ANTIMICROBIAL AND ANTIBIOFILM PERFORMANCE OF AN ULTRATHIN SYNTHETIC MATRIX CONTAINING SILVER AND GALLIUM

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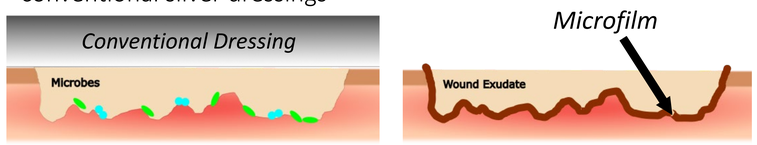
Abstract

Biofilms are implicated in delayed healing in chronic wounds, however there is no commercially available topical formulation effective in dispersal of biofilms in wounds.[1,2,3] Here we report the evaluation of antimicrobial, antimicrobial barrier, and antibiofilm performance of a synthetic matrix, made of polyvinyl alcohol with a polymeric multilayer coating impregnated with silver and gallium (microfilm-Ag/Ga), both *in vitro* and *in vivo*.

Microfilm Matrix

- The thin-film form factor (~20 µm) allows the microfilm to conform to the wound bed, providing intimate contact with the tissue surface
- Well-controlled loading of therapeutic bioactives
- Maintains moist wound microenvironment
- Due to the conformity of the microfilm to the wound bed, lower doses of compounds are usually needed for effectiveness
- Ex. Silver-loaded microfilms require 1000x less silver than conventional silver dressings

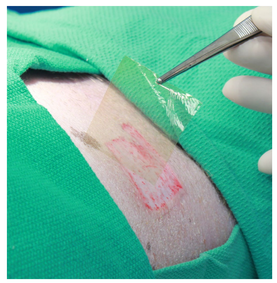


Microfilm-Ag/Ga

- Biofilms are bacteria encased in extracellular polymeric substance (EPS) and are 1000 times more resistant to antibiotics/ antimicrobials than planktonic bacteria[3,4]
- Both ionic silver and gallium have been shown to be both antimicrobial and biocompatible.[5]

Mode of Action

- Gallium blocks redox-driven biological processes of metabolism that reduces bacterial growth and biofilm formation.[6,7]
- Silver has been shown to possess many different modes of antibacterial action including the disruption of the bacterial cell wall, denaturation of bacterial ribosomes, and the interruption of ATP production.[8]
- Combination of antibiotic agents is an effective strategy for treating and combating multi-drug resistant organisms.[9]



Antimicrobial Performance *In Vitro*

Antimicrobial performance of the microfilm-Ag/Ga over 24 and 72 h was tested against 8 clinically relevant microbes (*K. pneumoniae, E. coli, C. albicans, C. tropicalis,* MRSA, *S. aureus, P. aeruginosa, and E. faecalis)* per ISO 22196. Its antimicrobial barrier performance was evaluated by pipetting 10 µL of bacterial inoculum of *P. aeruginosa*, *A. baumannii* or *K. pneumoniae* on the microfilm placed on an agar plate and incubating at 37 ± 2 °C for 3 days and quantifying the CFU subsequently.

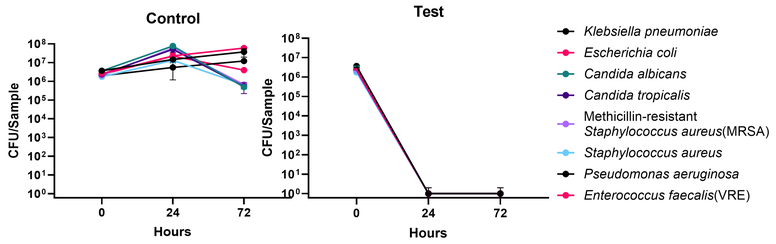
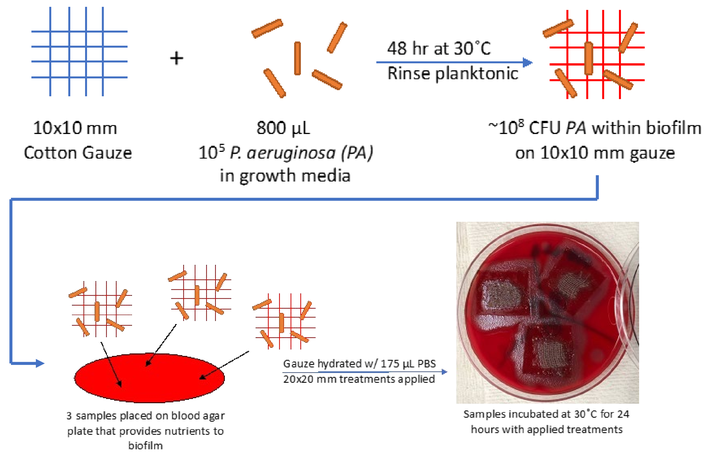


Figure 1: Antimicrobial Performance of the Matrix over 24 and 72 Hours

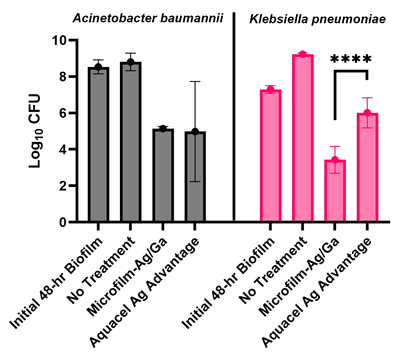
The microfilm killed > 4 Log10 CFUs of all microbes tested per ISO 22196, thus confirming its antimicrobial activity against planktonic bacteria (Figure 1). It also killed bacteria on its surface and prevented the breakthrough of microbes over 72 h thereby demonstrating its ability to serve as an antimicrobial barrier.

Antibiofilm Performance *In Vitro*

To evaluate the antibiofilm performance *in vitro*, robust biofilms containing 10^8 CFU of *A. baumannii* or *K. pneumoniae* were established on gauze specimens over 48 h and rinsed with saline to remove planktonic bacteria. Moist biofilm specimens were then treated with a single application the microfilm-Ag/Ga for 24 h, and the CFUs were determined relative to no-treatment controls.



Antibiofilm Performance *In Vitro* cont'd

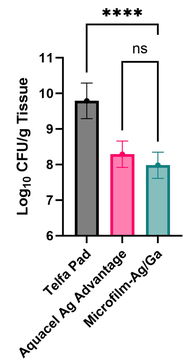


> 4 and 1.5 Log_{10} CFU reduction of *A. baumannii* and *K. pneumoniae* biofilms bacteria was achieved *in vitro* over 24 h with a single application of the microfilm (Figure 2).

Figure 2: Antibiofilm performance of the microfilm against *A. baumannii* and *K. pneumoniae.* **** = p < 0.0001

Antibiofilm Performance *In Vivo*

In vivo assessment was performed by transplanting pre-established biofilm in 1 cm dia. full thickness porcine wounds by placing the gauze specimens supporting 10^8 CFU *P. aeruginosa* biofilms for 24 h. Afterwards, the gauze specimens were removed, and wounds were treated with the microfilm-Ag/Ga once daily for two days. On day 3, biopsies of all wounds were minced and CFUs were determined



biopsies of all wounds were minced and CFUs were determined relative to no-treatment control.

The microfilm-Ag-Ga killed 1.5 Log_{10} CFU of *P. aeruginosa* biofilm bacteria in vivo, thus establishing its antibiofilm performance.

Figure 3: Antibiofilm Performance of Microfilm-Ag/Ga in a Porcine Wound Model - **** = p < 0.0001

Conclusion

The microfilm containing silver and gallium is effective in killing both planktonic and biofilm bacteria and is thus suitable for assessment in the treatment of biofilms in chronic wounds.

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