A wound dressing containing Ag Oxysalts™ has greater antibacterial activity than other types of silver

D. Pepin[1], J. Wilson[2*], L. Kalan[1**]
[1]Exciton Technologies Inc, Edmonton, AB, Canada. [2]Crawford Healthcare, Doylestown, PA, USA
*Presenter. **Corresponding author lkalan@exciton.com

Background

Approximately $35-45 Billion USD/year is spent on hospital acquired bacterial infections, many of which are "Multi-" or even "totally" drug resistant. Novel treatment approaches to combat resistance is required. Silver (Ag) is a metal used for centuries in medicine and is of renewed interest for treatment and prevention of infection. The antimicrobial activity of Ag is multi-faceted, thus decreasing its potential for resistance.

Currently, many Ag containing medical devices incorporate either metallic Ag or compounds such as AgCl and Ag_2SO_4. The differences re significant as ionic Ag^{1+} is essential to antimicrobial activity and the physiochemical properties of these compounds relates to efficacy. Furthermore, research into highly oxidized $Ag^{(2+ \text{ and } 3+)}$ is still in its infancy. This study aims to differentiate the antimicrobial efficacy of Ag species often found in commercially available medical products with focus on Ag Oxysalts™ ($Ag_7NO_{11(s)}$) containing Ag^{2+} and Ag^{3+}.

Methods

Wound dressings containing $AgCl_{(s)}$, $Ag_2SO_{4(s)}$, $Ag_{(s)}$, $Ag_2O_{(s)}$, or $Ag_7NO_{11(s)}$ were evaluated for chemical composition by X-ray diffraction (XRD) and Ag release profiles into reverse osmosis water and a simulated wound fluid.

Potentiometric titration and neutron activation analysis (NAA) were used to evaluate.

Log-reduction assays were performed for *Staphylococcus aureus* and *Pseudomonas aeruginosa* over 7 days, re-challenging with 106 cfu/mL every 24 h. Quantification occurred at 4 h., 1, 3, 5 and 7 days.

This study aims to differentiate the antimicrobial and antibiofilm efficacy of Ag species found in commercially available wound dressings with focus on Ag Oxysalts ($Ag_7NO_{11(s)}$) containing Ag^{2+} and Ag^{3+}.

In Vitro Methods

Previously, a *in vitro* study utilizing the Calgary Biofilm Device screened a panel of Ag compounds and reported that significantly less Ag Oxysalts are required to:

1. Kill planktonic cells
2. Inhibit biofilm formation and
3. Eradicate established biofilms

This was consistent for both Gram (+) and (–) bacteria including multidrug resistant isolates, 24hr biofilms and mature 4-6 d biofilms[1]. The study also found that Ag Oxysalts can disrupt and reduce the total biomass of mature biofilms (fig 3).

Wound dressings containing different Ag compounds were then evaluated for their ability to kill mature (72hr) biofilms in 4hrs. The model was adapted from2 and biofilms were confirmed by SEM (fig 2). When the biofilms were treated in a petri dish with saline both the Ag oxysalt dressing and the HF Ag/EDTA/BC dressing had a >4-log reduction. When the biofilms were treated on a nutrient agar only the Ag oxysalt dressing was able to reduce the biofilm significantly from the negative control (fig 1 and 4).

Fig. 1

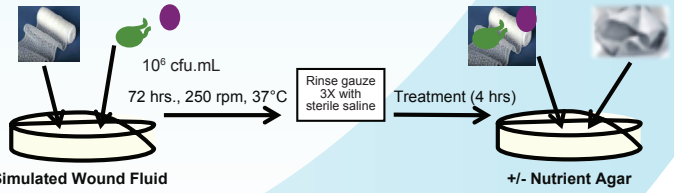

Figure 1: *In vitro* biofilm gauze model[2]. Biofilms are grown on fibers of sterile cotton gauze as shown above. The gauze is then rinsed in sterile saline to remove planktonic cells. The biofilms are then transferred to either a sterile petri dish or a plate of nutrient agar and test dressings applied. After a 4hr incubation at 37°C the biofilms and test dressings are vortexed in neutralization buffer for 1 min, serial diluted and plated to calculate log reduction values.

Fig. 2

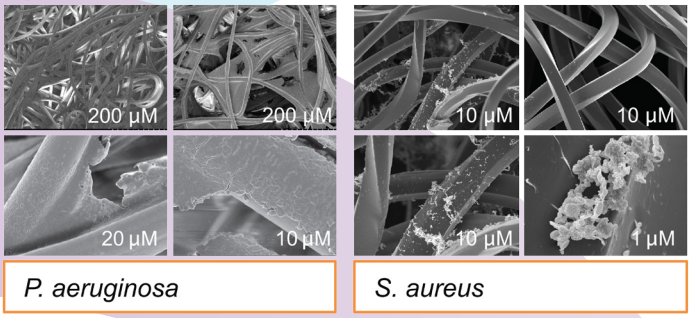

Figure 2: SEM images of 72 hr P. aeruginosa and S. aureus biofilms growing on cotton gauze in a simulated wound fluid (1:1 Fetal Calf Serum: Peptone Water)

Fig. 3

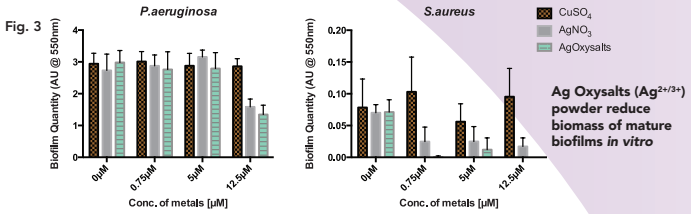

Figure 3: Ag_7NO_{11} reduces biomass. Biofilms of *E. coli, P. aeruginosa*, and *S. aureus* were established in the MBEC™ device for 24h and exposed to various concentrations of **A)** $CuSO_4$, **B)** $AgNO_3$ and **C)** Ag_7NO_{11} and stained according to O'Toole, 2011. –The biomass was quantified by measuring the absorbance at 550 nm. Values are represented as the mean ± the SD, n=15.

Ag Oxysalts (Ag2+/3+) wound dressings have a greater kill and log reduction of mature biofilms of carbapenem resistant P. aeruginosa in vitro

Fig. 4

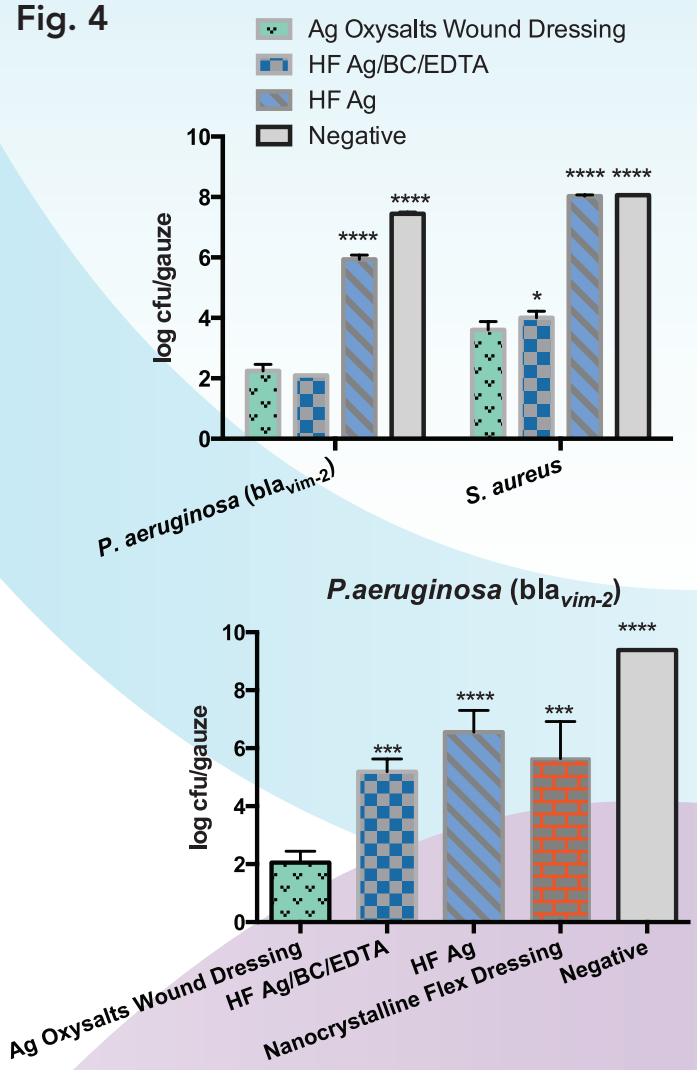

Figure 4: Log Reduction of *P. aeruginosa*$_{(bla\text{-}vim\text{-}2)}$ and *S. aureus* ATCC 6538 72hr biofilms grown on cotton fibers and treated with various Ag wound dressings for 4hrs. **A)** Treated in saline **B)** Treated on nutrient agar.

Results

- Release of Ag^{1+} was measured over the course of 7 days and the dressings with either $Ag_2O_{(s)}$ or $Ag_7NO_{11(s)}$ displayed a rapid and sustained release of Ag over time. (Data available, not shown).
- $AgCl_{(s)}$, or $Ag_2SO_{4(s)}$ released low levels of Ag. $Ag_2O_{(s)}$ and
- Ag7NO11(s) achieved a 4-log reduction after 4 hours, in addition to sustained 4-log reduction over 7-days.
- Ag Oxysalts disrupts biofilms.
- $AgC_{l(s)}$, $Ag_2SO_{4(s)}$, and $Ag_{(s)}$ wound dressings did not meet the 4-log reduction pass criteria.
- Dose was evaluated and 1.5 mg $Ag_2O_{(s)}/cm^2$ was required versus 0.4 mg Ag_7NO_{11}/cm^2 to maintain efficacy.

Conclusions

- The different properties of Ag complexes effect bioavailability as evaluated *in vitro*.
- Antimicrobial efficacy of Ag is dependent on release of Ag ions in a rapid and sustained manner.
- Ag_{2+} and Ag_{3+} provide rapid, sustained release, correlated to high antimicrobial efficacy against both Gram (+) and (-) organisms using a lower quantity of silver.

S. Davis.[2], Pepin, D.[1], Lemire, J.[3], Gil, J.[2], Valdes, J.[2], Solis, M.[2], Higs, A.[2], Turner, R.[3], and Kalan, L.[1]*

[1]Exciton Technologies Inc, Edmonton, AB, Canada, [2]Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, USA. [3]University of Calgary, Biofilm Research Group, Calgary, AB, Canada *lkalan@excitontech.com. [2] Rowlands et. al. 2013. ConvaTec Inc. SC-000387-GB.

Notes: 1) Ag oxysalt wound dressing: Kerracontact Ag **2)** HF Ag/BC/EDTA: Aquacel Ag plus **3)** HF Ag: Aquacel Ag **4)** Nanocrystalling Ag: Acticoat flex or Acticoat 7

References: [1]Lemire et al. 2015. AAC. 10.1128/AAC.05177-14; [2]Seth et al. 2015. *Wound Repair and Regeneration DOI*: 10.1111/wrr.12232; [3]Sullivan TP, et al. 2001. *Wound Repair Regen*. 2001 Mar-Apr; 9(2):66-76.