectiveness of such therapies on promoting wound healing exists
- the use of silver dressings has widely been promoted as a means of reducing the bioburden of wounds without concomitant development of resistant bacterial strains and preventing a wound infection
- silver alginate dressings in particular are known to have prolonged antimicrobial efficacy, sometimes as long as 21 days, indicating sustained availability of ionic silver and therefore the requirement of fewer dressings changes

an estimated 2–3% of the population suffering from them at some point in their lives (4). In addition to increased patient morbidity associated with chronic wounds, there is also a considerable financial cost to healthcare providers, with an estimated annual expenditure of $25 billion associated with chronic wound care in the USA alone (5).

The causes of chronic wounds are multiple, often involving underlying peripheral vascular diseases, uncontrolled diabetes, or prolonged pressure ulceration (6). In recent years, much attention has focussed on the role that microorganisms play in impairing wound healing (7) which could arise through the promotion of continuous inflammatory responses or the presence of microbial virulence factors that induce damage to the local tissue (8). For example, during wound infection, the number and virulence of infecting organisms are sufficient to overcome host defence mechanisms, thereby causing tissue damage and delayed healing.

Some researchers have postulated the concept of a 'critical colonisation' of wounds, which implies that at a given bioburden of microorganisms (i.e. 10^5 colony forming units (CFU) per gram of wound tissue) delayed healing is induced (9). This concept does not, however, take into consideration wound aetiology, the type of microbial species present, nor their relative virulence potential (1). In addition, there is increasing evidence to suggest that there is no direct correlation between wound bioburden and non healing in chronic wounds (7,10). Indeed, it seems more likely that synergy between bacteria present, producing enhanced virulence, is a significant contributing factor to non healing (7,11). Also, it has more recently been reported that microbial colonisation of wounds involves the formation of polymicrobial biofilms (12,13). Biofilms can be defined as communities of microorganisms, invariably attached to a solid surface, and encased within an extracellular polymeric substance, generated by the microorganisms themselves. Importantly, it has been shown that biofilms exhibit a much higher tolerance to both host immune defences, as well as to any administered antimicrobial agents (14,15).

To combat infection and wound biofilms, a number of strategies have been utilised. Topical antimicrobials including certain antibiotics, antiseptics and disinfectants have all been used with varying degrees of success. Povidone iodine is an antiseptic that has Food and Drug Administration approval for use on certain types of superficial and acute wounds (16). Topical antibiotics may eliminate surface bacteria and those not associated with the biofilm. Generally however, these topical agents are not deemed effective in the complete eradication of biofilms. Furthermore, prolonged use of topical antibiotics can potentially provide a selective pressure for bacterial resistance against the administered agent (17). Systemic antibiotic therapy is more frequently used for treating chronic wounds, although limited evidence on the effectiveness of such therapies on promoting wound healing exists (18,19). Frequent physical debridement of microbial biofilms can, at least in part, reduce the biofilm in wounds to levels that may be sensitive to administered antimicrobial agents (20).

A wide range of wound dressings are available to promote wound healing through the generation and maintenance of a moist environment. The types of dressings frequently used include low adherent dressings suitable for flat exudating wounds, semi-permeable flexible films for use at anatomically difficult sites, as well as hydrocolloid dressings, hydrogels, foams and alginates (21) which swell in the presence of moisture to fill the wound site. In addition, a number of antimicrobial dressings are also available that are often impregnated with agents such as iodine, silver or metronidazole gels. The use of silver dressings has widely been promoted as a means of reducing the bioburden of wounds without concomitant development of resistant bacterial strains (22) and preventing a wound infection (23). Silver alginate dressings in particular are known to have prolonged antimicrobial efficacy, sometimes as long as 21 days, indicating sustained availability of ionic silver and therefore the requirement of fewer dressings changes (24). Metallic silver is inert, but will release silver ions in an aqueous environment. It is these silver ions that exhibit antimicrobial effects through first absorption and then concentration within microbial cells. Inside the microbial cell, ionic silver denatures proteins, and also binds to nucleic acids, thus inhibiting protein function and cell replication. Ionic silver exhibits activity against a wide spectrum of microorganisms, although the extent of activity varies depending upon the ionic form used,

and also the possible interaction with complex organic molecules in the surrounding medium. Few studies have looked at the development of silver resistance following long term use of such dressings.

This present study aimed to examine the antimicrobial properties of one such silver alginate dressing (RESTORE silver alginate) against typical wound microorganisms. This activity was compared with an equivalent silver-free alginate control dressing (silver-free alginate). In addition to determining antimicrobial activity over time using $\log_{10}$ reduction and corrected zones of inhibition (CZOIs), a direct analysis of antimicrobial activity within the dressings was performed using live/dead-staining and CLSM of microorganisms.

MATERIALS AND METHODS

Microorganisms and test dressings

Test dressings were RESTORE silver alginate (Holister Woundcare, Libertyville, IL, USA) and a silver-free alginate (Advanced Medical Solutions Ltd., Winsford, UK). A total of nine microbial strains were used to assess the antimicrobial activity of the dressings (Figure 1). All aerobic species were subcultured at 24 hours intervals and maintained at 37°C using Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB). Strict anaerobic bacterial strains were cultured on fastidious anaerobe agar (FAA) supplemented with 5% (v/v) defibrinated horse blood (TCS Biosciences Ltd., Buckingham, UK) and in Brain–Heart Infusion broth (BHI). These anaerobic bacteria were cultured for 48 hours at 36–37°C in an anaerobic environment (10% v/v CO_2, 20% v/v H_2, 70% v/v N_2). All media, unless otherwise stated, was obtained from Lab M™ (International Diagnostics Group plc, Bury, UK).

Log_{10} reduction assay to measure antimicrobial efficacy (speed of kill)

The ability of the dressings to rapidly kill microorganisms was determined using a one-day $\log_{10}$ reduction assay adapted from Cavanagh *et al*. (25). Colonies from agar cultures were used to inoculate 20 ml of liquid medium, which was incubated for either 24 hours for aerobes or 48 hours for anaerobes, and then a portion of this culture (100 µl) was used to inoculate a fresh 20 ml liquid culture. The re-inoculated media was incubated under the same conditions, either 4–6 hours for aerobes, or 36–48 hours for anaerobes, until the organism was in log-phase growth and contained approximately 10^8 CFU/ml.

Test dressings were aseptically cut into 2 cm^2 pieces. The experimental dressing pieces were then immersed in sterile phosphate buffered saline (PBS) and allowed to saturate during 1 hour incubation in the dark, at room temperature. Control dressing pieces were similarly immersed in neutralisation buffer (NB), which inactivates ionic silver. NB consisted of PBS containing 1% (v/v) polysorbate 20 and 0·1% (w/v) sodium thioglycolate (Sigma-Aldrich Ltd., Gillingham, UK).

Key Points

- this present study aimed to examine the antimicrobial properties of one such silver alginate dressing (RESTORE silver alginate) against typical wound microorganisms
- a direct analysis of antimicrobial activity within the dressings was performed using live/dead staining and CLSM of microorganisms
- test dressings were RESTORE silver alginate (Holister Woundcare, Libertyville, IL, USA) and a silver-free alginate (Advanced Medical Solutions Ltd., Winsford, UK)
- a total of nine microbial strains were used to assess the antimicrobial activity of the dressings

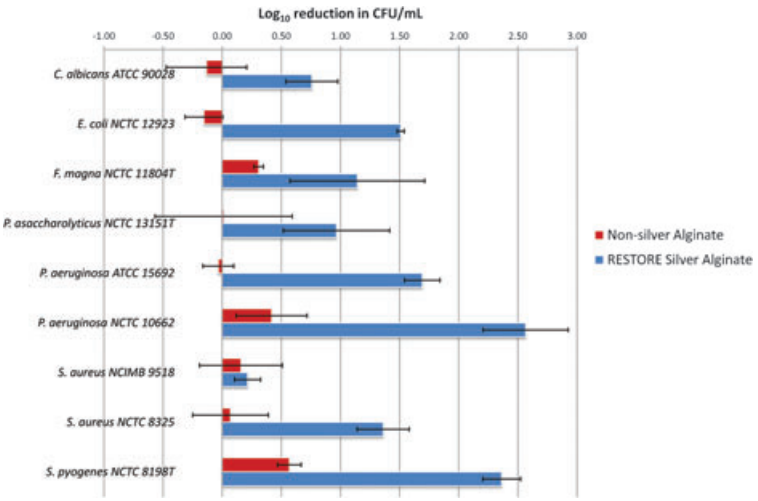

Figure 1. Log_{10} reduction assay measuring dressing antimicrobial activity after 2 hours exposure ($n = 3$; standard deviation from the mean was used to produce the error bars).

Saturated dressings were aseptically transferred, without squeezing, and allowed to drain for 10 seconds before being placed flat in a sterile container. Each dressing was then inoculated with 1 ml of the log-phase culture and incubated, in the dark and under the appropriate conditions, at 37°C for 2 hours. Following incubation, dressings were placed in NB (9 ml) to achieve a 1:10 dilution of the inoculum and vigorously vortexed for 10 seconds to re-suspend the microbial cells. The recovered microorganisms were then serially diluted in NB. Each dilution (50 µl) was spirally plated on to appropriate agar media using a Whitley Automatic Spiral Plater (WASP; Don Whitley Scientific Ltd., West Yorkshire, UK) and plates incubated either overnight for aerobes or for 48 hours for anaerobes. Subsequently, the resulting number of colonies was counted to calculate the number of CFUs from each dressing. The counts generated from the neutralised control dressing pieces were used to estimate initial microbial numbers CFU/ml in the original inocula, while the counts from the experimental dressing pieces were used to calculate the surviving number of CFU/ml. Log_{10} reduction values, representing the antimicrobial effect of the dressing were then calculated as the difference between the log_{10} values of the starting and surviving numbers of microorganisms.

CZOI assay to assess antimicrobial persistence of the wound dressings

The ability of the dressing to inhibit microbial growth over several days was also assessed using a day-to-day agar transfer and CZOI assay, adapted from Cavanagh *et al.* (25).

Fresh culture of each test microorganism (100 µl) was used to create a microbial lawn on agar, as per the The British Society for Antimicrobial Chemotherapy guidelines for disc diffusion testing (26). Triplicate pieces of the test dressing (2 cm^2) were saturated with sterile distilled water and aseptically placed on to the middle of the seeded agar plates. Prior to placement on the agar, the dressings were allowed to drain for approximately 10 seconds. The original placement of the dressing pieces was traced on to the base of the petri plate to allow correction for any dressing shrinkage over time. Plates were incubated with the dressing, either overnight for aerobic species or for 48 hours for anaerobes. The dressings were then transferred to a new agar plate, seeded as above, and incubated in the same manner. The zones of microbial growth inhibition and original dressing widths were measured in two perpendicular directions for each plate. These measurements were then used to calculate the CZOI value by dividing the inhibition zone width by the dressing width. This procedure was repeated for each microorganism for as many days that were required for all the dressing pieces to no longer inhibit growth.

CLSM to visualise death of sequestered microorganisms

Broth cultures of *Candida albicans* ATCC 90028, *Pseudomonas aeruginosa* ATCC 15692 and *Staphylococcus aureus* NCTC 8325 were cultured in a shaking incubator until mid-log phase growth, for 5–6 hours. Each culture (5 ml) was briefly centrifuged (13 000 *g*, 1 minute), the media gently aspirated, and the pellet of cells re-suspended in PBS (1·5 ml) containing working concentrations of live/dead *Bac*Light™ bacterial viability kit (Invitrogen Molecular Probes Inc., Eugene, OR). The stained microbial suspensions (100 µl) were pipetted on to fibres from each dressing, on glass slides with a cover slip sealed with petroleum jelly to prevent drying out. Preparations were viewed and analysed using a Leica TCS SP2 spectral confocal microscope and Leica confocal software (Leica, Heidelberg, Germany). Representative regions of dressing were scanned through their full depth at 20-minute intervals over a 15 hour period using a ×20 objective lens and appropriate scan parameters for simultaneous fluorescence recordings of live cells (Syto 9; green fluorescence; excitation filter (Ex) maximum 485 nm; emission filter (Em) maximum 500 nm) and dead cells (propidium iodide; red fluorescence; Ex maximum 536 nm; Em maximum 617 nm). The excitation lasers used for each probe (argon 488 nm and helium neon 543 nm, respectively) were used at their lowest possible power output to minimise potential phototoxic side-effects on the microorganisms. To provide context, individual fibres within the dressing were simultaneously imaged using Nomarski differential interference contrast (DIC) optics. Z-Stacks of optical sections taken at each time point were reconstructed using a maximum intensity projection algorithm and then presented as green/red bacterial overlays upon a greyscale DIC image of the fibres. Bacterial

viability curves were produced from analysing the relative ratio of representative green/red (live/dead) fluorescent signal intensities (i.e. voxel intensities 0–255) at each time point from within selected regions of interest.

RESULTS

Log_{10} reduction assays to measure antimicrobial efficacy

Compared with the silver-free dressing, the log_{10} reduction assays showed excellent antimicrobial activity with the RESTORE silver alginate dressing (Figure 1). No growth inhibition was evident using RESTORE silver alginate dressing previously immersed in NB. While all bacterial species tested were inhibited by ionic silver, this was in a strain dependent manner. In addition, distinct differences were seen between different isolates of the same species for example, *S. aureus*. Antifungal activity was similarly evident against the tested *C. albicans* strain. *P. aeruginosa* exhibited greatest sensitivity to the silver dressing, with *Streptococcus pyogenes* and *Escherichia coli* also appearing particularly sensitive. In contrast, *S. aureus* NCIMB 9518 exhibited greater tolerance to the silver containing dressing.

CZOI assay to assess antimicrobial persistence of silver dressings

Confirmation of the persistence of antimicrobial activity of the silver containing dressings was provided by the CZOI assay (Table 1). Strain-dependent variation was again apparent, but the RESTORE silver alginate dressing was able to produce a clearance zone for up to 5 days for each strain tested.

CLSM to visualise death of sequestered microorganisms

When the dressings were hydrated with stained microbial culture, the alginate fibres swelled quickly, causing sequestration and immobilisation of the microorganisms in the gel-like spaces between the fibres. With the silver-free dressing, all microbial species quickly formed aggregates of viable cells. Bacterial multiplication was not evident, indicating a possible microbiostatic effect inherent to this alginate dressing. In comparison, when the same organisms were added to the RESTORE silver alginate dressing, they progressively appeared red (i.e. dead) with some red cells evident within the first hour indicating a rapid speed of kill evident in real time. Figure 2 shows the rapid kill observed for both *P. aeruginosa* and *S. aureus* within the RESTORE silver alginate dressing. In the case of *P. aeruginosa*, total kill of the microbial bioburden in the dressing was evident within 16 hours, whereas for the *S. aureus* microbial load, 4 hours was sufficient time for a total cidal effect to be observed. The broad-spectrum antimicrobial effect of silver was evident when *C. albicans* was exposed to the dressing fibres.

An estimate of the relative viability of microbial cells at each time point could be made by comparing the ratio of green (live) and red (dead) fluorescence intensities. The shift in the ratio for each strain over the first 6 hours (Figure 3) was evidence of the relative rate of cell death for each microorganism on both dressings. With each microorganism there was a steady decline in living cells over time on the silver-free alginate samples, probably because of natural cell death and the phototoxic effect of the laser. However, for each species the cell death rate was notably greater over this initial time period on the RESTORE silver alginate fibres, indicating the microbicidal effect of that dressing.

DISCUSSION

Wounds are frequently contaminated by a mixed population of microorganisms that can lead to colonisation or infection, which in turn, has been associated with impaired wound healing. The concept of 'critical colonisation' implies that once a particular bioburden associated with infection is reached ($>10^5$ CFU/g of wound tissue), impaired wound healing occurs and the use of antimicrobial dressings is advocated (27,28). However, this premise does not take into consideration the wound type nor the microorganisms involved in wound colonisation, because impairment of healing can occur at lower microbial bioburden when opportunistic pathogenic strains are involved. A more scientifically and clinically accepted term, used instead of critically colonised, is biofilm infected (23,29). Interestingly, recent studies assessing the bioburden of chronic wounds showed no relationship between bacterial counts and healing rates (7,10,30).

While the application of topical antimicrobials can reduce the microbial burden within

Table 1 CZOI assay to measure antimicrobial longevity of wound dressings

		Mean CZOI ($n = 6$)												
Strain	Dressing	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Candida albicans ATCC 90028	Ag-free	**0·00** ± 0·00												
	RESTORE Ag	**1·19** ± 0·04	**1·28** ± 0·03	**1·26** ± 0·04	**1·19** ± 0·04	**1·15** ± 0·05	**1·12** ± 0·03	**1·08** ± 0·04	**1·00** ± 0·03	**0·96** ± 0·05	**0·00** ± 0·00			
Escherichia coli NCTC 12923	Ag-free	**0·00** ± 0·00												
	RESTORE Ag	**1·03** ± 0·03	**1·00** ± 0·00	**1·00** ± 0·03	**0·92** ± 0·04	**0·94** ± 0·02	**0·80** ± 0·12	**0·59** ± 0·16	**0·00** ± 0·00					
Finegoldia magna NCTC 11804^{T}	Ag-free	**1·00** ± 0·06	**0·00** ± 0·00											
	RESTORE Ag	**0·98** ± 0·04	**0·90** ± 0·06	**0·89** ± 0·03	**0·91** ± 0·05	**0·82** ± 0·10	**0·00** ± 0·00							
Peptoniphilus asaccharolyticus NCTC 13151^{T}	Ag-free	**1·03** ± 0·16	**0·68** ± 0·06	**0·00** ± 0·00										
	RESTORE Ag	**0·99** ± 0·05	**0·94** ± 0·08	**0·95** ± 0·04	**0·85** ± 0·05	**0·82** ± 0·07	**0·00** ± 0·00							
Pseudomonas aeruginosa ATCC 15692	Ag-free	**0·00** ± 0·00												
	RESTORE Ag	**1·28** ± 0·05	**1·25** ± 0·04	**1·26** ± 0·07	**0·94** ± 0·02	**0·97** ± 0·02	**0·00** ± 0·00							
Pseudomonas aeruginosa NCTC 10662	Ag-free	**0·00** ± 0·00												
	RESTORE Ag	**1·03** ± 0·02	**1·10** ± 0·02	**1·08** ± 0·05	**0·98** ± 0·03	**0·95** ± 0·04	**0·96** ± 0·01	**0·69** ± 0·13	**0·00** ± 0·00					
Staphylococcus aureus NCIMB 9518	Ag-free	**0·00** ± 0·00												
	RESTORE Ag	**1·16** ± 0·06	**1·66** ± 0·06	**1·03** ± 0·04	**0·97** ± 0·02	**0·95** ± 0·03	**0·00** ± 0·00							
Staphylococcus aureus NCTC 8325	Ag-free	**0·00** ± 0·00												
	RESTORE Ag	**1·13** ± 0·06	**1·12** ± 0·02	**1·09** ± 0·03	**1·06** ± 0·02	**1·00** ± 0·02	**0·96** ± 0·02	**0·92** ± 0·03	**0·75** ± 0·07	**0·00** ± 0·00				
Streptococcus pyogenes NCTC 8198^{T}	Ag-free	**1·22** ± 0·22	**1·18** ± 0·04	**1·25** ± 0·34	**1·0** ± 0·14	**0·00** ± 0·00								
	RESTORE Ag	**1·46** ± 0·04	**1·63** ± 0·04	**1·55** ± 0·04	**1·43** ± 0·04	**1·39** ± 0·02	**1·46** ± 0·08	**1·31** ± 0·08	**1·25** ± 0·07	**1·23** ± 0·06	**1·16** ± 0·09	**1·07** ± 0·05	**0·91** ± 0·05	**0·00** ± 0·00

Ag-free, silver-free alginate dressing; CZOI, corrected zone of inhibition; RESTORE Ag, RESTORE silver alginate dressing.

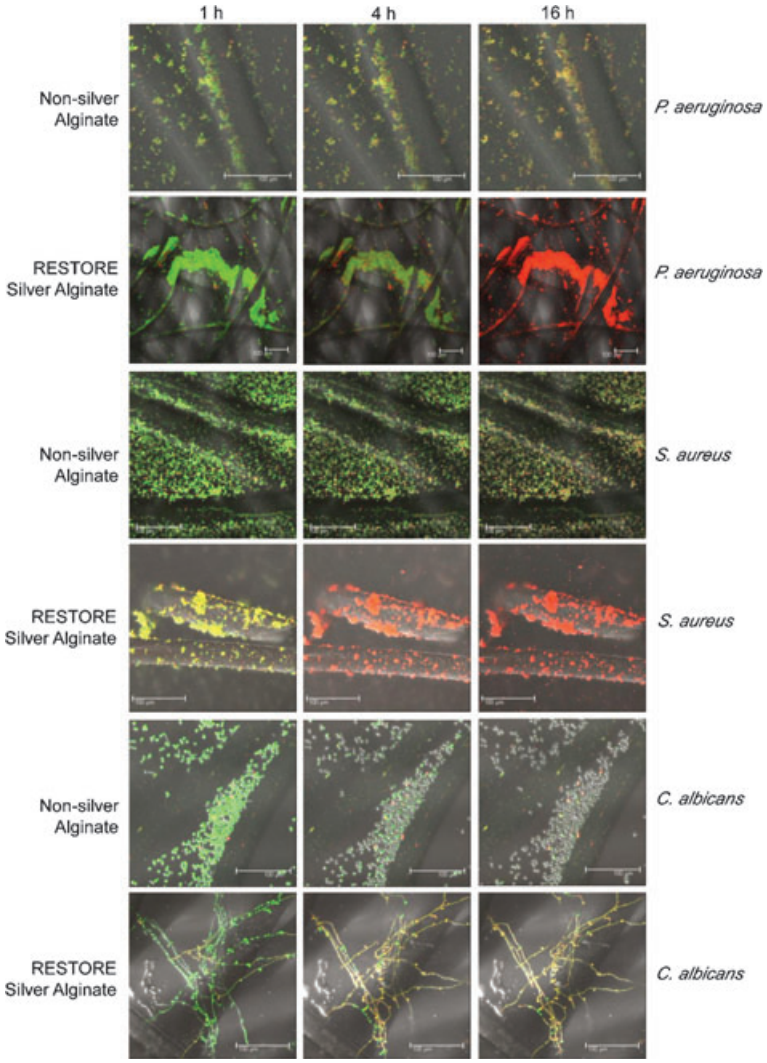

Figure 2. Confocal laser scanning microscopy images of live/dead stained microorganisms on alginate dressing fibres.

a wound dressing, the eradication of biofilm microorganisms is generally not reported on. A wide range of antimicrobial dressings are available that promote reduction in planktonic microbial levels and enhance wound repair. In recent years, much focus has been given to the potential role of silver dressings in inhibiting wound microflora (22,25,30,31) and the aim of this study was to investigate the performance of a commercially available silver-containing alginate dressing against prevalent wound microorganisms.

The findings of the study showed that the RESTORE silver alginate dressing had activity against all of the tested wound isolates including Gram-positive and Gram-negative bacteria, as well as fungi. These test isolates had been selected for based on their prevalence within wounds and their pathogenic associations. This broad activity spectrum of ionic silver is widely recognised for silver alginate dressings, in particular against burn wound (32) and chronic wound isolates (33) although, as with several silver containing wound dressings, activity has been reported to be greater against Gram-negative bacteria compared with Gram-positive bacteria (22,34). Indeed, in this present study, a 2 hours exposure of the silver dressing to one of the *S. aureus* strains did appear to have limited effects in terms of a $\log_{10}$ reduction, although the more extensive overnight exposure used in the CZOI assay proved effective against this strain. The spectrum of activity of RESTORE silver alginate did vary with species and strains, as

Key Point

- the findings of the study showed that the RESTORE silver alginate dressing had activity against all of the tested wound isolates including Gram-positive and Gram-negative bacteria, as well as fungi

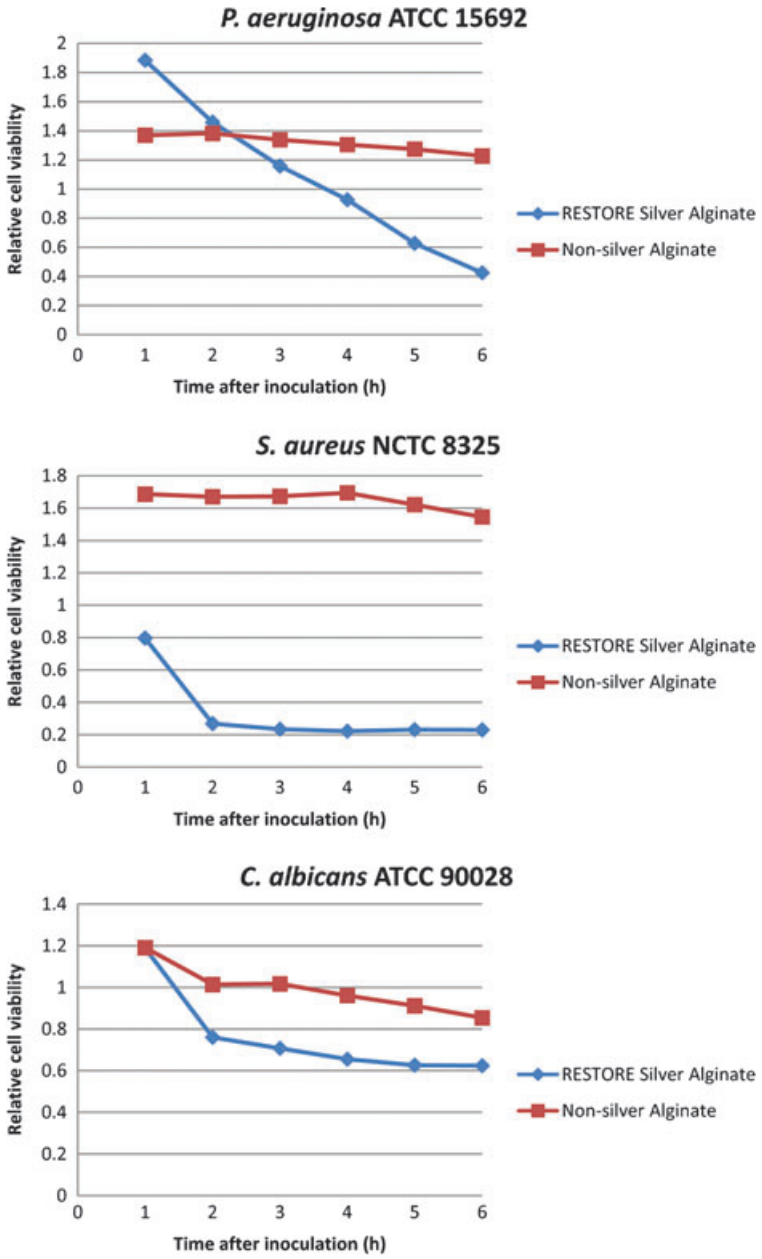

Figure 3. Relative cell viability on inoculated wound dressing fibres, as showed by the change in ratio of live and dead-stained cells measured by Confocal laser scanning microscopy imaging and relative green and red fluorescence intensity analysis.

Key Points

- the absence of silver resistance in this study was, however, reassuring given that there are now several reports of isolates exhibiting tolerance and inherent resistance to ionic silver
- a loss of antimicrobial efficacy may be anticipated as the silver concentration reduces in the dressing over time, because of the demands of the microbial bioburden
- this is important in situations where wound dressings may be in place for several days, with silver alginate dressings recently being shown to demonstrate efficacy for up to 21 days

has been previously reported (32,33,35). The absence of silver resistance in this study was, however, reassuring given that there are now several reports of isolates exhibiting tolerance and inherent resistance to ionic silver (36–38).

Dressings exert their activity, at least in part, by making ionic silver available to the local wound dressing environment. Hence, a loss of antimicrobial efficacy may be anticipated as the silver concentration reduces in the dressing over time, because of the demands of the microbial bioburden. This is important in situations where wound dressings may be in place for several days, with silver alginate dressings recently being shown to demonstrate efficacy for up to 21 days (24). For this reason, to examine the antimicrobial persistence of the test dressing, the CZOI assay was designed to monitor performance over time, up to 13 days, until the antimicrobial effect was no longer apparent. It was therefore significant to see that even when challenged daily, the efficacy

of the silver dressing was maintained for several days, particularly against prevalent wound pathogens such as *P. aeruginosa*, *S. pyogenes*, *C. albicans* and strict anaerobic bacteria.

The use of live/dead viability staining with CLSM to observe microbial kill within the dressing environment is a novel approach, although a method had previously been described by Newman *et al*. in a study examining the antimicrobial properties of a silver salt-containing Hydrofiber wound dressing (39). However, the method by Newman *et al*. did not take into account the full extent of inadvertent microbial killing induced by the confocal lasers. The effect of kill by the lasers was fully validated by including the control dressing in this study to evaluate the antimicrobial activity of the silver containing wound dressing. Using relative intensity measurements to calculate the change in total cell viability is not ideal as it does not take into account the potential movement of the microorganisms. This may take cells out of the focus of the field being scanned and change the total number of organisms counted. Nor does it take into account possible bleaching of the fluorescent signal. However, despite these limitations, the technique was able to provide good quantification of relative microbial viability in the image from each time point and, therefore, the rate of cell death in real time. This method proved to be a useful way of visualising the effect of the dressing fibres on the microorganisms and could certainly be used to investigate the antimicrobial efficacy of other such wound care products in the future.

As shown in Figure 3, calculating the relative death rate for the first 6 hours after dressing inoculation showed an overall decline in total viability for both silver and silver-free dressings. The general trend of decline presumably reflects the 'natural' death rate under these conditions. Regardless, the significantly greater losses of viability in the 6 hours after inoculation of the silver alginate fibres compared with the control indicate a rapid microbiocidal effect because of the presence of the ionic silver. The importance of this rapid killing effect within the RESTORE silver alginate wound dressing cannot be understated, because not only will microorganisms be removed from the wound dressing environment but the death of these microorganisms will effectively prevent further proliferation within the dressing and subsequent recontamination of the wound. While further assessment of the performance of this dressing against true biofilms is warranted, these studies highlight the potential value of RESTORE silver alginate in reducing the microbial bioburden within the dressing and thus creating an environment conducive to wound healing.

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Key Points

- the effect of kill by the lasers was fully validated by including the control dressing in this study to evaluate the antimicrobial activity of the silver containing wound dressing
- this method proved to be a useful way of visualising the effect of the dressing fibres on the microorganisms and could certainly be used to investigate the antimicrobial efficacy of other such wound care products in the future
- the importance of this rapid killing effect within the RESTORE silver alginate wound dressing cannot be understated, because not only will microorganisms be removed from the wound dressing environment but the death of these microorganisms will effectively prevent further proliferation within the dressing and subsequent recontamination of the wound
- while further assessment of the performance of this dressing against true biofilms is warranted, these studies highlight the potential value of RESTORE silver alginate in reducing the microbial bioburden within the dressing and thus creating an environment conducive to wound healing

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