EPP025, Abstract ID no. 628, EWMA 2018

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In vitro evaluation of a silver foam dressing with and without silicone adhesive against biofilms and a broad range of microorganisms

Cecilie Christiansen, Gitte Bell Huniche, Marie Allesen-Holm, Coloplast A/S, Humlebæk, Denmark

Introduction

Studies indicate that biofilms can be found in 60-100% of non-healing wounds. Biofilms are known to cause infection, inflammation and delayed wound healing[1,2]. Therefore, evaluation of antimicrobial wound dressings should include biofilm tests as well as standard antimicrobial tests.

Aim

In this study two different microbiological test methods were employed for evaluation of silver foam dressings with and without silicone adhesive*; a standard antimicrobial test and an *in vitro* wound biofilm model.
It has been shown that biofilms in non-healing wounds are heterogeneously distributed, including in the deeper tissue of the wound bed[3]. The test dressings were evaluated against mature biofilms and in the prevention of biofilm formation in an *in vitro* wound biofilm model that specifically addresses the problematic biofilms heterogeneously embedded in the wound environment.

Method

ASTM E2149-13a was performed over a 7-day period[4]. In short, dressing samples were incubated for 24 hour in flasks containing a microbial monoculture. The dressing samples were then transferred to fresh cultures of the same microorganism every day. Six different microorganisms were tested, covering the most prevalent wound pathogens, fungi and antibiotic resistant strains (*P. aeruginosa S. aureus*, VRE, MRSA, *A. brasiliensis, C. albicans*). The efficacy was evaluated by log reduction in CFU**/ml.

An *in vitro* wound biofilm model (WBM) based on a study by S. Crone et al[5] developed at University of Copenhagen consisted of biofilm aggregates (*P. aeruginosa or S. aureus*) embedded in semi-solid agar. The microorganisms were inoculated into the semi-solid agar and either 1) grown to mature biofilms for 24 hours or 2) treated shortly after inoculation to demonstrate biofilm prevention. In both test setups, the microorganisms/biofilms were subsequently exposed for 24 hours to samples of the test dressings or comparable dressings without silver.

Results

Standard antimicrobial test

The results of the ASTM test demonstrated that the test dressings reduced all tested microorganisms, including antibiotic resistant strains, by more than log 3 (1000-fold). The efficacy was similar on day 1 and 7 (figure 1, A&B). The results indicate a sustained and effective release of silver up to 7 days according to the log 3 reduction requirements described in prEN16756[6].

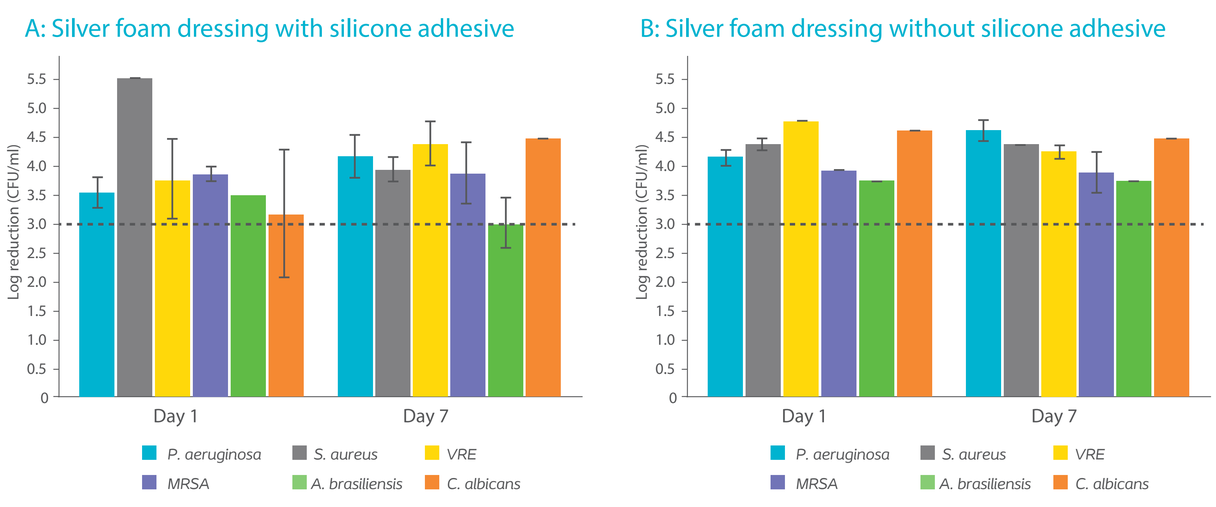

Figure 1. Antimicrobial efficacy tested according to ASTM E2149-13a against a broad range of microorganisms. The results are shown as mean log reduction ± standard deviation (SD). N=3 samples. Log reduction was calculated based on start inoculum. All log reductions were ≥ log 3, which is the current standard for antimicrobial efficacy according to prEN16756.

Killing of mature biofilms

In the WBM, both test dressings showed statistical significant effect against mature biofilms of both *S. aureus and P. aeruginosa*, compared to their respective control dressings (Figure 2, A&B). Both test dressings reduced mature *P. aeruginosa* biofilms by >99.99% and mature *S. aureus* biofilms by 99.3% (A) and 99.93% (B), respectively. The variation in results between different bacterial strains is expected and caused by the differences in susceptibility of microorganisms to silver.

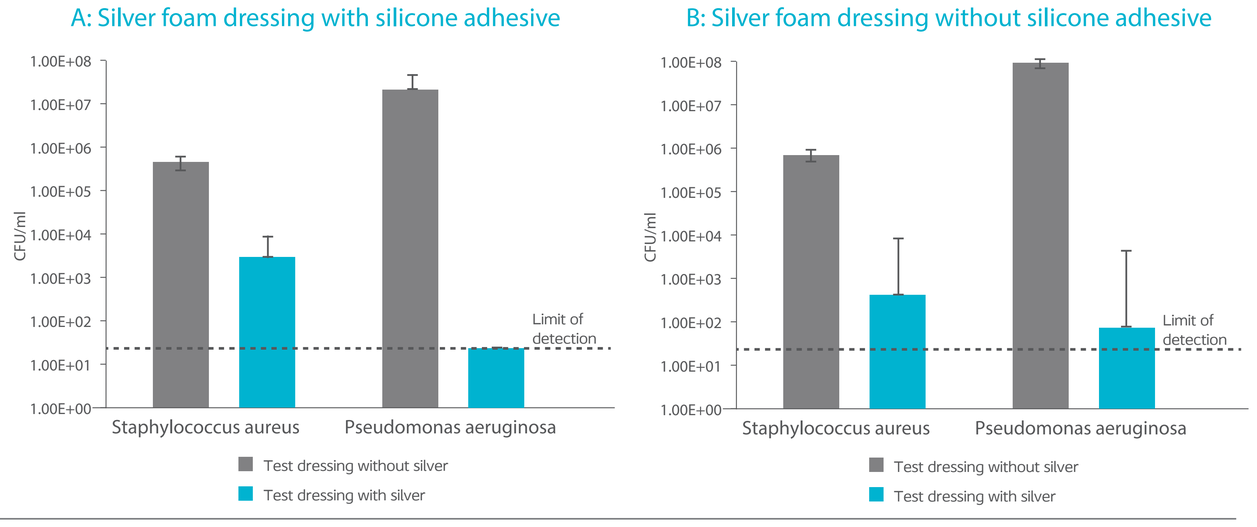

Figure 2. Killing of mature biofilms tested in the WBM. The results are shown as geometrical mean of CFU/ml ± standard deviation (SD). N=20 samples. The horizontal line represents limit of detection at 25 CFUs.

Prevention of biofilm formation

In the WBM, both test dressings prevented growth of both biofilms of *S. aureus* and *P. aeruginosa* to the limit of detection which was set to 25 CFUs (Figure 3, A&B).

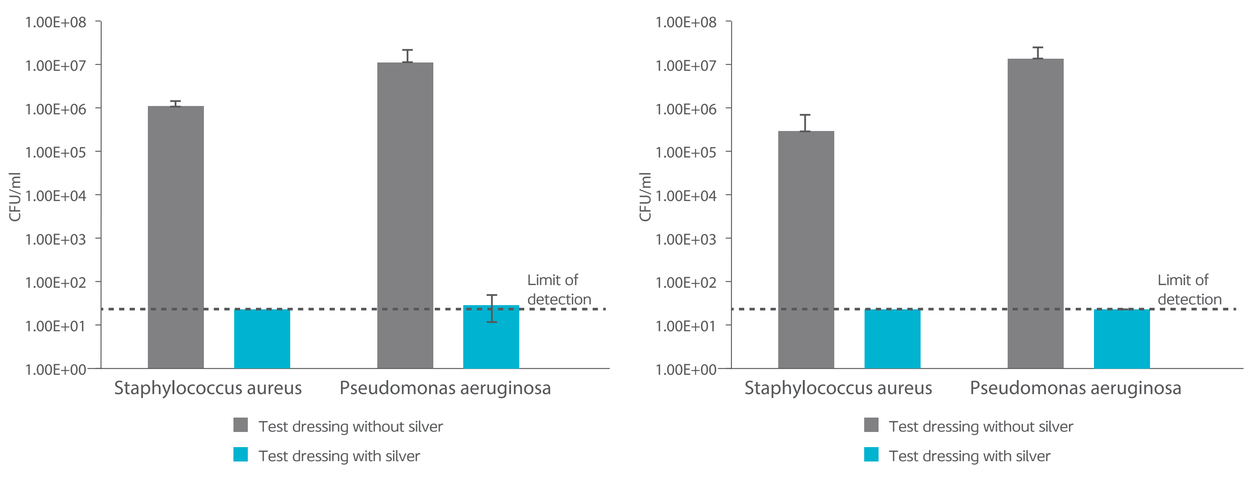

Figure 3. Prevention of biofilm formation tested in the WBM. The results are shown as geometrical mean of CFU/ml ± standard deviation (SD). N=20 samples. The horizontal line represents limit of detection at 25 CFUs.

Conclusion

The silver foam dressings, with and without silicone adhesive, both demonstrated significant efficacy against a broad range of microorganisms in a standard test as well as against mature biofilms and in the prevention of biofilm formation in an embedded wound biofilm model.

Both treatment of mature biofilms and prevention of biofilm formation are essential strategies in the framework for the treatment of biofilms[2].

References

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6. prEN16756 (draft). Antimicrobial wound dressings – Requirements and test methods.

***Biatain® Silicone Ag, Biatain® Ag (Coloplast). **Colony forming unit**