Experiment Demonstrates Synergistic Actions of Novel Technologies Within AQUACEL® Ag+ Extra™ Dressing that Drive Product Efficacy

An *In Vitro* Test of the Efficacy of an Anti-Biofilm Wound Dressing

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Key Highlights:

- This *in vitro* study showed that the addition of an ingredient to disrupt biofilm structure to a dressing containing an antimicrobial compound can eradicate biofilm, which is the formulation strategy behind AQUACEL® Ag+ Extra™ dressings
    - A wound dressing containing silver alone might be less effective against a biofilm culture, since the biofilm formation adds a significant degree of protection against antimicrobial agents.
    - Compared with AQUACEL® Ag Extra™ dressings, AQUACEL® Ag+ Extra™ dressings (AAg+E) include two new ingredients that enhance the delivery and efficacy of ionic silver: ethylenediaminetetraacetic acid [EDTA] and benzethonium chloride [BC].
- When AAg+E was tested *in vitro* versus un-medicated dressing, silver-containing dressing, and silver nitrate solution ($AgNO_3$) using a biofilm model of *Staphyloccocus Aureus*. (*S. aureus)*, the biofilm was only eradicated in the presence of AAg+E. In all other cases, the biofilm remained viable.
- The effect of EDTA has been attributed to its properties as a metal chelator, with a dual role of disrupting biofilm matrix integrity and facilitating delivery of silver to bacteria; whereas BC acts to reduce surface tension, affecting biofilm architecture and cell-cell interactions, thus promoting the activity of silver when delivered with EDTA.
- Control experiments showed that neither EDTA nor BC alone had a bactericidal effect, indicating that it is the synergistic action of EDTA and BC to disrupt biofilm that is driving the efficacy of AAg+E.

Methods:

- The biofilm model was developed in an isothermal microcalorimeter. The challenge organism, *S. aureus*, was grown for 16 hours in nutrient broth, harvested, washed, and re-suspended. Ampoules were prepared containing agar, Tryptone soya broth and *S. aureus* to a density of 1 x 10^6 cfu/ml, and placed in a calorimeter. When required wound dressing, EDTA, BC, or $AgNO_3$ were also added.
- The dressings used to test against AAg+E were un-medicated AQUACEL® (AH), silver-containing AQUACEL® Ag or AQUACEL® Ag Extra™ (AAgH) dressings.
- Cell counts were determined after each calorimetric experiment.Data were captured using Digitam 4.1 and analysed with Origin 8.1

Results:

Figure 1. Growth curves for biofilms in the presence of AH, AAgH and AAg+E[3]

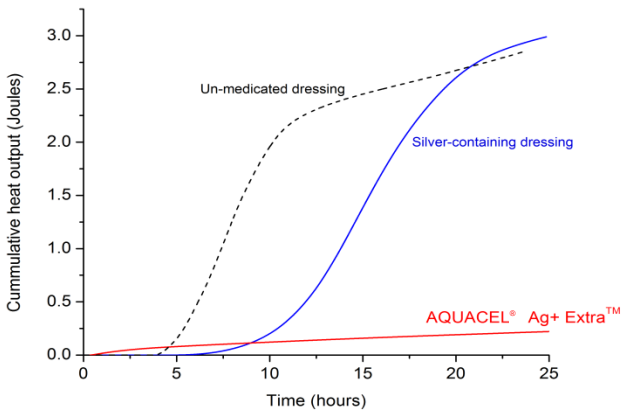
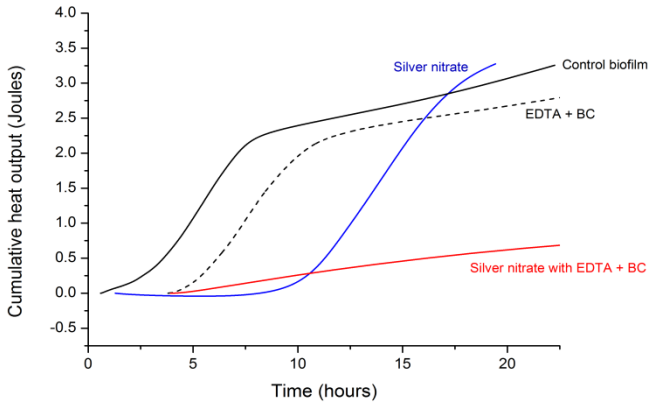


Figure 2. Growth curves for biofilm alone and in the presence of $AgNO_3$, EDTA+BC, and $AgNO_3$+EDTA+BC[3]



AH=dressing alone; AAgH=silver-containing dressing; AAg+E=AQUACEL® Ag+ Extra™

- ***Figure 1*** shows dressing alone did not prevent biofilm growth and silver-containing dressing only delayed it. However, a reduction was seen with AAg+E: the average cell count following exposure was 2x10^4 cfu/mL, so the dressing can be considered bactericidal because it induced a 3 log reduction in viable cells relative to the control.
- ***Figure 2*** demonstrates that the individual components of AAg+E alone cause an initial delay, but then growth is seen that is identical to the control; however when added in combination, a reduction in biofilm growth is seen. This confirms the hypothesis that it is the combination of EDTA, BC, and silver working in synergy that is necessary for efficacy.

References:

1. Said, J., Walker, M., Parsons, D., Stapleton, P., Beezer, A., Gaisford, S. An *in vitro* test of the efficacy of an anti-biofilm wound dressing. *International Journal of Pharmaceutics*, 2014; 474, 177-181.

For more details please refer to the original published article.