



## Wound Bed Preparation

### It's About TIME

Wound bed preparation is a new approach that integrates proven concepts to build a platform for the treatment of chronic wounds. It organizes currently approved medical procedures and products into a holistic approach that can be used to evaluate and remove barriers to the wound-healing process. Removal of such barriers allows for optimal wound repair and healing. To address these barriers, a systematic approach to the management of chronic wounds has been developed. The approach goes by the acronym TIME (tissue: non-viable or deficient; infection or inflammation; moisture imbalance; and edge of wound: non-advancing or undermined) — the goal is to establish a well-vascularized wound bed that facilitates the effectiveness of other therapeutic measures.

Clinical studies have shown that wounds that do not heal often have a high bacterial burden. This implies a connection between high bacterial burden in the wound bed and the failure of chronic wounds to close. Decreasing this bacterial burden is a vital element of wound bed preparation. The use of silver dressings deserves careful consideration in this endeavor.

This is the third of 12 supplements that discuss various aspects of the TIME principle.



83 General Warren Boulevard, Suite 100  
Malvern, PA 19355  
Phone (800) 237-7285 FAX (610) 560-0502  
[www.hmpcommunications.com](http://www.hmpcommunications.com)

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## The Problem — How to Correct Chronic Wound Abnormalities to Enable Healing

When wounds do not heal in a timely manner, bacterial burden is one culprit. Factors that must be considered include the type and amount of bacteria present and whether synergy, even between lower levels of organisms, is exacerbating reasons for delayed healing. In addition, determining where the wound exists along the bacterial continuum (contamination, colonization, critical colonization, infection) will influence wound management decisions, including dressing selection.

Interest in silver and its role in wound healing have been rekindled in recent years. Understanding the history and evolution of silver use in wound care products allows wound care clinicians to make better informed choices for their patients.

## The Solution — Weighing the Value of Silver: Effectiveness and Potential for Bacterial Resistance

**Silver as an antimicrobial agent.** Silver as an antimicrobial agent has an impressive spectrum of use — from disinfecting hospital and hotel water sanitization systems to inhibiting bacterial and fungal growth in food products to sterilizing recycled water aboard the MIR space station and the NASA space shuttle. Silver is used as a preservative in cosmetics and toiletries and incorporated into various forms of plastics to protect against microbial contamination; colloidal silver has been used as a health food additive.

Silver's long medicinal history dates back to ancient Greece and Rome when silver coins dropped into receptacles of water acted as a disinfectant. In 1884, Crede, a German obstetrician, used a 1% silver nitrate solution to eliminate blindness caused by postpartum infection in newborns. In 1887, von Behring used the same compound to treat typhoid and anthrax. von Nägeli coined the term *oligodynamic* to describe silver's mode of activity in 1893. In 1964, Moyer, Monafó, and Burke first used silver in the burn arena; 4 years later, Fox developed silver sulfadiazine (SSD). Medically, silver-coated catheters are used to prevent infection.

**Silver's modes of action.** Slawson and Trevors<sup>1,2</sup> describe silver as generally more toxic than most other metals. The antimicrobial activity of silver involves complex interaction with membranes, enzymes, nucleic acids, and other cellular components. Silver ions (Ag<sup>+</sup>) interact strongly with electron donor groups containing nitrogen, oxygen, and sulfur<sup>3</sup> present in microbial cells as amines, hydroxyl groups, phosphates, and thiols. It has been postulated that thiol group-containing enzymes such as lactate dehydrogenase are inhibited by the binding of Ag<sup>+</sup>.<sup>4</sup> Silver also has been shown to inhibit the respiratory chain at two sites; hypothetically, silver exerts its toxicity at multiple sites.<sup>5,6</sup> Potential sites of action for silver associated with energy-yielding reactions of the respiratory chain, collapse of proton motive force, and interference with phosphate uptake have been investigated. Indirect toxicity may arise from salt formation with silver ions that results in a chloride or anion limitation within the cell.<sup>7</sup> Silver also may compete for cellular entry with an essential copper transport system. Intracellular metal accumulation results in a greater uptake of silver than simply silver binding to the cell surface.<sup>8</sup>

Once uptake occurs, silver may bind to various cellular structures. Silver introduced into *Pseudomonas aeruginosa* is (in majority) complexed to DNA.<sup>9</sup> In addition, silver binds to adenosine in a stoichiometric ratio of 2:1.<sup>10</sup> Silver also complexes with various proteins and nucleic acids.<sup>12</sup> From a toxicity

standpoint, silver likely penetrates microbial species sensitive to the metal. For instance, SSD binds to the cell wall and membrane of *P. aeruginosa*, producing bleb-like structures.<sup>12</sup> Silver accumulation by non-growing *Escherichia coli* cells is due to both surface binding and intracellular uptake. Silver is accumulated by an energy-independent process and initially bound to the specific structures within the cell. Once these sites are saturated with silver, binding to the cell surface occurs<sup>7</sup>; therefore, metal accumulation may occur in two stages — a rapid, reversible, and metabolically independent surface binding followed by metabolically dependent, irreversible, intracellular accumulation.<sup>6</sup>

**Silver resistance.** The historical and current broad use of silver has prompted concern over silver resistance. Gupta et al<sup>13</sup> state that silver resistance is important to monitor because modern technology has developed a wide range of products that depend on silver as a key microcidal component. This is a common theme in the world of microbial resistance and is well exemplified by the appearance of triclosan-resistant organisms as a result of over use of this well known antimicrobial agent. Levy<sup>14</sup> states that the widespread use of silver could result in more bacteria developing resistance, analogous to the emergence of antibiotic- and biocide-resistant bacteria. Ultimately, development of silver-resistant bacteria has led to decreased effectiveness for silver-based treatments.

Researchers who have spent careers studying this issue seem to place significant emphasis on silver resistance. Silver resistance has been associated with invasive burn infections, as well as other wound types. Silver-resistant bacterial strains (eg, *Acinetobacter baumannii*, *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Pseudomonas stutzeri*, and *Salmonella typhimurium*) have been isolated from both clinical and environmental sources.<sup>15,16</sup> Why, then, is knowledge of silver resistance limited? One reasonable explanation is the fact that only silver nitrate and SSD have been available in wound care. These two forms each deliver more than 3,000 ppm of Ag<sup>+</sup> over a 24-hour period — levels of silver ion that result in a rapid bacterial kill rate (bactericidal in the extreme); thus, minimizing the chances for survival, mutation, and resistance. Newer “advanced” dressings that deliver low levels of silver are worthy of further consideration.

**Mechanisms of silver resistance.** The rapid influx of a wide variety of silver-based antimicrobial dressings into the wound care arena has increased concern regarding silver resistance. Although it seems highly unlikely for a microbe to develop resistance to all of the modes of action, Gupta et al<sup>17</sup> point out that a resistance determinant has been cloned and sequenced from a burn isolate of *Salmonella* known for other metal resistance systems. Much of the heavy metal resistance results from a protective mechanism that pumps the silver ion from the cell and/or from chelation by metal-binding proteins (often using cysteine or histidine residues) where the sulfur complexes the Ag<sup>+</sup> to form Ag<sub>2</sub>S (silver sulfide), an insoluble silver salt. The formation of this salt negates the antimicrobial activity of Ag<sup>+</sup>. The availability of the genes for silver resistance has allowed for the identification of closely related genes in bacteria from various areas of the environment including the clinic.<sup>18</sup>

Slawson et al<sup>19</sup> investigated silver resistance and accumulation in Ag<sup>+</sup>-resistant *P. stutzeri* strain AG259 and Ag<sup>+</sup>-sensitive *P. stutzeri* strain JM303. Both strains exhibit a similar pattern of silver accumulation although to different final concentrations. X-ray analysis revealed dense silver deposits with the silver-resistance strain but not the silver-sensitive strain. Little is

known about the mechanism of silver resistance in Gram-negative bacteria.<sup>19</sup> When they contain silver-resistant plasmids, silver-resistant bacteria sometimes have been found to accumulate less silver than susceptible strains.<sup>14,20,21</sup> In Gram-negative bacteria, at least two general mechanisms effectively block drug access — the outer membrane (OM) and active efflux systems.<sup>22,23</sup> Mutants of *E. coli* lacking OM porins (microscopic pores) were more resistant to silver. Silver-resistant strains of Gram-negative bacteria (such as *Citrobacter freundii*, *E. coli*, and *K. pneumoniae*) selected on silver-containing agar plates often lacked major OM proteins.<sup>24</sup>

Li et al<sup>24</sup> demonstrated that *E. coli* microbes may develop resistance to silver in a stepwise fashion. By exposing microbes to low (bacteriostatic) levels of silver, silver-resistant strains may evolve that are tolerant to quite high levels of silver. In another study involving *P. aeruginosa*,<sup>25</sup> silver-resistant mutants were selected by stepwise selection on tryptic soy broth agar plates containing increasing concentrations of either AgNO<sub>3</sub> or SSD without the use of mutagens. Minimum inhibitor concentration (MIC) values were determined for several silver-susceptible strains and the respective resistant strains. Silver-susceptible strains of *E. coli* studied included 116, 496 and B1; silver-resistant strains included 116AgNO<sub>3</sub>R, 496AgNO<sub>3</sub>R, 496SSDR, B1AgNO<sub>3</sub>R, and B1SSDR. The MIC values for all of the silver-susceptible strains were 8 ppm; whereas, for the silver-resistant strains, values were >1, 024 ppm. These results clearly show that by gradually increasing the level of silver exposure of silver-susceptible strains of *E. coli*, resistant strains with remarkably increased MIC values could be produced. Li et al<sup>25</sup> also measured radioactive AgNO<sub>3</sub> accumulation by intact cells. Silver-resistant mutants of *E. coli* were obtained by stepwise exposure of the parent strains to increasing concentration of AgNO<sub>3</sub> and SSD. Analyses of the OM and cytoplasmic membrane proteins of the silver-resistant mutants showed all five silver-resistant mutants were essentially deficient in either OmpF porin or both OmpF and OmpC porins. The quantitative results of the OM permeability assay indicated that the permeability of the laboratory-selected silver-resistant mutants was five times lower than that of the parent strains. Silver accumulation studies were carried out mainly using one pair of strains, *E. coli* 116 and 116 AgNO<sub>3</sub>R. The laboratory-selected silver-resistant mutants of *E. coli* were deficient in their major porins. This finding was supported by the lower OM permeability in the mutants. However, the absence alone cannot explain the silver resistance because various well-characterized OmpF- and/or OmpC-dependent mutants showed almost identical susceptibilities to silver ions. Silver ions are believed to be actively pumped out by resistant (and even susceptible) cells of *E. coli* and that lowered OM permeability acts synergistically with this efflux mechanism to raise the level of resistance.

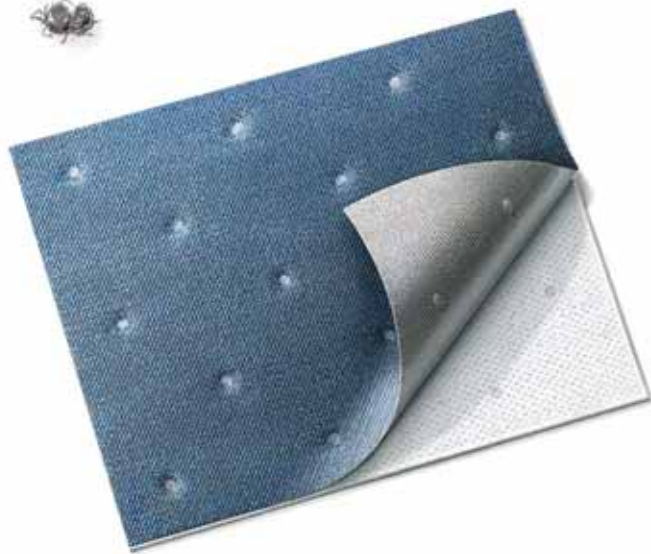
Active efflux is well known as a major mechanism of both antibiotic and heavy metal resistance.<sup>22,23</sup> Silver-resistant strains accumulated much lower steady-state levels of silver than the susceptible parent strains. This cannot be explained by differences in permeability alone and requires the assumption that silver is extruded out of the resistant cells. Likely, the efflux system for these strains is encoded by chromosomal genes because the parent strains used were fully silver susceptible and plasmid DNA could not be detected in either 166 or 496. The low level of accumulation of silver ions reported for plasmid-containing silver-resistant strains<sup>15,18</sup> is also due to the active efflux of this ion.

## Conclusion

Numerous strains of Ag<sup>+</sup>-resistant bacteria have been identified in the clinic and other healthcare settings. The strategies for silver resistance are numerous and varied. Studies have shown that the use of sublethal (to bacteria) doses of silver could initiate a process potentially leading to resistant strains. However, if bactericidal levels are used, the potential of selecting for resistance would likely be reduced. With the rapid influx of a wide variety of silver-based antimicrobial dressings into the wound care arena, the time is right to address this issue.

## References

1. Slawson RM, Lee H, Trevors JT. Bacterial interactions with silver. *Biol Metals*. 1990;3:151–154.
2. Trevors JT, Oddie KM, Belliveau BH. Metal resistance in bacteria. *FEMS Microbiol*. 1985;32:39–54.
3. Grier N. Silver and its compounds. In: Block SS, ed. *Disinfection, Sterilization and Preservation*. Philadelphia, Pa.: Lea and Febiger;1977.
4. Rogers K.S. Variable sulfhydryl activity toward silver nitrate by reduced glutathione and alcohol, glutamate and lactate dehydrogenase. *Biochem Biophys Acta*. 1972;263:309–314.
5. Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Escherichia coli*. *Can J. Microbiol*. 1973;20:883–889.
6. Slawson RM, Van Dyke MI, Trevors JT. Germanium and silver resistance. In: *Accumulation and Toxicity in Microorganisms*. Philadelphia, Pa.: Academic Press/Elsevier;1992.
7. Ghandour W, Hubbard JA, Diestrung J, Hughes MN, Poole PK. The uptake of silver ions by *Escherichia coli* K12: toxic effects and interaction with copper ions. *Appl Microbiol Biotechnol*. 1988;28:559–565.
8. Hughes, MN, Poole, RK. *Metals and Microorganisms*. London, UK: Chapman and Hall;1989:287–291.
9. Modak SM, Fox CL, Jr. Binding of silver sulfadiazine to the cellular components of *Pseudomonas aeruginosa*. *Biochem Pharmacol*. 1973;22:2391–2404.
10. Phillips R, George P. Metal-ATP bindings. I. Thermodynamic data for adenosine-silver binding. *Biochem Biophys Acta*. 1968;162:73–78.
11. McNeilage LJ, Whittingham S. Use of bio-rad silver strain to identify gel-purified RNA components of small nuclear ribonucleoprotein antigens. *J Immunol Methods*. 1984;66:253–260.
12. Coward JS, Carr HS, Rosenkranz HS: Silver sulfadiazine: effect on the ultrastructure of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 1973;3:621–624.
13. Gupta A, Silver S. Silver as a biocide: will resistance become a problem? *Nature Biotechnology*. 1988;16:888.
14. Levy SB. The challenge of antibiotic resistance. *Scientific American*. 1998;(278(3)):32–39.
15. Deshpande LM, Chopade RA. Plasmid mediated silver resistance in *Acinetobacter baumannii*. *Biometal*. 1994;7:49–56.
16. Henry AT, Stewart IO. Silver-resistant *Enterobacteriaceae* from hospital patients. *Can J Microbiol*. 1979;25:915–921.
17. Gupta A, Matsui K, Lo F, Silver S. Molecular basis for resistance of silver cations in salmonella. *Nature Med*. In press.
18. Slawson RM, Trevors JT, Lee H. Silver accumulation and resistance in *Pseudomonas stutzeri*. *Arch. Microbiol*. 1992;158:398–404.
19. Solioz M, Odermatt A. Copper and silver transport by CopB-ATPase in membrane vesicles of *Enterococcus hirae*. *J Biol Chem*. 1995;270:9217–9221.
20. Starodub ME, Trevors JT. Silver resistance in *Escherichia coli*. *RI J Med Microbiol*. 1989;29:101–110.
21. Starodub ME, Silver JT. Silver accumulation and resistance in *Escherichia coli*. *RI J Inorg Biochem*. 1990;39:317–325.
22. Silver S, Phung LT. Bacterial heavy metal resistance mechanism: new surprises. *Ann Rev Microbiol*. 1996;50:753–789.
23. Nikaido H. Prevention of drug access to bacterial targets: role of permeability barrier and active efflux. *Science*. 1994;264:382–388.
24. Li X-Z, Nikaido H, Williams KE. Silver-resistant mutants of *Escherichia coli* display active efflux of Ag<sup>+</sup> and are deficient in porins. *J Bacteriol*. 1997;179 (19):6127–6132.
25. Li X-Z, Nikaido H, Poole K. Role of MexA-MexB-OprM in antibiotic efflux in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 1995;39:1948–1953.



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1. Rubin RJ, Harrington CA, Poon A, Dietrich K, Greene JA, Molduddin A. The economic impact of *Staphylococcus aureus* infection in New York City hospitals. *Emerg Infect Dis*. 1999;5:9-17.

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