Effective Antibiofilm Bioresorbable Microfilm™ Matrix Containing Gallium and Silver

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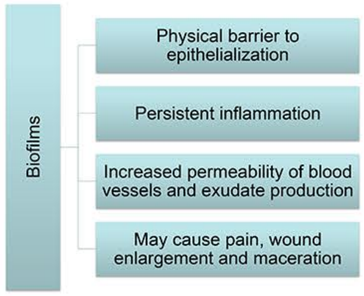
INTRODUCTION

The health care costs associated with treatment of chronic and burn wounds exceeds $25 billion annually in the U.S., involving 8.6 million patients (1). Biofilms play a significant role in rendering a wound chronic as evidenced by James *et al*. who showed **that 60% of chronic wound debridement samples contained biofilm, whereas only 6% of acute wound biopsies contained biofilm (2)**.

Biofilms are structured communities of bacteria encased and protected in extracellular polymer matrix (EPM), which comprises 80% volume (3).



Due to restricted penetration of antimicrobial agents through EPM, presence of persister cells and other mechanisms, **bacteria in biofilms are 1000 times more difficult to kill than planktonic bacteria (4,5)**.

Many wounds have complex surfaces and debridement can be challenging, leaving biofilm fragments that remain resistant to antimicrobial therapy and act as a nidus for recrudescence of biofilms. **There is currently no commercially available wound dressing that is clinically indicated for dispersal of biofilms in a wound bed.** Commercial antimicrobial dressings have been shown to only be marginally effective against biofilms delivering 1-2 Log_{10} CFU reduction (6).

INNOVATION

Several studies have shown that gallium (Ga) prevents bacterial growth, biofilm formation and disperses established biofilms (7,8). However, at non-toxic concentrations Ga^{3+} is not effective at killing bacteria. On the other hand, Ag^{+} is a broad spectrum antimicrobial effective in killing planktonic bacteria but not the bacteria encased in biofilms.

We are the first to demonstrate that synergy between non-cytotoxic levels of Ag^{+} and Ga^{3+} disperses biofilm and kills the bacteria encased in it (9). Specifically, we determined that non-cytotoxic concentrations of 1.5 µg/ml Ag^{+} (14 µM) and 10 µg/ml Ga^{3+} (143 µM), when used independently, lowered biofilm CFU by only 20% and 25%, respectively. However, an aqueous solution containing 1.5 µg/ml of Ag^{+} and 10 µg/ml of Ga^{3+} lowered biofilm CFU by 85%.

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METHODS

Microfilm matrix was prepared as follows,

An interpenetrated 300 nm thick nanofilm was assembled by alternating deposition of oppositely charged polyelectrolytes (polyacid and polyamine). Post-assembly, nanofilm was impregnated with Ag^{+} ions via ion exchange and Ag nanoparticles (5-20 nm) were formed in-situ via reduction. Nanofilm was further impregnated with Ga^{3+} ions via ion exchange, as needed. A bioresorbable 20 µm thick polyvinyl alcohol hydrogel w/wo Ga^{3+} ions was laminated to nanofilm.

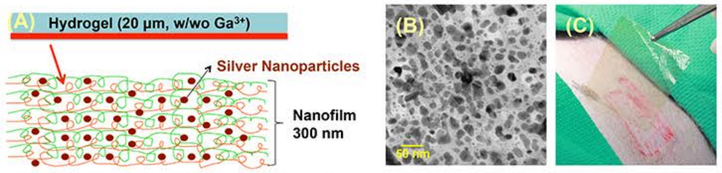


Fig. (A) Schematic of bioresorbable matrix containing silver and gallium, (B) TEM image showing silver nanoparticles embedded into nanofilm, (C) Bioresorbable matrix

P. *aeruginosa* biofilm growth,

Robust biofilms of P. *aeruginosa* were grown on 15 mm squares of Kendall Curity Gauze Bandage (Tyco Healthcare, KDL1143) by plating 800 µl suspension of P. *aeruginosa* (10^{8} CFU/ml) for 48 h at 30 ºC. Post incubation, gauze specimen was rinsed 3x with sterile 1x PBS to remove loosely adhering planktonic bacteria. The presence of biofilm was confirmed with crystal violet (CV) staining which binds specifically to EPM. Biofilm mass was quantified as absorbance of CV at 595 nm using a spectrophotometer.

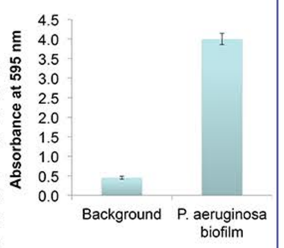


Fig. CV absorbance of 48 h P. *aeruginosa* biofilm relative to background confirms robust biofilm growth on gauze

Antibiofilm testing,

Gauze specimen supporting biofilm were placed on tryptic soy agar contact plate (w 5% sheep blood), hydrated with 175 µl of 1x PBS and treated with 20 mm x 20 mm test matrix for 24 h at in a humidified incubator at 30 ºC.

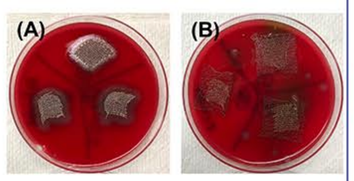


Fig. (A) Untreated gauze supporting biofilm (control), (B) Microfilm matrix applied over gauze supporting biofilm

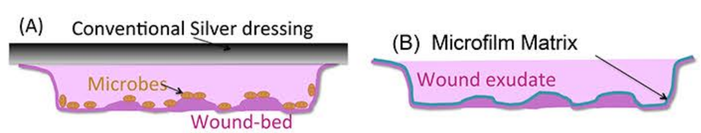


Fig. Schematic showing that unlike (A) conventional silver dressings, (B) Microfilm matrix adheres intimately to wound bed resulting in a targeted and localized action

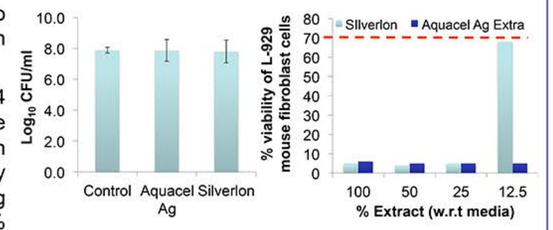
Post treatment, gauze specimens were transferred to 24 well plates and rinsed 3x with sterile PBS. One set of specimens was homogenized and serial plated to determine biofilm CFU, and another set was treated with CV to determine total biofilm mass.

RESULTS

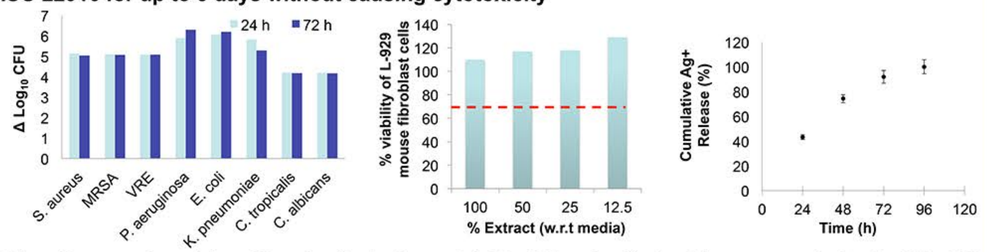
Commercial antimicrobial dressings Silverlon® and Aquacel® Ag Extra containing high level of silver, 5460 and 120 µg/cm² respectively, are not only ineffective against P. *aeruginosa* biofilms *in-vitro* but also cytotoxic

Silverlon and Aquacel Ag Extra showed no reduction in P. *aeruginosa* biofilm CFU over 24 h relative to untreated control.

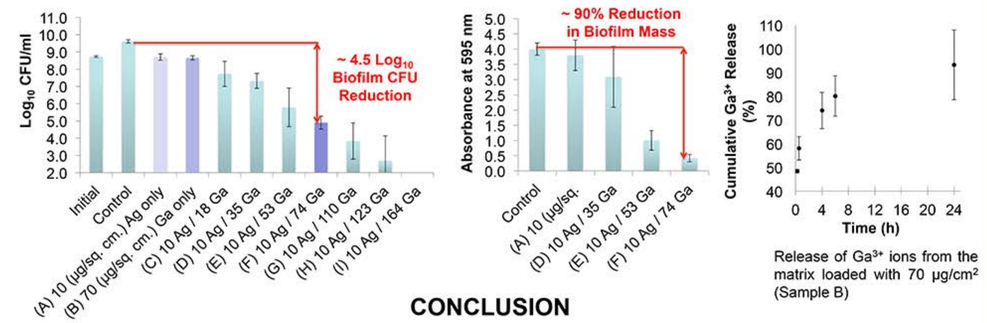
Dressings were extracted in growth media for 24 h at 37°C; extracts and their dilutions were incubated with L-929 mouse fibroblasts cells in growth media (5% FBS) for 24 h; Cell viability assessed by NAMSA (Northwood, OH) using MTT cell metabolic activity assay. At least 70% cell viability indicates non-cytotoxicity.



Microfilm matrix containing only 10 µg/cm² of silver kills 4 Log_{10} CFU of planktonic bacteria per ISO 22916 for up to 3 days without causing cytotoxicity



Microfilm matrix pairing 10 µg/cm² of silver with 70-165 µg/cm² of gallium synergistically kills 4-9 Log_{10} CFU of P. *aeruginosa* biofilm bacteria and disperses > 90% biofilm mass



Release of Ga^{3+} ions from the matrix loaded with 70 µg/cm² (Sample B)

CONCLUSION

Microfilm matrix amplifies the efficacy of silver and gallium ions 1000 times compared to topical aqueous formulations for combating biofilms.

FUTURE WORK

We will evaluate the efficacy of microfilm matrix containing Ag and Ga in the healing of splinted mice wounds inoculated with mixed species (S. *aureus* and P. *aeruginosa*) biofilm, which impairs wound healing (Fig. on right), as shown by Dr. Jonathan F. McAnulty.

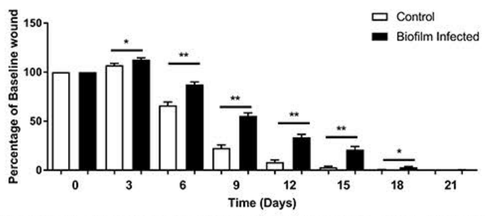


Fig. Healing of wounds covered for 3 days with gauze with a *S. aureus* biofilm. Wounds were rinsed on day 3 and then onwards treated with fresh gauze. Biofilm wounds had significantly slower healing over 21 days compared to control wounds with no biofilms (* p<0.05; ** p<0.0001).

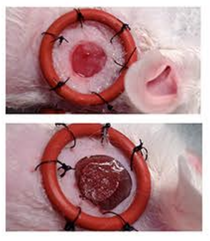


Fig. (Top) A splinted full-thickness wound-model in mice, (Bottom) Microfilm Matrix placed on a contaminated moist wound.