Challenging Silver – Influence of Extraction Medium on the Release of Silver from Commercial Silver Dressings

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ABSTRACT

A number of antimicrobial silver dressings based on different substrates and silver technologies have been introduced recently. Initial in vitro testing of a new cost-effective silver dressing* showed a strong dependence between silver release and the ionic strength and organic content of the extraction solution. To answer the question if all silver dressings show similar dependence on extraction medium, a number of commercial dressings were tested using simulated wound fluid (SWF =142mM sodium chloride and 2.5mM calcium chloride in water) and SWF plus 5% bovine serum albumin (BSA) as the extraction media. Silver elution as a function of solution composition and time was measured using Atomic Absorption Spectroscopy. The source of silver and the composition of the extraction medium were found to strongly affect the release of silver. Apparent equilibria established between silver and halide-containing salts (e.g. chloride ions) were disrupted by the presence of 5% BSA.

* Tritec™ Silver by Milliken Healthcare Products LLC, Spartanburg, SC

BACKGROUND

As many wound care practitioners can attest, the use of silver-treated wound dressings is becoming a standard practice for reducing the risk of infection in chronic wounds. Despite the growing use of silver dressings, there still remains a great deal of confusing information regarding the dose, release and antimicrobial activity of silver. While no single study could answer all the questions regarding the use of silver as an antimicrobial, there are still opportunities to understand more about the kinetics of silver release and efficacy during typical conditions of use.

Silver ions are written as Ag⁺, the “+” representing the ionic nature of the atom. This appears simple enough, yet silver ions are so reactive, the release and efficacy can be surprisingly complex. Although there are some variations to the main theme, it is generally accepted that the most reactive and efficacious form of silver is solubilized Ag⁺. Many types of silver dressings are treated with silver ions either in the form of silver salts or silver ion-exchange compounds. For these types of treatments, silver release requires moisture or moisture plus ions to solubilize and facilitate release of Ag⁺. In the case of dressings treated with silver
metal, Ag\textsuperscript{0}, the metal must oxidize and form soluble silver ions to be effective. It has been reported that nanocrystalline silver treatments are unique and can release Ag\textsuperscript{0}/Ag\textsuperscript{+} nano-clusters as well as other solubilized forms of silver\textsuperscript{1}.

It is known that silver must be released into the wound fluid to exhibit antimicrobial activity in the dressing and on the surface of the wound so understanding the kinetics of release is critical to understanding the efficacy of silver dressings during use. Many previous studies of silver release kinetics from wound dressings have measured release into plain water or high ionic-strength solutions\textsuperscript{2,3}, possibly because of the difficulties in measuring silver in the presence of organics. Release of silver into an ionic solution such as normal saline may be relevant for understanding the interaction of saline-based wound cleansers with silver dressings; however, these studies are not as relevant for understanding the release of silver in the presence of complex mixtures of organic and non-organic molecules and ions that are prevalent in actual wound exudate. It is well known that complex organics such as proteins can affect the efficacy of solubilized silver ions\textsuperscript{4,5}; however, less information is available regarding the effect of biological components on the release of soluble silver from wound dressings.

While it is difficult for an in vitro model to completely simulate the actual use of a wound dressing, there are several critical parameters that should be included: exposure of the dressing to wound fluids at an appropriate volume to surface ratio; repeated daily exposures, and exposure to wound fluids with relevant levels of both ionic and organic components. In the studies described below, the dressing area to fluid volume ratio was based on the amount of exudate that would be produced per day from a highly draining venous leg ulcer (1.2g/cm\textsuperscript{2}/24hr).\textsuperscript{6} Serum albumin (the most abundant protein in human plasma\textsuperscript{7,8}) was a logical choice to simulate the organic load in wound fluid in a simple in vitro model. In addition, the concentration of protein in chronic wound fluid has been reported to range from ca. 2.6 to 5.1%, with a mean of 3.8 ±1.3%.\textsuperscript{9} To further simplify the laboratory studies, it was reasonable to substitute the readily-available Bovine Serum Albumin (BSA) for human serum albumin (HSA) as BSA is very similar to HSA with respect to both chemical and physical properties.\textsuperscript{10}

**OBJECTIVES**

The purpose of this study was to analyze release of silver from wound dressings in a medium that simulates proteinaceous wound fluid at a volume to surface area ratio corresponding to a heavily exuding wound over a period of several days. These analyses will allow us to better understand the kinetics of release and the antimicrobial performance of commercial silver dressings during use.
MATERIALS

Dressings tested in this study include several different formats of silver dressings, including composites, foam and hydrofiber-based dressings. The descriptions and loadings of silver in the dressings have been reported in several publications, including product literature and brochures.\textsuperscript{11,12}

- “Bilayer Composite” = Tritec\textsuperscript{TM} Silver by Milliken Healthcare Products is a bilayer contact dressing incorporating a silver ion-exchange compound and Active Fluid Management\textsuperscript{TM} technology.
- “Charcoal Composite” = Actisorb\textsuperscript{®} Silver 220 by Systagenix is silver-impregnated activated charcoal fabric contained in a non-woven sachet.
- “Foam” = Biotane Ag Foam by Coloplast\textsuperscript{™} is a hydrophilic polyurethane foam treated with a silver ion-exchange compound.
- “Mesh” = 3M\textsuperscript{™} Tegaderm\textsuperscript{™} Ag Mesh Dressing with Silver consists of silver sulfate deposited onto a non-woven cotton fabric.
- “Nanocrystalline Composite” = Acticoat\textsuperscript{TM} 7 with Silcryst Nanocrystals\textsuperscript{™} by Smith & Nephew\textsuperscript{®} is a multi-layer contact dressing. The silver treatment is a physical vapor deposition process.
- “CMC Hydrofiber” = Aquacel\textsuperscript{®} Ag Hydrofiber Dressing with Silver by ConvaTec\textsuperscript{®}, ionic silver-impregnated sodium carboxymethylcellulose, consists of AgCl combined with hydrocolloid (sodium carboxymethylcellulose).

METHODS

The goal of this study was to determine the kinetics of silver release from wound dressings into a simulated wound fluid (SWF; NaCl (142mM), CaCl\textsubscript{2} (2.5mM)) that is isotonic to actual wound fluid. Release kinetics were determined in this fluid with and without the addition of an organic component over several days with daily exchange of simulated wound fluid. Samples (19.6cm\textsuperscript{2} = surface area of wound contact side of dressing) of each dressing were placed into SWF at room temperature.\textsuperscript{2,3} For the study of release into wound fluid without organic components, each dressing sample was placed into 100ml of solution. For seven consecutive days, the dressings were removed each day and placed into fresh solution. Each day, the solution was collected and measured for silver concentration using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

For a subsequent study, samples (19.6cm\textsuperscript{2}) of each dressing were placed into 100ml of SWF + 1.0, 2.5 or 5.0% Bovine Serum Albumin (BSA; Fraction V, Sigma-Aldrich) at room temperature for seven days. In a similar study, samples (19.6cm\textsuperscript{2}) of each dressing were placed in 24g of SWF + 5% BSA. For seven consecutive days, the dressings were removed each day and placed into fresh solution to mimic the continuous exposure of exudate to the wound dressing over time. For each study, a sample of the solution was collected daily and measured for silver concentration using Atomic Absorption Spectroscopy (AAS). This instrument is better suited to measure silver in a solution containing protein than ICP-OES.
RESULTS

Silver Release in Simulated Wound Fluid with No Organic Load

In the absence of an organic loading, the chloride levels typically present in wound fluid have a dramatic effect on silver release from wound dressings. Regardless of dressing type or type of silver treatment, silver release reaches an apparent equilibrium at ca. 0.5ppm (Fig 1). Because of this equilibrium, the concentration of silver released does not depend on the amount of silver in the dressing as long as the dressing is capable of releasing enough silver to achieve equilibrium. For example, the charcoal composite dressing in Figure 1 did not release enough silver to achieve equilibrium during any of the seven daily exposures. The apparent equilibrium can be explained by a number of factors related to the solubility of AgCl, including the solubility product constant (k_sp), Le Châtelier’s principle (common ion effect), and the lyophobic colloidal nature of the solution.13

Figure 1. Average release of silver from commercial wound dressings into simulated wound fluid with no organic components. Dressings were immersed in 100ml of fresh NaCl/CaCl_2 solution every 24hr. Each day, the solution was collected and measured for silver concentration by ICP-OES. Studies were run in triplicate. Error bars represent the standard deviation of the mean value.
Silver Release in Simulated Wound Fluid with Organic Load

Although release of soluble silver from wound dressings into a wound fluid based on salts alone is interesting and important for understanding the chemistry of silver ions (and relevant to wound cleansers and saline used to irrigate wounds), this situation is not clinically relevant to a silver dressing placed onto an exuding wound.

Addition of an organic “load” as would be found in wound fluid dramatically increases silver release from commercial silver dressings. For example, daily release of silver from a new bilayer contact silver dressing, Tritec™ Silver, increased substantially with increased levels of bovine serum albumin (BSA) (Fig. 2). This phenomenon was observed for other commercial dressings as well, although the magnitude of the effect varied (Fig. 3).

![Figure 2](image1.png)

**Figure 2.** Release of silver from a bilayer composite silver dressing into simulated wound fluid supplemented with 1, 2.5 or 5% BSA after 24hr at room temperature. Samples of dressings were immersed in 100ml of solution and analyzed periodically over seven days for the concentration of soluble silver using AAS.

![Figure 3](image2.png)

**Figure 3.** Release of silver from several commercial dressings into simulated wound fluid supplemented with 0, 1, 2.5 or 5% BSA. Samples of dressings were immersed in 100ml of solution for 24hrs at room temperature, and then analyzed for the concentration of soluble silver using AAS.

These results indicate that silver release from silver dressings is enhanced by the presence of a protein similar to those commonly found in blood and wound fluids. Because it is well known that proteins like BSA can bind to and inactivate released silver ions, the next phase of these studies will be to examine the biological efficacy of silver dressings with different levels of BSA.
Another important consideration is the duration of silver release under clinically-relevant conditions. As described previously, we have attempted to simulate several critical parameters involved with the use of silver wound dressings, including the exposure of dressings to simulated wound fluid over seven days and mimicking the composition and hydrodynamics (volume to surface area; fresh fluid each day) of wound fluid in a heavily exuding wound. Using these conditions of exposure, we measured the release of silver over seven days and determined the percentage of silver remaining in the dressings based on the amount of silver released and the dose of silver contained in the dressing samples (based on literature values). The most efficient silver dressing would release silver at an effective level throughout the duration of use and would release all of the silver by the end of normal use. If a dressing exhausts its silver too quickly, the dressing may not be protected from contamination for the entire duration of use. If a dressing exhausts its silver too slowly, the dressing may never reach an effective level of silver release and/or a portion of the expensive silver treatment is wasted because the dressing will be removed and discarded before all the silver is utilized. The levels of daily silver release from several commercial silver dressings are shown in Figure 4. All dressings exhibited the same "bolus" effect with the highest silver release occurring on the first day of exposure. After the high initial release rate, the rate of release decreases substantially for all dressings, resulting in a lower slope and thus more sustained release. The percentage of silver remaining at the end of seven daily exposures reveals a wide range of release "efficiency". In the worst case, one type of dressing exhausted most of its silver reservoir after only one day of exposure (Figure 5).

![Graph](image)

Figure 4. Average release of silver from commercial silver wound dressings into simulated wound fluid supplemented with 5% BSA. Samples of dressings were immersed in 24g of fresh solution every day for 7 days. After each day, solutions were analyzed for the concentration of silver using AAS. Error bars represent the standard deviation from triplicate samples and in many cases are smaller than the symbols.
Further analysis of the efficiency of silver release is shown in Figure 5. Results from this study indicate there is no "perfect" dressing in terms of effective and complete release of silver through seven days of use. Some dressings readily release 50-100% of the silver reservoir in the first two days of exposure. Other dressings are able to sustain a high release of silver, but still contain at least 30% of the silver reservoir at the end of the target duration of use. The most efficient dressing in terms of these two parameters was the bi-layer composite dressing (Tritec Silver), which sustained silver release for seven consecutive daily exposures and released >60% of its silver reservoir.

![Figure 5. Percentages of silver remaining in wound dressings through multiple exposures over a seven-day period. The percentage remaining was calculated based on the amount of silver released and the dose of silver contained in the dressing samples (from literature values).](image)

**CONCLUSIONS**

- In the presence of halides (e.g., Cl⁻) and absence of biological components, the silver released from most silver dressings peaked at ca. 0.5ppm of soluble silver. One dressing released too little silver to reach the apparent equilibrium level.
- Apparent equilibria established between silver and halide-containing salts (e.g., Cl⁻) were disrupted by the presence of protein (BSA) resulting in a dramatic increase in silver release over ionic solutions with no protein.
- All dressings tested responded similarly to increasing concentrations of protein regardless of different dressing formats and different forms and amounts of silver.
- It is clear from this study and other previous reports that organics such as protein act as a sink to disrupt and shift the equilibrium of soluble silver. Further studies will focus on the efficacy of released silver from different dressings and whether microbes themselves can act as a “sink” for silver ions.
- Dressings differed dramatically in the “efficiency” of silver release. The percentage of silver depleted from the dressings after exposure to clinically-relevant amounts of exudates ranged from 100% (silver reservoir was depleted after only one day of exposure) to ≤60% after 7 days.
REFERENCES

8. Mallick, P. http://www.biochemistry.ucla.edu/biochem/faculty/Mallick
12. a. AFM™ Ag product bulletin, Tegaderm™ Ag product bulletin, MSDS for Acticoat™ 7, AquaCel™ Ag product bulletin.
c. Thomas, S., McCubbin, P. J of Wound Care 2003; 12:8, 305-308.