Antimicrobial activity of a novel silver dressing against wound pathogen biofilms *in vitro* and in an *in vivo* animal model

Prof Richard White

Anti-biofilm activity was measured by determining the minimum biocidal concentrations at 4h, minimum biofilm inhibition concentration and minimum biofilm eradication concentrations (MBEC) of biofilm populations of *S. aureus*, *E. coli*, and *P. aeruginosa* at 24h.

Methodology

1) Grow biofilms for 4h in the presence of metal treatments
2) Establish biofilms for 24h. Then treat with metals for 4 and 24h

*Media used:*
1. Peptone water (0.1% w/v peptone; 0.9% NaCl)
2. Simulated wound fluid (50% fetal calf serum/50% peptone water)

*Grown at 37°C @125rpm

Treatments (in serially diluted concentrations):
1. AgNO₃ ([Ag⁺])
2. CuSO₄ ([Cu²⁺])
3. Ag-oxy-salts (Exsults™) [Ag⁺, Ag₂⁺, Ag³⁺]

**note: Ag-oxy-salts were used at equivalent Ag concentrations as AgNO₃*

Ref: Kalan L et Al. Antimicrobial Silver in Medical Devices: Composition and Efficacy. Poster SAWC 2013
Organisms were grown in the Calgary Biofilm Device using peptone or simulated wound fluid media (SWF)- 50:50 peptone water and foetal calf serum to represent more closely the chronic wound environment. Silver and copper salts were used as control. The MBEC assay and confocal microscopy vital staining techniques were used to determine effect.

- Media influences both Ag release and antimicrobial efficacy against both planktonic cells and those growing as a biofilm.
- Charged species and proteins in complex medium bind ionic Ag dramatically reducing bioavailability.
- The MBC 4h/MBEC 24h Ag oxy-salt for P. aeruginosa was 36/156µM and, for AgNO3, 58/1250µM. The corresponding values for E. coli are 16µM/42µM and 8µM/38µM; and for S. aureus 250µM/1260µM.

- The concentration of Ag2+/3+ ions required to eradicate an established P. aeruginosa biofilm is 10x less than Ag+ as represented by silver nitrate.
- Ag2+/3+ species are able to maintain greater antimicrobial efficacy regardless of media type due to higher oxidative power.

This data has been corroborated *in vitro* at Perfectus labs UK.

- Twenty-four hour cultures of *Pseudomonas aeruginosa* were harvested from a Tryptone Soya Agar (TSA) plate using a sterile swab and re-suspended in 20ml of Tryptone Soya Broth (TSB)
- The bacterial suspension was then diluted corresponding to a bacterial concentration of $10^8 \pm 5 \times 10^7$ cfuml$^{-1}$
- CDC reactors were incubated for 24 hours and 72 hours at 37°C, shaking at 50rpm in order to encourage biofilm growth
- The silver oxysalt dressing gave a Log 7 reduction for both 24h and 72h biofilms.

![Graph showing quantity of viable *Pseudomonas aeruginosa* recovered from a pre-formed 24 hour biofilm after 72 hour treatment with test agents.](image)
In Vivo Biofilms

- In a pig Pseudomonas biofilm model the silver oxysalt dressing gave a 4 log reduction by day 6 Superior to some silver hydrofiber dressings with biofilm claims.

Conclusion

• Silver in higher oxidation state in the silver oxysalt dressing

• Disrupts and breaks down biofilms exposing bacteria to be killed

• Does this at far lower concentrations of Ag+

• Eradicates biofilms even in hostile environment of SWF

• In animal models sustained effect reduced bacterial load in wounds over 6 days.

• Refer to clinical