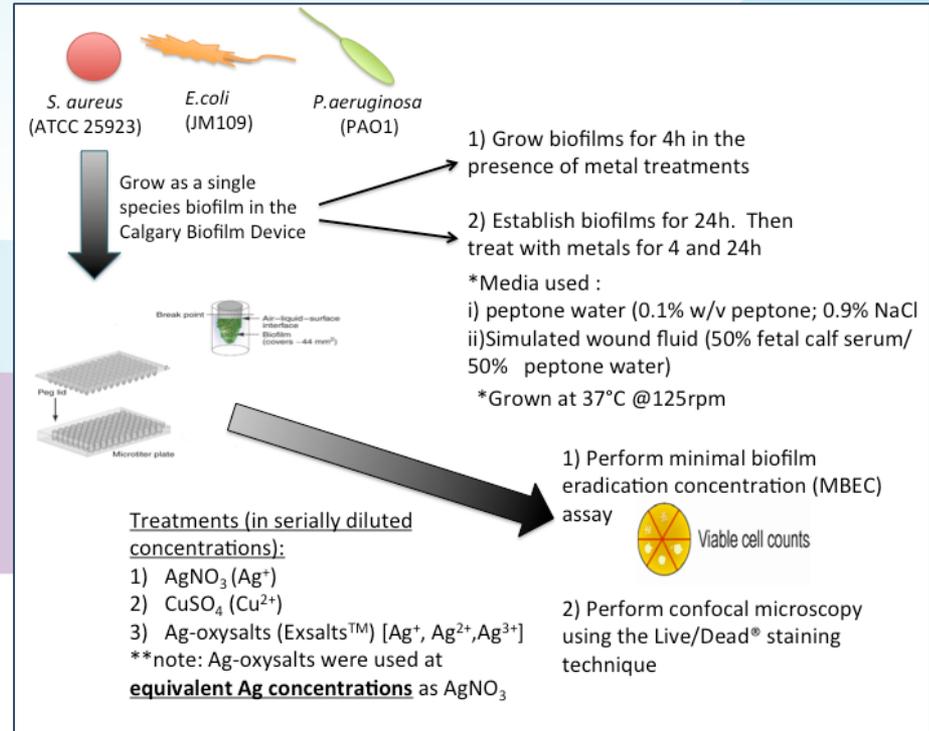


# Antimicrobial activity of a novel silver dressing against wound pathogen biofilms *in vitro* and in an *in vivo* animal model

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.

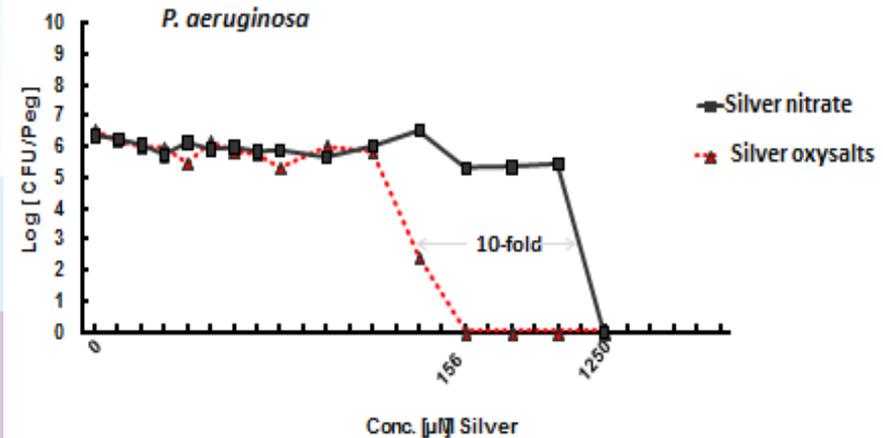
## Methodology



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.

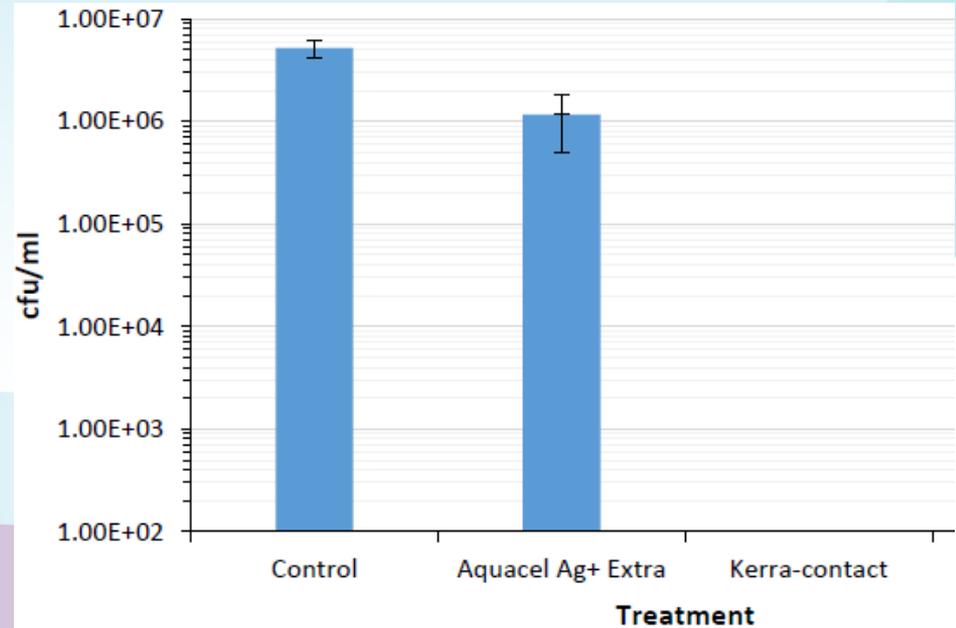
- Media influences both Ag release and antimicrobial efficacy against both planktonic cells and those growing as a biofilm.
- Charged species and proteins in complex medium bind ionic Ag dramatically reducing bioavailability.
- The MBC 4h/MBEC 24h Ag oxy-salt for *P. aeruginosa* was 36/156 $\mu$ M and, for AgNO<sub>3</sub>, 58/1250 $\mu$ M. The corresponding values for *E. coli* are 16 $\mu$ M/42 $\mu$ M and 8 $\mu$ M/38 $\mu$ M; and for *S. aureus* 250 $\mu$ M/1260 $\mu$ M.
- **The concentration of Ag<sup>2+</sup>/3<sup>+</sup> ions required to eradicate an established *P. aeruginosa* biofilm is 10x less than Ag<sup>+</sup> as represented by silver nitrate.**
- **Ag<sup>2+</sup>/3<sup>+</sup> species are able to maintain greater antimicrobial efficacy regardless of media type due to higher oxidative power.**

## Ag Oxysalts Eradicate Established Biofilms *in vitro*



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

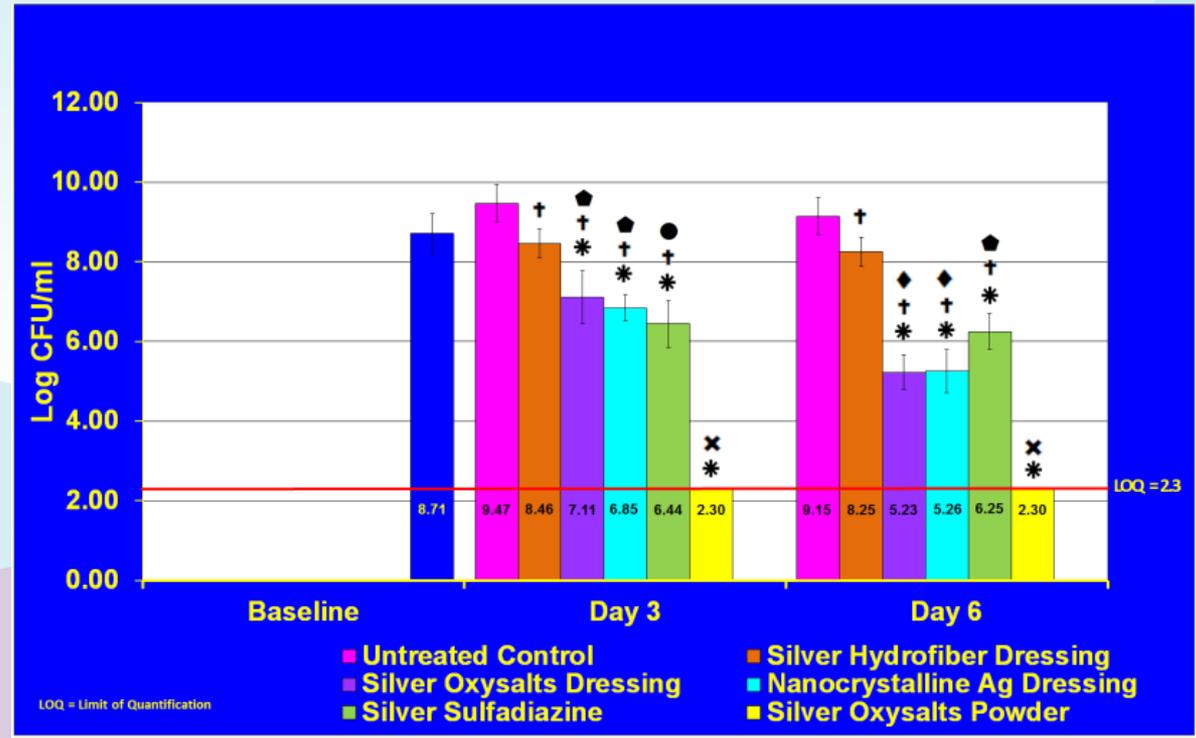
- Twenty-four hour cultures of *Pseudomonas aeruginosa* were harvested from a Tryptone Soya Agar (TSA) plate using a sterile swab and re-suspended in 20ml of Tryptone Soya Broth (TSB)
- The bacterial suspension was then diluted corresponding to a bacterial concentration of  $10^8 \pm 5 \times 10^7$  cfu/ml<sup>-1</sup>
- 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.



Quantity of viable *Pseudomonas aeruginosa* recovered from a pre-formed 24 hour biofilm after 72 hour treatment with test agents.

# 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.



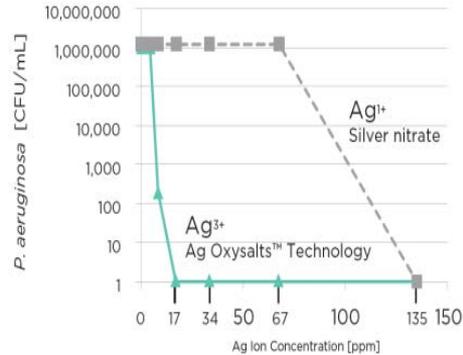
Davis S C. Effective Method to Remove Wound Bacteria: Comparison of Various Debridement Modalities in an In Vivo Porcine Model. *Journal of Surgical Research* 176, 701–707 (2012)

# 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

## DISRUPTS AND BREAKS DOWN *in vitro* BIOFILM TO EXPOSE BACTERIA<sup>2</sup>

Anti-bacterial Activity of Ag Oxysalts™ Technology within Established *in vitro* Biofilm



⊕ *in vitro* studies show Ag Oxysalts™ Technology disrupts and kills *P. aeruginosa* biofilm.

Molar concentrations of Ag Oxysalts™ Technology were used to measure kill rates and to determine efficacy vs. biofilms after 24 hour exposure using the Calgary biofilm device evaluating MBEC and following the device manufacturer's protocol; biofilms were grown for 24 hours, then confirmed and visualized using microscopy and staining; efficacy was demonstrated vs. *P. aeruginosa*, *S. aureus*, and *E. coli* biofilms.