Ultrathin Hydrogel Dressings Containing Gallium Ions and Metallic Silver for the Elimination of Biofilms

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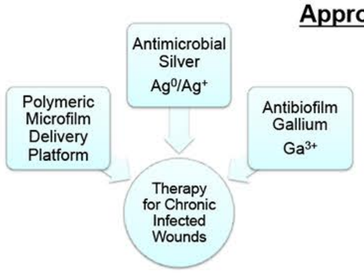
Introduction

Every year more than 6.5 million patients in the U.S. are treated in hospitals for chronic wound infections. Bacteria in chronic wounds are believed to grow in biofilms. Continued persistence of bacterial biofilms delays the healing of chronic wounds resulting in prolonged hospital stays, painful dressing changes and in some cases, death from sepsis.

Biofilms are the structured communities of bacteria encased in extracellular polymeric matrices. Clearing bacteria in biofilms in chronic wounds is challenging because extracellular polymeric matrix limits the penetration of antimicrobial agents, thus rendering the antimicrobial treatment ineffective.

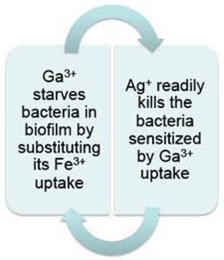
Approach



Here we show that synergistic action of silver and gallium delivered through polymeric microfilm kills 4 Log_{10} CFU of bacteria in biofilms and disperses ~100 % biofilm mass.

Synergy Between Gallium and Silver

Ga^{3+} is taken up by bacteria through binding with iron siderophores due to the chemical similarity of Ga^{3+} to Fe^{3+}, halting the redox biological processes required for bacterial growth and biofilm formation.



Silver is a broad-spectrum antimicrobial, which is effective in killing planktonic bacteria, including MRSA and VRE, but not the bacteria encased in biofilms at non-cytotoxic concentrations.

Ag^+ and Ga^{3+} together kill bacteria encased in biofilms at non-cytotoxic concentrations

Antibiofilm Activity of Ag^+ and Ga^{3+} Solutions

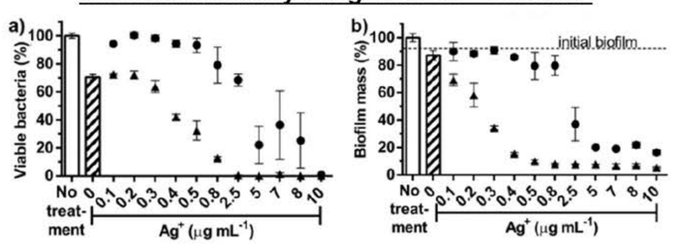


Fig. Ga^{3+}: 10 μg/ml (striped column), Ag^+: 0.1 – 10 μg/ml (circles), Ga^{3+} and Ag^+ Mixtures (triangles) (a) % viable bacteria, (b) Biofilm mass dispersal

Ag^+ and Ga^{3+} act synergistically, e.g. a mixture of 0.8 μg/ml of Ag^+ and 10 μg/ml of Ga^{3+} killed 87% (~1 Log_{10}) bacteria and dispersed 92% biofilm.[1,2]

Ref: (1). Herron et al., Adv. Healthcare Mater., 2015. (2). Herron et al., Appl. Mater. Interfaces., 2016.

ACKNOWLEDGEMENT

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Polymeric Microfilm Delivery Platform

Polymeric microfilm dressings are a robust bilayered composite of

- A 20-μm thick film of dissolvable polyvinyl alcohol hydrogel
- A 200-nm thick polymeric multilayer nanocoating
- Gallium or silver are incorporated in the nanocoating layer and in polyvinyl alcohol layer

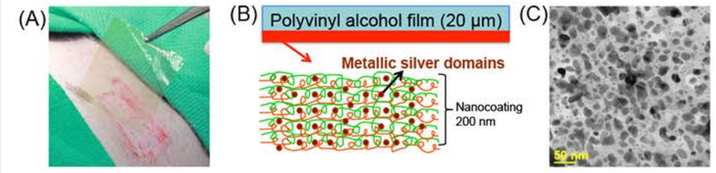
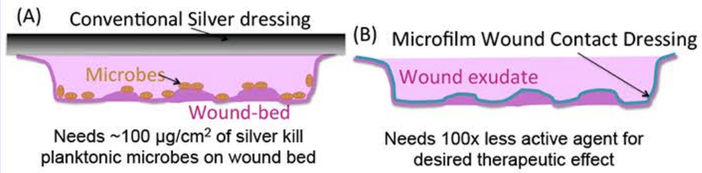


Fig. (A) Polymeric microfilm dressing containing (Ag^0) and gallium ions (Ga^{3+}), (B) Schematic of the bilayered hydrogel-nanocoating construction of polymeric microfilm dressing, (C) TEM image showing metallic silver (Ag^0) domains in nanocoating.

MEASURED PHYSICAL CHARACTERISTIC	MEASUREMENT (UNITS)
Thickness (ASTM D6988-13)	12.5 - 25 μm
Water Uptake Capacity (wet / dry weight ratio)	11.0 ± 3.1
Tensile Strength / % Elongation (ASTM D882-12)	4763 ± 633 psi / 59 ± 21%
Oxygen Transmission Rate (ASTM D3985-05)	975 ± 165 cc/(m^2-day)
Water Vapor Transmission Rate (ASTM F1249-13)	1407 ± 80 g/(m^2-day)

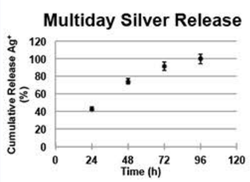
Mode of Action

- Polymeric microfilm dressings absorb wound fluid to form a soft gel that conforms intimately to the wound surface and maintains a moist wound healing environment.
- Compared to conventional dressings, the microfilm construct allows active agents to be effective at significantly lower concentrations, thus reducing potential of tissue toxicity and irritation.

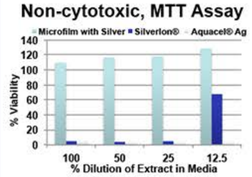


Polymeric Microfilm Dressing with Silver

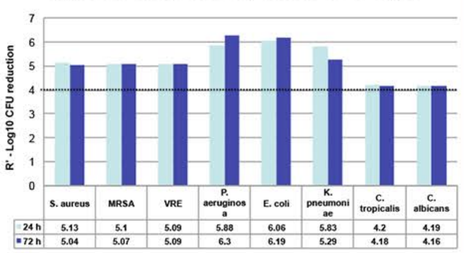
Multiday Silver Release



Non-cytotoxic, MTT Assay



9.1 μg/cm^2 silver in nanocoating, no gallium
Killed 5 Log_{10} of clinically relevant microbes within 24 h and maintained antimicrobial activity for up to 3 days



Polymeric Microfilm Dressing with Silver and Gallium

Microfilm Layers	Silver (Ag^0) μg/cm^2	Gallium (Ga^{3+}) μg/cm^2
Nanofilm	12.1	4.8
Dissolvable Hydrogel	0.0	68.0
Total	12.1	72.8

- **Non-cytotoxic levels of silver (MTT assay)**
- **Non-cytotoxic levels of gallium:** Microfilms produced < 0.7 mM gallium ions in 800 μL media in the biofilm tests, which is below the reported cytotoxicity limit*.

**Ref: Chandler et al., J. Dental Res., 1994, reported that <1 mM gallium ions were non-cytotoxic to L929 mouse fibroblast cells in an MTT assay.*

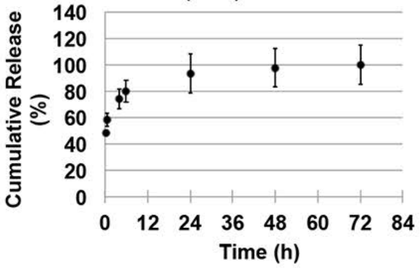
- **In-vitro, killed 4 Log_{10} CFU of *P. aeruginosa* encased in a 48 h old biofilm within 24 h**
  - Biofilms were grown on the surface of a collagen dressing Biobrane® (S&N) in 24-well plates by incubating them with 10^6 CFU of *P. aeruginosa* in growth media for 48 hours.
  - Subsequently, biofilms were rinsed off 3x with PBS to remove planktonic bacteria. Microfilms were placed over biofilms and incubated in fresh growth media at 30°C for another 24 h. Post incubation, all samples were rinsed off to remove Microfilms and planktonic bacteria. Biobrane® with biofilms were homogenized in PBS and serial dilutions plated on agar plates for CFU count.
- **In-vitro, dispersed >99.99% mass of a 48 h old biofilm of *P. aeruginosa* within 24 h**
  - In one set of 24-well plates described above, biofilms on Biobrane® were stained with crystal violet to measure total biofilm mass. The absorbance of dye indicating biofilm mass was quantified using a spectrophotometer at 595 nm.

Reduced microbial colonization and prevented sepsis in full-thickness 6 mm dia. splinted wounds in mice

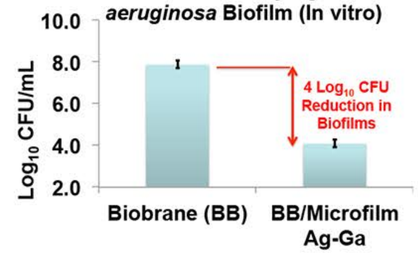
-Wounds inoculated with 2x10^5 CFU of *S. aureus* post-surgery. One group of mice (n=7 per treatment) were covered with Microfilm Ag-Ga after inoculation. All wounds were subsequently covered with a collagen dressing Biobrane® (S&N), and with secondary dressings. On day 3 post-surgery, all wounds were harvested and homogenized to measure total microbial burden. Wounds treated with Microfilm Ag-Ga had significantly less microbial burden, with >3Log_{10} difference compared to control group under the biologic dressing Biobrane® ($p<0.05$, one-way Anova).

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

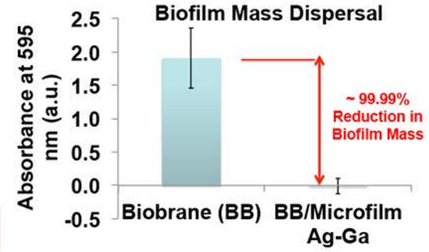
Gallium (Ga^{3+}) Release Profile



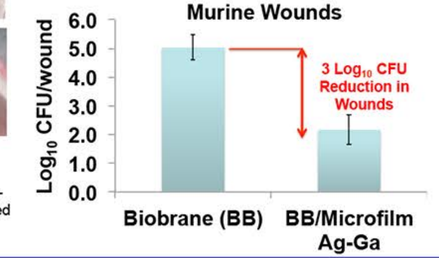
Antibiofilm Activity Against P. *aeruginosa* Biofilm (In vitro)



Biofilm Mass Dispersal



Treatment of Excisional Murine Wounds



CONCLUSION: Features of Microfilm Wound Contact Dressing

Design	Application	Performance
Conformable	Robust for handling	Broad-spectrum
Transparent	Non Staining	Non-cytotoxic
Adhesive	Compatible with biologic dressings	Allow re-epithelialization
Low silver/gallium	Long term release	Reduce microbial burden
Intimate wound contact		