FRESH VIEWS ON SILVER
Unique Chemical Structure and Clinical Efficacy of Ag Oxysalts™

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Introduction

Chronic wounds affect large numbers of patients globally and produce a substantial socioeconomic burden. Cost containment relies on timely healing of wounds and management of infection. Appropriate treatment of chronic wounds to promote timely healing is therefore a key focus for clinicians treating wounds. Antimicrobial silver dressings are used in the management of wound infections. The formulation of silver used in these dressings will influence antimicrobial activity and clinical effectiveness. So, clearly, clinicians must have a thorough understanding of the properties of a product to ensure effective use. Ag Oxysalts™ (silver oxynitrate) is a silver compound with unique properties that produce rapid, sustained, broad-spectrum antimicrobial activity. Ag Oxysalts™ is currently the only silver compound used in dressings to release Ag1+, Ag2+, and Ag3+ ions.

This supplement to Ostomy Wound Management was developed for the following objectives:

- Discuss how the chemical properties of Ag Oxysalts™ relate to its antimicrobial activity and clinical performance
- Describe how Ag Oxysalts™ effect the wound environment and promote healing independent of infection
- Explore the potential impact of Ag Oxysalts™ dressings such as KerraCel™ Ag (Crawford Healthcare) on wound healing through the presentation of clinical cases
- Understand how a Healthcare Professional (HCP) might explore the cytotoxicity, bacterial resistance, and biofilm disruption claims of the Ag Oxysalts™ technology

A Scientific Perspective

Luna, argentum, silver (Ag). As a metal of antiquity, the initial discovery of Ag is lost to history, but in the 21st century, its uses continue to evolve. Dr. Kalan was first introduced to Ag as she was immersed in research on the creation of new antibiotics that target drug-resistant microbes. She was asked to evaluate Ag Oxysalts™, a unique silver compound (Ag7NO11) and approached this as a scientist would with a small molecule antibiotic, beginning with the question: How does chemistry influence antimicrobial efficacy?

Ag is mined from the earth as pure metallic Ag⁰(s) or the salt AgCl(s), neither of which has antimicrobial activity. This is because the soluble Ag ion (Ag⁺) is required to capture electrons from bacterial cells, allowing Ag to be reduced back to a stable form (Ag⁰).¹² Electrons are like currency within a bacterial cell. They enable the cell to generate the energy required for DNA replication, protein synthesis, and cell division. If electron transfer processes are disrupted, the cell cannot survive. Ag Oxysalts™ are different from other Ag compounds in many ways; this summary will focus on solubility and a higher reduction potential.

Where do soluble Ag ions come from? A salt is an ionic complex composed of equal numbers of positively and negatively charged ions. Ag can exist as different salt compounds such as silver chloride (AgCl), silver sulfate (Ag2SO4), or silver oxynitrate (Ag7NO11). The formulation of Ag salts can greatly influence stability, solubility, and subsequent antimicrobial activity.¹³ Some compounds such as AgCl are virtually insoluble (remember this stable compound represents a major source of mined silver). Other silver compounds, including Ag Oxysalts™, are less stable and have high solubility in fluid, quickly releasing Ag ions to exert their activity. This is the

Figure 1. Ability to acquire electrons. Ag Oxysalts™ has 3 missing electrons, allowing it pull more electrons from bacteria to disrupt their function faster.
when oxygen is the anion used to stabilize positively charged Ag ions. Oxygen also is able to complex and stabilize more reactive Ag ions that exist in a higher oxidation state. This is a property that sets Ag Oxysalts™ apart from other Ag salts. Oxygen molecules stabilize the high reduction potential Ag²⁺ and Ag³⁺ ions, releasing oxygen alongside these highly reactive Ag ions when in contact with fluid.⁵⁻⁹

What does a higher reduction potential mean? Reduction potential measures the ability to acquire electrons. The higher the reduction potential, the stronger the affinity for capturing electrons. To put it simply, Ag²⁺ and Ag³⁺ are “stronger” than Ag⁺, allowing these ions to pull more electrons from different metabolic reactions and processes within a microbial cell (Figure 1). For example, Ag²⁺ has a reduction potential of +1.80V compared to the +0.8V for Ag⁺ (Table 1),⁴ and it needs to gain three electrons to get back to its stable state of Ag⁰ compared to only one electron for Ag⁺.

Speaking strictly about chemistry, solubility and reduction potential are two properties that influence antimicrobial activity. Compared side-by-side, Ag Oxysalts™ have the lowest minimum inhibitory concentration and minimum biofilm eradication concentration compared to other ionic Ag salts, resulting in less overall Ag use but greater efficacy.³,⁴

Clinically speaking, a highly soluble compound allows for bacteria to be killed faster. A higher reduction potential leads to higher efficacy. When you combine a highly soluble compound with high reduction potential, you have a compound that has the speed and strength to kill bacteria within a biofilm without cytotoxicity. Other properties and the effects of Ag Oxysalts™ on the wound environment are also currently being studied.

Inflammation is a crucial part of wound healing. When a wound occurs, neutrophils and macrophage are recruited to the site to remove pathogens, foreign material, and devitalized tissue. Once the wound is cleared of these substances, inflammatory cell levels diminish until healing is complete. However, in chronic wounds inflammation is heightened and prolonged. Increased numbers of neutrophils and macrophage persist within the wound. These inflammatory cells

<table>
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<th>Table 1. Standard Reduction Potentials</th>
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<td>Reaction</td>
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<tr>
<td>Ag³⁺ + e⁻ ⇌ Ag²⁺</td>
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<tr>
<td>Ag²⁺ + e⁻ ⇌ Ag⁺</td>
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<tr>
<td>Ag₂O + 2H⁺ + 2e⁻ ⇌ 2Ag⁺ + H₂O</td>
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<tr>
<td>Ag⁺ + e⁻ ⇌ Ag⁰</td>
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<tr>
<td>Ag₂SO₄ + 2e⁻ ⇌ 2Ag⁺ + SO₄²⁻</td>
</tr>
<tr>
<td>Ag₂O + H₂O + 2e⁻ ⇌ 2Ag⁺ + 2OH⁻</td>
</tr>
<tr>
<td>AgCl + e⁻ ⇌ Ag⁰ + Cl⁻</td>
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Figure 2. Ag Oxysalts™ promote many aspects of wound repair. The potent antimicrobial efficacy of Ag Oxysalts™ effectively kills bacteria within a wound biofilm. Despite the powerful antimicrobial action, dressings containing Ag Oxysalts™ promote healing of uninfected mouse wounds, reducing wound area, promoting reepithelialization, and dampening inflammation. The unique ability of dressings containing Ag Oxysalts™ to directly generate oxygen and to catalyze the breakdown of hydrogen peroxide to oxygen and water may be sufficient to create a more favorable wound environment to stimulate healing.
secrete a cocktail of proteinases, proinflammatory cytokines, and reactive oxygen species (ROS), including superoxide anions (O$_{2}$.-) and hydrogen peroxide (H$_{2}$O$_{2}$), that sustain inflammation and cause tissue damage. In turn, tissue damage supports microbial growth; consequently, infection is a frequent complication associated with chronic wounds. In an attempt to clear infection, additional inflammatory cells are recruited to the wound, leading to a cycle of elevated inflammation and causing tissue damage that supports microbial growth which, in turn, increases inflammation, exacerbating the hostile wound environment and inability to heal.

A wound stuck in the inflammatory stage of repair depletes the tissue of vital oxygen, an essential element in the generation of energy. During healing, demand for energy and subsequently oxygen to generate this energy are increased. Inflammatory cells consume high levels of oxygen; during phagocytosis, these cells utilize oxygen in the production of superoxide ions and hydrogen peroxide. Infec- tion exacerbates the problem, because aerobic bacteria consume oxygen within the wound. Thus, stalled or infected wounds often are deprived of vital oxygen. Therefore, antimicrobial therapies are paramount in the management of wound infections; however, concerns have been raised over their cytotoxicity and the effects they have on healing when infection is not present. Recently, the effects of Ag Oxysalts™ on healing independent of infection were assessed. Ag Oxysalts™ were added to the culture media of fibroblast and keratinocyte scratch wound assays. No effect was observed on the closure of fibroblast scratch wounds with the addition of Ag Oxysalts™; however, keratinocyte scratch wounds treated with Ag Oxysalts™ healed faster. When Ag Oxysalts™ dressings were applied to uninfected mouse wounds, the wounds healed faster, showing a reduction in wound area, increased reepithelialization, and decreased inflammation.

An examination was conducted of how Ag Oxysalts™ promote healing when infection is not present. Ag Oxysalts™ exposed to fluid are thought to break down and generate oxygen. These silver compounds were added to serum and the level of oxygen within the serum measured. Of the silver compounds tested, only Ag Oxysalts™ generated oxygen. In the wound environment, this oxygen may provide vital energy to the numerous cell types that require additional energy for effective repair. In a chronic wound, the addition of oxygen directly to the wound tissue may be sufficient to shift the wound out of a nonhealing state.

Ag Oxysalts™ also may promote healing through the ability to breakdown H$_{2}$O$_{2}$. Silver is a known catalyst of H$_{2}$O$_{2}$, breaking it down into oxygen and water. H$_{2}$O$_{2}$, produced by neutrophils and macrophage, is the primary mechanism for killing bacteria; however, this process causes severe tissue damage. During normal wound healing, enzymes rapidly detoxify H$_{2}$O$_{2}$. In chronic and infected wounds, levels can become unregulated. The ability of a variety of silver dressings to catalyse the breakdown of H$_{2}$O$_{2}$ was assessed. Only dressings containing Ag Oxysalts™ were able to catalyze the breakdown of H$_{2}$O$_{2}$ to oxygen and water. In recent years, the regulation of ROS through antioxidants and antioxidative enzymes has been examined as a mechanism to reduce oxidative stress-induced tissue damage and promote healing. The ability of Ag Oxysalts™ to breakdown H$_{2}$O$_{2}$ may rebalance redox signalling and create a less hostile wound environment to support repair.

These recent studies have shed light on the mechanisms of action of Ag Oxysalts™. Despite their potent antimicrobial efficacy, Ag Oxysalts™ do not adversely affect healing independent of infection. On the contrary, Ag Oxysalts™ promoted many aspects of wound repair when infection is not present.
Clinical Cases

Case One
A 70-year-old female presented with medical and surgical history of PAD, renal transplant on immunosuppressants, renal insufficiency, peripheral neuropathy, aortic stenosis, hypertension, cardiac stents x 3, ABI of 0.6, and multiple LE wounds over several months to years. Previous treatments included enzymatic debriding agents, 1+ silver ionic dressings, and alginate. She was on a course of intravenous antibiotics before the use of Ag Oxysalts™. She first presented to the clinic on August 26, 2016. The wound size was 15.1 x 15.1 x 0.3 cm at its greatest size in early April 2017 (Figure 3). The peri-wound tissue was hardened and edematous. The wound bed had 90% slough and was malodourous. Ag Oxysalts™ gel dressing was applied to her wound on her April 24, 2017 visit with no compression due to severe PAD and low ABI. Full closure of the wound was achieved on September 11, 2017 (Figure 4).

Case Two
A 75-year-old female presented with a past medical and surgical history of hypertension, Crohn's disease, proctocolectomy, osteoarthritis, and DVT with venous insufficiency ulceration involving the left lateral leg. She had different treatments used, including serial debridement, dressings that included honey-based dressings, silver foam, collagen-based dressings, and 1+ silver alginate dressings without much improvement. Compression was done using Coban wraps. The wound was biopsied. The pathology was suggestive of venous insufficiency. The patient underwent vein evaluation and subsequent laser treatment for venous insufficiency.

On April 26, 2018 (Figure 5), patient had a wound involving the left lateral leg measuring 6.2 x 3.2 x 0.1 with 60% necrotic tissue and 40% granulation tissue. Ag Oxysalts™ gel dressing was initiated. The wound was then covered with a secondary absorptive dressing and compression wraps were then applied. On July 26, 2018 (Figure 6), the wound measurements improved to 0.4 x 2.0 x 0.2 with 70% granulation tissue and 30% necrotic tissue.
Elemental silver in the Ag₀ format is a stable molecule and is relatively nonreactive. During the 1990s, for medical and healthcare purposes, elemental silver was developed into an ionic silver, a more reactive version and the only form of antimicrobial silver. This technology provided a way for silver compounds to be delivered in dressings and broken down into ions when exposed to wound fluid or an aqueous solution. The ions are an Ag⁺ electron format and can kill bacteria by rupturing the cell wall, inhibiting vital enzymes, destroying cells using free radicals, or interacting with the bacteria’s DNA. The mechanism of action (MOA) of newly engineered Ag Oxysalts™ technology makes highly oxidative states of silver ions (Ag¹⁺, Ag²⁺, Ag³⁺) available; thus, the silver is more powerful and can work faster while using a fraction of the amount of silver compared to other ionic silver formats³⁴ (Figure 7).

From an individual HCP perspective and after reviewing the MOA, the following questions can be asked to help one analyze the value of this new technology. These questions are based on experiences using silver dressings in clinical practice and teaching the antimicrobial properties of silver in pre- and post-professional coursework. Because of the historical challenges regarding the use of silver dressings in the literature, the questions were related to cytotoxicity and healthy tissue, resistance of microbes to silver, and the ability to disrupt a biofilm, not just planktonic bacteria.
Is this technology cytotoxic to healthy tissue and will it impair healing? Thomason et al. presented in vivo and in vitro data to support use of the Ag Oxysalts™ technology without impaired healing or cytotoxicity. Improved healing was demonstrated by significant decreases in wound area and increased epithelialization in full-thickness mouse wound models when the Ag Oxysalts™ technology was compared to a nonantimicrobial control. There also was evidence that treatment with Ag Oxysalts™ promoted wound healing and accelerated closure in human keratinocyte scratch wounds compared to untreated control scratch wounds.

Is there bacterial resistance to this technology? Bacteria have been around for millennia and have made some extraordinary adaptations to environment and in response to repeated exposure to lethal substances. This adaptive ability is evidenced among all the multidrug-resistant (MDR) bacteria known today. Using a time-kill analysis, Kalan et al. found that a panel of MDR pathogens isolated from wound specimens remained susceptible to Ag$_2$NO$_{11}$ over a period of 7 days, even with repeated inoculations of $1 \times 10^6$ CFU/mL to the dressing. Finley et al. found that both Klebsiella pneumonia (SRKP) and Enterobacter cloacae (SREC) expressing silver-resistant genes were highly resistant to 7 of 9 silver dressings; silver oxysalt dressing was among the silver dressings to which the SRKP and SREC remained sensitive.

Does this technology effectively disrupt biofilms? Dressings that contain higher oxidative states of silver (Ag$^{+}$, Ag$_{2}^{+}$, Ag$_{3}^{+}$) with Ag Oxysalts™ were found to be more effective against planktonic bacteria and bacteria within wound biofilms than another Ag$^{+}$ dressing. Mature Pseudomonas aeruginosa biofilms in a porcine ex vivo model were found to have statistically significantly less bacteria ($P < .05$) than the control 24 hours after application of Ag Oxysalts™ (Figures 8–10).

HCPs and educators who have evaluated many wound care products can conclude that the unique chemical qualities of the Ag Oxysalts™ compound allow it to perform as an effective antimicrobial, disrupting biofilm and improving wound healing processes without cytotoxicity and with minimal to no bacterial resistance. Upon evaluation of the MOA, published peer-reviewed literature, and clinical case support from other trusted HCPs, one could reasonably be encouraged to use this technology in clinical practice.

References
KerraCel® Ag dressing combines a carboxymethyl cellulose (CMC) gelling fiber dressing with Ag Oxysalts™ technology. Ag Oxysalts™ (Ag3+), provides **UP TO 6 TIMES MORE POWER** than traditional silvers (Ag1+) to reduce barriers that create a hostile wound environment.¹, ²

- **Quickly kills at least 99.999%** (5 log) of a broad spectrum of bacteria *in vitro*³,⁴,⁵
- **Effectively kills bacteria within a biofilm and prevents biofilm re-formation *in vitro*⁶,⁷
- **Reduces inflammation (in vivo) and H₂O₂ (in vitro) that cause tissue damage and support microbial growth.¹**

**References:**
3. Antibacterial efficacy of KerraCel® Ag against planktonic species over 7 days in vitro. CHC R539. 2017.

*As demonstrated in vitro

**Ag Oxysalts visualized by scanning electron microscopy.**

**Oxygen (O₂) is shown on the surface of Ag Oxysalts™. This O₂ is produced from the breakdown of Hydrogen Peroxide (H₂O₂) and the reaction of Ag Oxysalts™.¹**