

Antimicrobial Ovine Forestomach Matrix Prevents Biofilm Formation

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Introduction

Biofilm management continues to be a challenge in wound care. Clinical consensus indicates that biofilms are present in the majority of chronic wounds and contribute to delayed healing outcomes and infection risk¹.

Biofilms are typically polymicrobial² and notoriously resilient to treatment by antibiotic and antiseptic agents, thus debridement is the primary approach to controlling biofilm in the wound. Sharp debridement is highly effective in removing biofilm, however biofilm can rapidly regrow from any remnants or form anew from contaminating microbes³. Thus adjunctive measures to debridement such as topical antiseptics and antimicrobials are often used to control biofilm regrowth and facilitate the progression of healing.

We have previously described the antimicrobial Ovine Forestomach Matrix termed 'OFM-Ag'⁴, a native extracellular matrix scaffold functionalized with ionic silver. The present study sought to quantify biofilm prevention of OFM-Ag using an *in vitro* model of polymicrobial biofilm compared with Collagen/ORC/Ag⁵, Collagen/Ag⁶ and gauze⁷.

Conclusions

- Quantifiable biofilm formation occurs *in vitro* as rapidly as 1 h following microbial exposure.
- OFM-Ag demonstrated significant prevention of polymicrobial biofilm formation *in vitro*.
- Further studies are required to evaluate the potential for OFM-Ag to prevent wound biofilm when used as an adjunct to debridement.

Reference and Disclosures

- James, G.A., Swogger, E., Wolcott, R., deLancey Pulcini, E., Secor, P., Seistrich, J., Costerton, J.W. and Stewart, P.S., 2008. Biofilms in chronic wounds: Wound Repair and regeneration, 16(1), pp.37-44.
- Dalton, T., Dowd, S.E., Wolcott, R.D., Sun, Y., Watters, C., Growold, J.A. and Rumbaugh, K.P., 2011. An *in vitro* polymicrobial biofilm wound infection model to study interspecies interactions. PloS one, 6(11), p.e27317.
- Carver, C. How to identify biofilm in the wound. WoundSource. <http://www.woundsource.com/blog/how-to-identify-biofilm-in-wound>. Published August 18, 2015. Accessed October 24, 2018.
- Endoform™ Antimicrobial (Aroa Biosurgery Ltd, New Zealand). Silver concentration data held on file.
- Promogran® Prisma Matrix (Acelyl, United States). Silver concentration derived from product instructions for use.
- Puracol® Plus Ag+ (Medline Industries, United States). Silver concentration derived from product instructions for use.
- ES-Kompressen Gauze Dressing (Paul Hartmann Ag, Netherlands)
- Mepitel® Film (Mölnlycke Health Care, Sweden)
- Merritt, J.H., Kadouri, D.E. and O'Toole, G.A., 2011. Growing and analyzing static biofilms. Current protocols in microbiology, 22(1), pp.18-1.

Methods

Figure 1. Biofilm Formation Assay

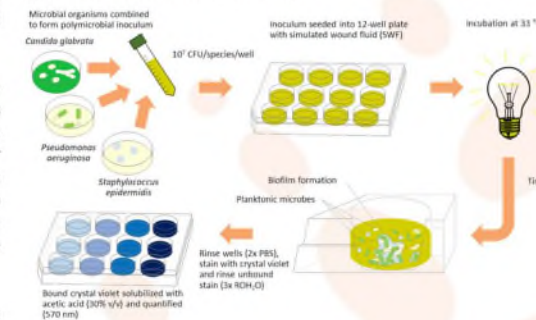


Figure 2. Biofilm Prevention Model

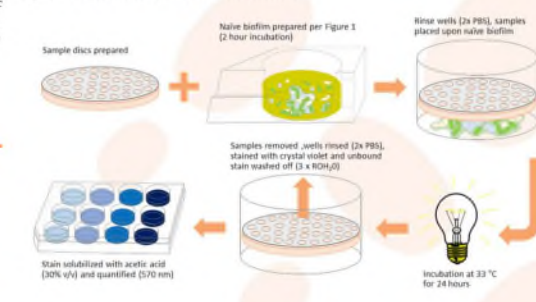
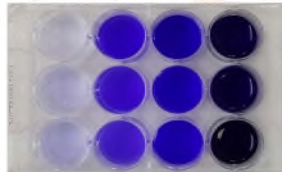
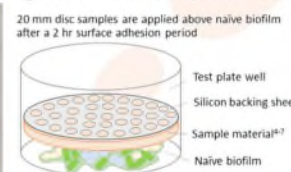


Figure 3. Crystal Violet



Crystal violet stain solubilized in acetic acid (30% v/v)

Figure 4. Biofilm Sample Contact



Results

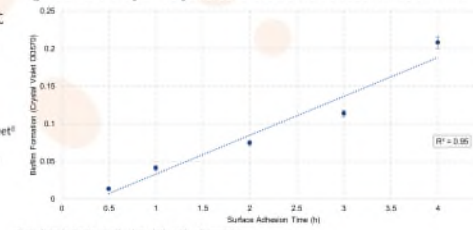
Crystal violet stain was used to quantify biofilm due to its affinity towards staining viable and dead microbes in addition to extracellular polysaccharide biofilm matrices⁹ (Figures 1 – 4).

The biofilm formation kinetics of the model demonstrated dependency on surface adhesion time (Figure 5 and 6). Biofilm increased as a linear function of adhesion time (0.5-4 h). Interestingly, there was no significant difference in biofilm formation over adhesion times of 0-30 minutes (student t test, p=0.686).

As expected, the non-antimicrobial gauze⁷ dressing exhibited high biofilm formation (Figure 7). In contrast, OFM-Ag was significantly more effective in preventing biofilm formation (p<0.05) compared to all other treatment groups. Collagen/ORC/Ag⁵ was also more effective in biofilm prevention compared to gauze and Collagen/Ag⁶ (p<0.05) but less effective compared to OFM-Ag (p<0.05). Collagen/Ag was the least effective antimicrobial dressing in preventing biofilm formation, exhibiting less biofilm formation than gauze however, this result was not statistically significant (p>0.05).

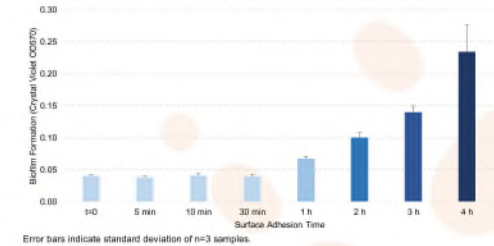
Interestingly, Collagen/Ag has the highest silver concentration (1.2% w/w AgCl or 0.9% Ag w/w)⁶ compared to Collagen/ORC/Ag (0.25% w/w)⁵ and OFM-Ag (0.3% w/w)⁴ as portrayed in Figure 8. Therefore it is surprising that Collagen/Ag was the least effective in preventing biofilm formation. This may be due the dressing design which utilizes AgCl rather than ionic silver.

Figure 5. Linearity of Polymicrobial Growth Vs Surface Adhesion Time



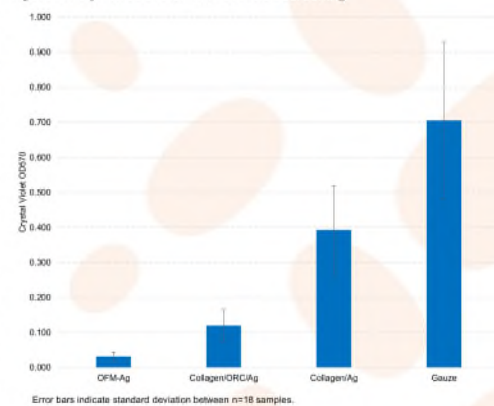
Error bars indicate standard deviation of n=3 samples.

Figure 6. Polymicrobial Biofilm Growth Vs Surface Adhesion Time



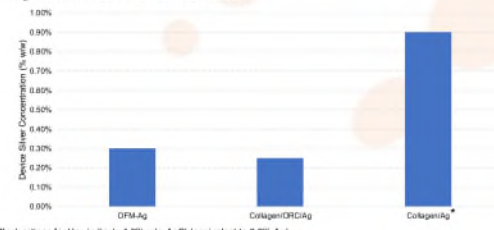
Error bars indicate standard deviation of n=3 samples.

Figure 7. Polymicrobial Biofilm Prevention Screening



Error bars indicate standard deviation between n=18 samples.

Figure 8. Device Silver Concentration



*Instructions for Use indicate 1.2% w/w AgCl (equivalent to 0.9% Ag)