Resistance to the biocidal activity of silver in burn wound dressings – is it a problem?

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Severe burn injuries are commonly associated with significant mortality and morbidity. A burn injury of 30% of the body surface area is associated with generalised depression of the immune system[1]. Survival from these injuries is due to many factors, including the control of bacterial colonisation and infections leading to sepsis. Many of the organisms commonly recovered from infected patients in the burn ICU are members of the ESKAPE[2] (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) group of pathogens recognised as the most challenging bacteria carrying multidrug (MDR) resistance facing clinicians today[2-4]. Efforts to control wound burn sepsis is routinely managed by the topical application of dressings containing silver. There is concern that some microorganisms can develop resistance to the biocidal activity of silver and that this may increase due to the widespread commercial use of silver[5].

Background

Silver's biocidal activity dates back to Ancient Greece and Rome when silver coins dropped into receptacles of water acted as a disinfectant. The silver ion has been the biocide of choice in the topical control of burn wound infections[5]. One of the earliest uses of silver was the application of 0.5% silver nitrate to the burn wound and this was continued until recent years in some burn units, but it was eventually discontinued due to its staining property[6].

Silver sulphadiazine cream was first described by Fox *et al*.[7] in 1968 and is still widely used throughout the world. It has recently been superseded by the development of a fabric coated with nanocrystalline particles of silver, approximately 15 microns in size[8]. Despite some drawbacks, this material is the most popular wound dressing due to the ease of usage and reduction in nursing time to apply complex wound dressings. However, in some cases this fabric does not conform well to the body surface and the burn wound, potentially leaving some area without total contact with the silver coating.

Mechanical debridement of the burn wound is an essential part of daily wound management in which removal of loose, necrotic debris is carried out prior to the application of wound dressing[1]. Instructions on the use of the silver nanocrystalline dressings require that the dressing be moistened with water prior to its application and that saline is contraindicated as the silver reacts with the chloride ion, reducing the availability of the active silver ion. As the burn wound exudes tissue fluid containing chloride, particularly in the early post-burn period, it is likely that the surface effectiveness of silver may be reduced during this time. Other dressing materials (Actisorb™) are also available which contain low-release silver and in addition have the ability to absorb any wound exudate and thus form a close bond with the wound surface[9]. Bacterial and fungal biofilm formation and the inability of various antimicrobials to penetrate the burn wound is a problem and this forms the basis of bacterial colonisation, which may lead to systemic infections[10].

Biocidal action of silver

In the literature few have looked at the mechanism(s) of bactericidal action or discussed how or why different organisms exhibit varying sensitivity to the silver ion. Microbiological studies[5, 11] show that the "activated" silver ion can exert its lethality through action on the bacterial cell membrane (envelope) or binding to and inactivating intracellular proteins/enzymes and nuclear DNA. More substantive information on the bactericidal action of silver relates to its accumulation in bacterial cells and its opportunity to interact with the cytosolic proteins, mitochondrial enzymes nuclear DNA or RNA synthesis. *In vitro* studies have shown that the bactericidal effect is attributable largely to the binding of the silver ion to free sulphydryl groups in the bacterium or on its surface. Silver sulphadiazine and two other silver-containing products were shown to inhibit the growth of *Candida albicans* or *E. coli* through the inactivation of the enzyme phosphomannose isomerase[11,12]. Where the enzyme was mutated to replace the free cystine moiety with alanine (lacking-SH groups), inhibition was not seen[12]. Sodium chloride has been shown to inhibit the antibacterial action of silver nitrate by precipitating the silver as insoluble silver chloride while ethylenediaminetetraacetic acid (EDTA) or ethylene glycol tetraacetic acid (EGTA), has been shown to enhance the biocidal effect of silver nitrate, possibly through chelating silver binding surfaces[11].

Microbiology of the burn wound

In contraindication to most other wounds, the burn injury may involve a large surface area in excess of 80% of the body surface area. This area may comprise superficial burns which may spontaneous heal, those which are deep and may require skin grafting and areas of ulceration. The burn wound thus provides an ideal medium for microorganisms to colonise and proliferate[1,3,13]. Significant Gram-positive organisms such as *Staphylococcus aureus* (Figure 1) are usually noted in the first few days following injury, whilst Gram-negative organisms appear in the second week. Fungal elements are usually not prominent until the third week post-injury, *Candida* spp. and filamentous fungus such as *Aspergillus* spp., *Fusarium* spp., the Phycomycetes can also invade[1]. Prolonged healing and chronicity of the burn wound may result in the development of a biofilm in the wound and the presence of resistant microorganisms[10]. The changing pattern of flora in the burn wound often reflects changing methods of therapy more than any other factor, and although the ecology of the burn-wound flora can be altered by therapy, the burn wound cannot be sterilised. Figure 2 is an electron micrograph showing cocci and rod-shaped bacteria, some which are viable and some non-viable. Administration of antimicrobial agents merely provides environmental pressure for a change in the burn wound flora to more resistant organisms[1]. This is illustrated by positive blood cultures and surveillance of infected wound swabs of patients who survive following burns to 80% of the body. Multidrug-resistant organisms (MRSA, vancomycin-resistant enterococci (VRE), and Gram-negative bacilli (*P. aeruginosa, Acinetobacter baumannii*) occur despite single room accommodation, patient and unit microbiological surveillance, meticulous infection control measures and the isolation of infected patients[12]. In addition, the severely injured burn patient is at risk from biofilm associated with the presence of implantable devices such as central and urinary catheters as well as endotracheal tubes[10,14,15].

Silver resistance and burn wound infections – time for action?

There is abundant evidence that the use of silver dressings reduces septic episodes in the burn patient and is associated with an improved outcome[9]. Laboratory tests also show that silver is an effective biocide to a wide variety of organisms in the concentrations associated with and released by the dressing material. Despite this, a recent comprehensive review of 26 random control trials (involving 2066 participants) by the Cochrane Group[16] has found that the use of dressing materials containing silver does not prevent burn wound infection. Concern has been raised regarding the potential for development of bacterial resistance and an association with cross-resistance to antibiotics has been implied. Cross-resistance is of concern because plasmids encoding silver-resistant genes also may encode for antibiotic resistance. Silver-resistant organisms have been reported in clinical and environmental samples[17]. The genetic basis for silver resistance was first reported by McHugh *et al.*[18] underscoring that silver resistance was plasmid-encoded.

Studies[18,19] on the silver-resistant determinant plasmid pMG101 established the molecular basis of silver resistance. Plasmid pMG101 is a 182kb transferable plasmid on the bacterial chromosome encoding resistance to silver (nine open reading frames [ORFs] in three transcriptional units), mercury, tellurite, ampicillin, chloramphenicol, tetracycline, streptomycin, and sulphonamide. It confers resistance in bacteria at silver concentrations six or more times the concentration of what a sensitive enteric bacteria can tolerate. Functions assigned to the genes are based on homologous systems encoding resistances to other metals. The silver-resistance system encodes two silver efflux pumps (one an ATPase and the other chemical osmotic) and two periplasmic Ag^+ ion binding proteins. For a more detailed description of how these genes function, the reader is directed to the paper by Silver[19], where Figures 3A and 3B give a brief diagrammatic representation of a model on how all these genes carried on this plasmid are associated and function in conferring silver resistance. The question remains, could the use of 'new' silver dressings delivering sub-lethal (to bacteria or fungi) doses of silver in the long process initiate a development leading to resistant strains[19-22]? The literature contains inconsistencies with respect to silver release and the resulting antimicrobial efficacy. The existence of silver-resistant pathogens and that of a potential mechanism promoting resistance has been demonstrated[18]. Silver-resistant bacterial strains have been isolated from both clinical and environmental sources and these resistances have not been followed with further research[17,21]. Further work is

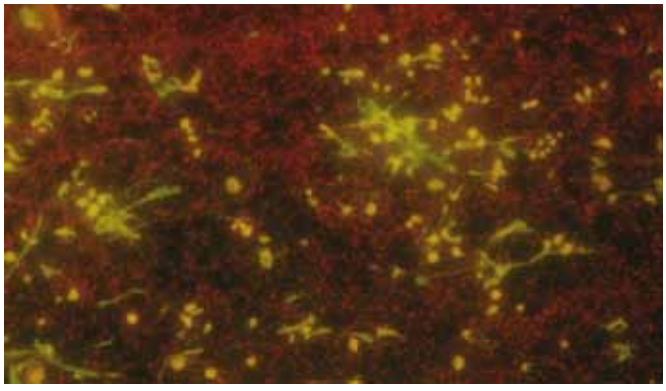

Figure 1. Acridine orange stain showing staphylococcal colonisation (orange microorganisms) from a burn skin specimen under UV light microscopy.

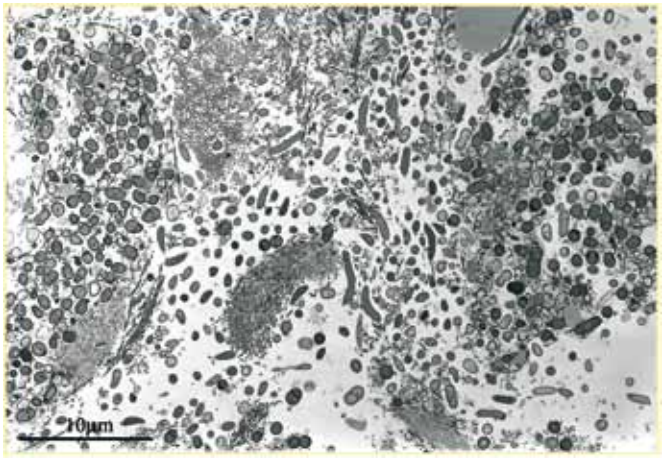

Figure 2. Electron micrograph showing bacterial skin colonisation (bacteria cocci and rod shapes) from a burn patient. Some of these microorganisms are not viable in normal bacterial cultures yet can be identified by EM microscopy[15].

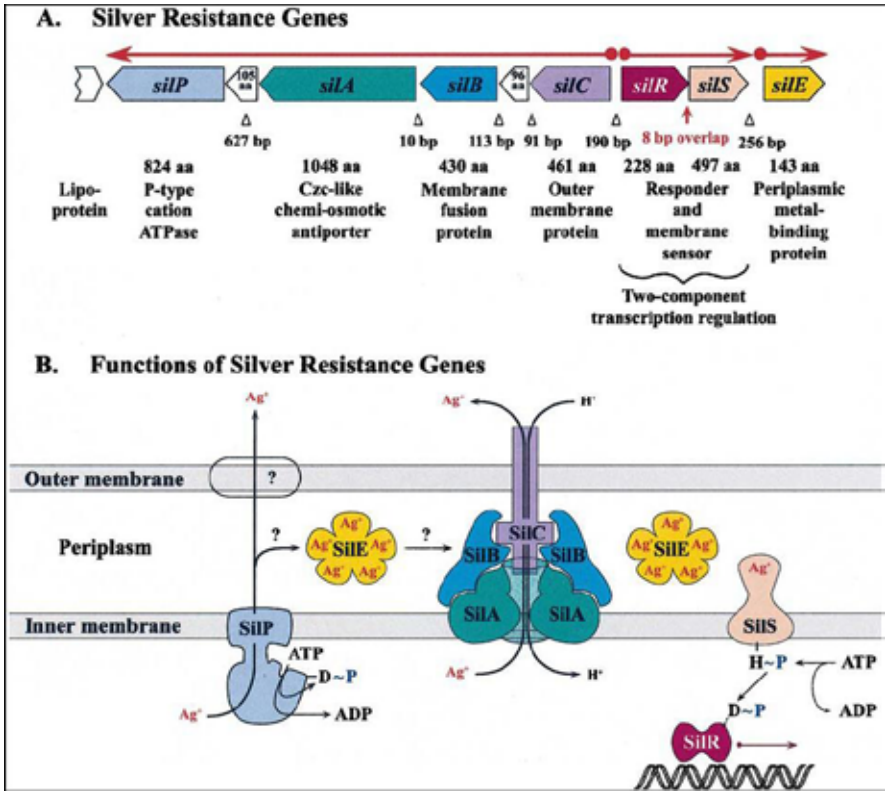

Figure 3. Diagrammatic representation of silver resistance mechanisms.
A: The pMG101 plasmid on the bacterial chromosome and the associated silver-resistant genes and functions.
B: Model of the functions of silver genes and protein products adapted from article from Silver [18].

urgently needed to examine mechanisms and prevalence of silver sensitivity in bacterial and fungal strains found in the burn wound[23].

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Dr Peter Kennedy AM MDS MB BS FRACS has a distinguished career and was made a Member of the Order of Australia for "Service to medicine, particularly in the fields of severe burns management and emergency medicine". He first commenced management of burns injuries in 1976 and in mid 1980's was appointed as Staff Head and Neck Surgeon and Director of the Burns Unit of Concord RGH and later as a Commander in Disaster Response by the New South Wales Health Department. During bushfires in 1993 he was responsible for the overall management and placement of several hundred evacuees from three hospitals and nursing homes. Dr Kennedy has now limited his practice to the care of Burn Injuries and is currently a VMO to the Burn Unit at Concord Hospital, Sydney.