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Antimicrobial activities of silver dressings: an *in vitro* comparison

Margaret Ip,[1] Sau Lai Lui,[1] Vincent K. M. Poon,[2] Ivan Lung[2] and Andrew Burd[2]

Department of Microbiology[1] and Department of Surgery[2], Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

Correspondence
Margaret Ip
margaretip@cuhk.edu.hk

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A range of silver-coated or -impregnated dressings are now commercially available for use but comparative data on their antimicrobial efficacies are limited. The antibacterial activities of five commercially available silver-coated/impregnated dressings were compared against nine common burn-wound pathogens, namely methicillin-sensitive and -resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris*, *Acinetobacter baumannii* and a multi-drug-efflux-positive *Acinetobacter baumannii* (BM4454), using a broth culture method. The rapidity and extent of killing of these pathogens under *in vitro* conditions were evaluated. All five silver-impregnated dressings investigated exerted bactericidal activity, particularly against Gram-negative bacteria, including *Enterobacter* species, *Proteus* species and *E. coli*. The spectrum and rapidity of action, however, ranged widely for different dressings. Acticoat and Contreet had a broad spectrum of bactericidal activities against both Gram-positive and -negative bacteria. Contreet was characterized by a very rapid bactericidal action and achieved a reduction of $\geqslant$10 000 c.f.u. ml^{-1} in the first 30 min for *Enterobacter cloacae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. Other dressings demonstrated a narrower range of bactericidal activities. Understanding the characteristics of these dressings may enable them to be targeted more appropriately according to the specific requirements for use of a particular dressing, as in for prophylaxis in skin grafting or for an infected wound with MRSA.

INTRODUCTION

The emergence and spread of antibiotic resistance is an alarming concern in clinical practice. Increasingly, agents with 'antimicrobial' effects are being coated on materials and medical devices (Darouiche, 1999) as a prophylaxis to prevent bacteria from growing or for therapeutic use. The new technology of impregnation of silver nanoparticles (Furno *et al.*, 2004) is enabling a wider range of these medical products to be available to clinicians.

The use of metallic silver as an antimicrobial agent has long been recognized (Klasen, 2000; Lansdown, 2002a). Dilute solutions of silver nitrate had been used since the 19th century to treat infections and burns before the introduction of silver sulphadiazine cream (Fox, 1968). Of the commonly used forms of topical silver applications, silver-coated dressings have been demonstrated to be effective at killing a broader range of bacteria than the cream base, were less irritating than the silver nitrate solution and were better tolerated (Wright *et al.*, 1998). Silver-coated dressings are used extensively for wound management, particularly in burn wounds (Ross, *et* al., 1993; Caruso *et al.*, 2004), chronic leg ulcers (Karlsmark *et al.*, 2003), diabetic wounds (Hilton *et al.*, 2004) and traumatic injuries. These dressings vary in containing compounds of silver nitrate or sulphadiazine, to sustained silver-ion release preparation (White, 2001) and silver-based crystalline nanoparticles (Klaus *et al.*, 1999). The dressing component also varies, as nylon, mesh, hydrocolloid or methylcellulose. A range of silver-impregnated dressings are now commercially available for use. However, there has been little comparative analysis as to the 'antimicrobial' effect of each of the dressings and the spectrum of bacterial killing that each dressing provides.

Silver has the advantage of having broad antimicrobial activities against Gram-negative and Gram-positive bacteria and there is also minimal development of bacterial resistance. The use of these compounds and the mechanisms of silver resistance have been reviewed (Silver, 2003). One major advantage of its use is the limited side effects of topical silver therapy; silver toxicity or argyrosis can be resolved with cessation of therapy (Lansdown, 2002b; Marshall & Schneider, 1977). The incorporation of silver for topical dressings or as coating on medical products may therefore play an important role in the era of antibiotic resistance.

Table 1. Content of six commercially available dressings

Dressing	Content	Website
Aquacel (ConvaTec)	Dressing with hydrofibre composed of sodium carboxymethylcellulose (Hydrocolloid)	http://www.convatec.com
Aquacel Ag (ConvaTec)	Silver-impregnated dressing with hydrofibre composed of Hydrocolloid and 1·2 % ionic silver	http://www.convatec.com
Acticoat (Smith & Nephew)	Three-ply gauze dressing consisting of an absorbent polyester inner core sandwiched between outer layers of silver-coated, polyethylene net (nanocrystalline silver)	http://wound.smith-nephew.com
Urgotul SSD (Urgo)	Hydrocolloid dressing consisting of a polyester web, impregnated with carboxymethyl cellulose, vaseline and silver sulphadiazine	http://www.urgo.com/en/index.php
PolyMem Silver (Ferris)	Polyurethane membrane matrix containing F68 surfactant, glycerol, a superabsorbent starch copolymer and silver (minimum 124 μg cm^{-2}, generating at least 10^7 ions)	http://www.ferriscares.com
Contreet antimicrobial foam (Coloplast)	Foam dressing with ionic silver (silver sodium hydrogen zirconium phosphate)	http://www.us.coloplast.com

This study compared the antibacterial activity of five commercially available silver-impregnated dressings against nine common burn-wound pathogens. The rapidity and extent of killing of these pathogens under *in vitro* conditions were evaluated.

METHODS

A bacterial broth method (Fraser *et al.*, 2004) was adapted and modified to study the inhibition of bacterial growth by the silver-impregnated dressings. The properties of the five silver-impregnated dressings and one without silver (Aquacel; dressing control) are listed in Table 1.

Preparation of dressings. Squares of 1 cm of each dressing were prepared in an aseptic manner. Each square was placed in a sterile vial and the dressing subjected to pretreatment with 800 μl distilled water for 10 min (according to a previously established protocol for absorbency test for the volume required and duration required for pretreatment). Tryptone soy broth (2·2 ml) was then added to each vial to make up to a total volume of 3 ml.

Preparation of bacterial cultures. Nine bacterial strains were used: *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC BAA-43, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, *Enterobacter cloacae* ATCC 13047, *Proteus vulgaris* ATCC 6380, *Acinetobacter baumannii* ATCC 19606 and a multi-drug-efflux-positive *Acinetobacter baumannii* strain (BM4454; Magnet *et al.*, 2001).

A suspension of each organism was prepared from fresh colonies on blood agar plates after overnight incubation and the turbidity was adjusted to 0·5 McFarland standard ($\sim 1 \times 10^8$ c.f.u. ml^{-1}). An aliquot (10 μl) of the bacterial suspension was added to each vial containing the dressing. Control broths with and without bacterial inoculation were also included. The vials were then incubated with agitation at 35 °C, 220 r.p.m. Aliquots of 10 μl bacterial broth were sampled from each vial at specific time intervals (0, 30 min and 2, 4, 6 and 24 h) and serial tenfold dilutions for each aliquot were prepared in broth. Duplicate aliquots (50 μl) of each of the serially diluted samples were spread on to plates. The plates were incubated overnight at 35 °C and bacterial counts (c.f.u. ml^{-1}) were performed (producing counts ranging from 0 to 10^6 c.f.u.). The dilution that allowed quantification (of 1–100 c.f.u.) was counted and the mean counts calculated. Eight vials, containing the six dressings as well as the culture and broth controls, were included in each experiment for each organism. Plate counts were measured in duplicate and each experiment was repeated twice and mean c.f.u. counts obtained. Bactericidal activity was defined as a reduction of greater than 10^3 c.f.u. in a 10^5 c.f.u. ml^{-1} inoculum.

RESULTS

The bactericidal activities of the silver-impregnated dressings against the nine bacteria studied are shown in Fig. 1. Bactericidal activity was indicated by a reduction of bacterial counts in $\log_{10}$ c.f.u. ml^{-1} over time. These curves also indicated the rate of bacterial killing and provide an additional index of efficacy against the described isolates. The growth rate of each organism was represented by the growth control and that of the Aquacel dressing, which contained no silver.

For *Staphylococcus aureus* (Fig. 1a, b), the Contreet and Acticoat dressings exerted maximal bactericidal activity, achieving $>10\,000$ c.f.u. ml^{-1} reduction of bacterial growth at 24 h. Interestingly, the killing effect was more rapid and marked with MRSA than with the methicillin-susceptible strain. Maximal killing of MRSA was achieved at 4 h with Contreet and the reduction in bacterial counts was sustained, whilst a slower, gradual reduction occurred with the methicillin-sensitive strain. However, the bactericidal activities of the other dressings against *S. aureus* were similar and marginal. For Aquacel Ag and PolyMem Silver

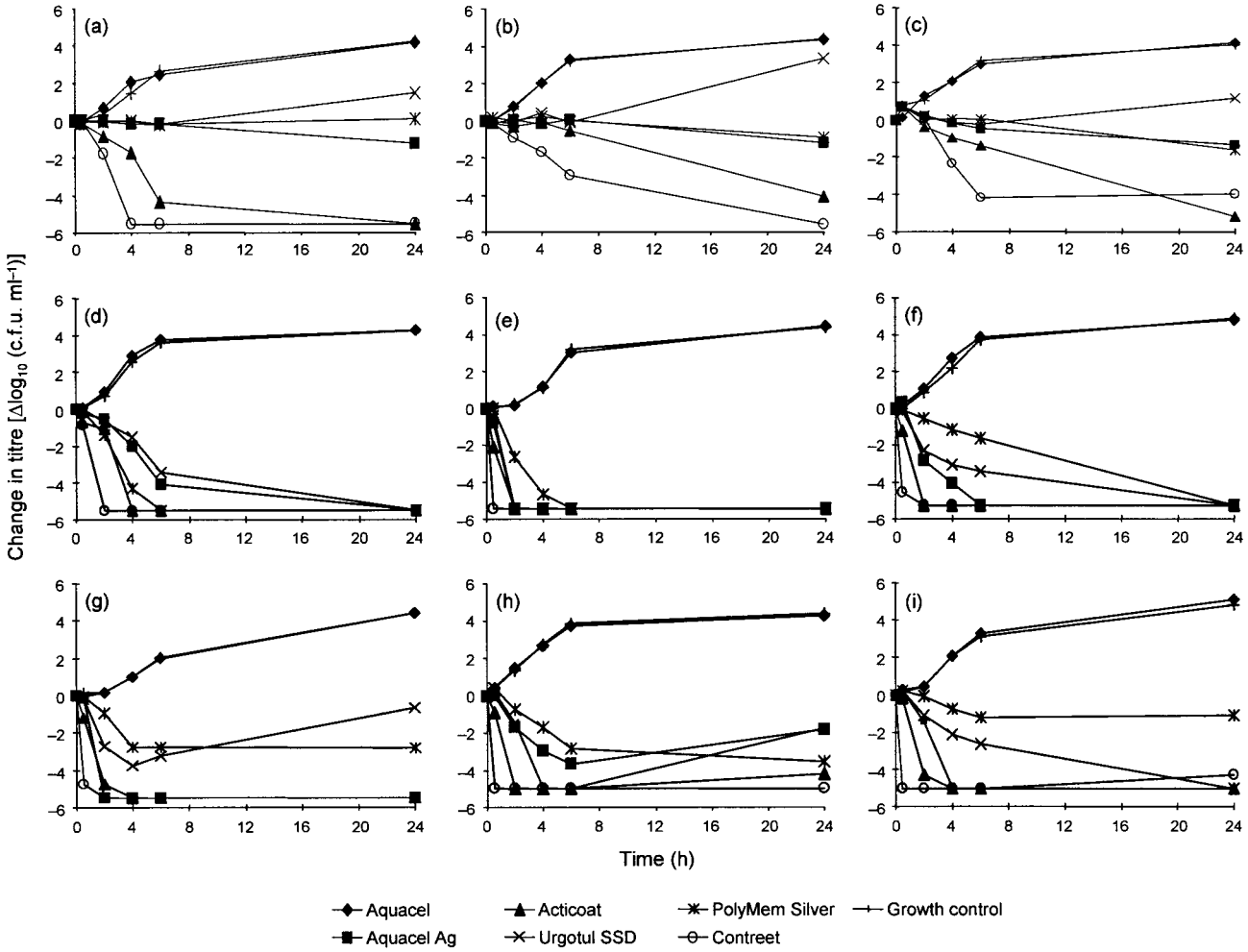

Fig. 1. Bactericidal activities of silver-impregnated dressings against Gram-positive and Gram-negative bacteria. Values are means of two experiments performed in duplicate. $\Delta\log_{10}$ c.f.u. ml^{-1} is the difference in $\log_{10}$ c.f.u. ml^{-1} at the time of bacterial inoculation, starting from $t=0$. Strains: (a) methicillin-resistant *S. aureus* (MRSA) ATCC BAA-43; (b) methicillin-sensitive *S. aureus* ATCC 29213; (c) *Enterococcus faecalis* ATCC 29212; (d) *E. coli* ATCC 35218; (e) *Proteus vulgaris* ATCC 6380; (f) *Enterobacter cloacae* ATCC 13047; (g) *Acinetobacter baumanii* ATCC 19606; (h) *Acinetobacter baumannii* BM4454; (i) *P. aeruginosa* ATCC 27853.

dressings, little or only up to 10 c.f.u. ml^{-1} reduction of *S. aureus* was obtained, whereas with Urgotul silver, an increase in bacterial growth was observed at 24 h. With *Enterococcus faecalis*, again Contreet gave the maximal bactericidal activity at 6 h, whilst Acticoat also gave a reduction of $>10\,000$ c.f.u. ml^{-1} in bacterial growth at 24 h. Again, the other dressings gave similar effects to those with *S. aureus*, with a maximal reduction of 100 c.f.u. bacteria ml^{-1} using Aquacel Ag and PolyMem Silver dressings. Bacterial growth was observed for the Urgotul silver dressing.

All the silver-impregnated dressings were bactericidal on the coliforms, and achieved $>100\,000$ c.f.u. ml^{-1} reduction of *E. coli* (Fig. 1d), *Proteus vulgaris* (Fig. 1e) and *Enterobacter cloacae* (Fig. 1f) between 30 min and 24 h. Contreet achieved the most rapid killing, with $>100\,000$ c.f.u. ml^{-1} reduction in the first 30 min for *Enterobacter cloacae*, *Proteus vulgaris*, *P. aeruginosa* and *Acinetobacter baumanii*. Acticoat exerted bactericidal action on all the Gram-negative bacteria, with a reduction of $>10\,000$ c.f.u. ml^{-1} after 6 h of exposure. Aquacel Ag also exerted bactericidal effects on all Gram-negative bacilli at 6 h, although regrowth of *Acinetobacter baumanii* BM4454 occurred after 24 h. PolyMem Silver was less satisfactory and was bacteriostatic for *P. aeruginosa* and, although it reduced the growth of the two acinetobacters by >1000 c.f.u. ml^{-1} at 6 h, regrowth occurred at 24 h. Urgotul silver showed variable antibacterial effects. It achieved a reduction of $>100\,000$ c.f.u. ml^{-1} with *Proteus vulgaris*, *E. coli*, *Enterobacter cloacae* and *P. aeruginosa* at 24 h, but regrowth occurred for the two acinetobacters. The action on Gram-positive bacteria was least satisfactory, with bacterial growth after 24 h.

DISCUSSION

All five silver-impregnated dressings investigated exerted bactericidal activity, particularly on Gram-negative bacteria, including *Enterobacter* species, *Proteus* species and *E. coli*. The spectrum and rapidity of action, however, ranged widely for the various dressings. Both Acticoat and Contreet were very effective and had rapid and wide spectrum of bactericidal activities on the bacteria tested. Acticoat proved to be very active for *S. aureus* and its effect was also supported in a recent infected animal model (Heggers *et al.*, 2005). Contreet was also active for the Gram-positive bacteria, but was characterized by a very rapid bactericidal action, particularly against the Gram-negative bacteria. An advantage of a rapid bactericidal action may be that it permits wound healing to proceed without bacterial interference and reduces the likelihood for resistance to develop.

This method gave consistent and reproducible results for comparisons. It may represent the sustained effect of the silver ions that leach from a dressing to inhibit bacterial growth in the wound exudates in the clinical setting. One limitation of this study was that the study was not extended for more than 24 h, as some of the dressings may have the property of sustained effects for a number of days.

Tryptone soy broth was chosen as the medium for study. An initial assessment of the methodology also included different broth media containing saline or sera which gave variable results for inhibition (data not shown). Halide ions, e.g. Cl^-, have been shown to have profound effects on silver and alter its 'bioavailability' by acting as both a precipitating agent and soluble forms of silver complexes (Gupta *et al.*, 1998). *In vivo*, a wound is often compounded with sera, blood and tissue fluid, which may interfere with the composition of active silver ions, and it is likely that the interaction between the dressing and the wound would be more complex. The concentration and rate of 'bioavailable' silver ions that are released from the surface of the dressing to the wound exudates will be an important factor. Urgotol silver and PolyMem Silver contain a petroleum jelly matrix and glycerol, respectively, in the composition of the dressings, and this might affect the results of these experiments. An animal wound model might be employed to reflect more realistically the effect of these dressings *in vivo*. The efficacy of these dressings will need to be correlated with comparisons in clinical trials for a particular wound type or setting.

A wide array of silver-based dressings is available in the market and this is encouraging their much wider application in acute and chronic wound care, such as in diabetic ulcers. Our study confirmed the effectiveness of topical silver against a broad range of bacterial pathogens, and identified differences between these commonly available dressings. With the enhanced bacterial killing effects, there is also concern clinically that too much silver could be delivered into the tissue, resulting in adverse effects on the recovery of wounds. Poon & Burd (2004) demonstrated that silver was toxic to keratinocytes and fibroblasts and affected wound healing. As the characteristics of these dressings are further understood, it may be possible to target the specific requirements for a particular circumstance, e.g. a dressing for prophylaxis would need to inhibit bacterial growth adequately, yet exhibit minimal silver toxicity to enhance wound healing, whereas another form of dressing would be more appropriate for use in a wound infected with MRSA.

We utilized an *in vitro* culture broth method to compare the antimicrobial effects of different silver-impregnated dressings on commonly encountered pathogens. Other methods, employing solid culture media to examine for zones of inhibition of bacterial growth, have been used previously (Thomas & McCubbin, 2003; Jones *et al.*, 2004). A standardized methodology should be established in order to examine and compare the efficacies of the antimicrobial effects of these commercially available 'antimicrobial' coated medical products. Further methods of assessment, including the use of infected animal models and clinical studies, will be necessary to gain a better understanding of the antimicrobial efficacies of these dressings.

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