GelATA™ Wound Care Dressing Against Hostile Wound Infection by Hyperbiofilm Forming Bacteria

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BACKGROUND

Early intervention to manage combat wounds particularly burn wounds is a necessity especially if immediate evacuation for skilled interventions is not possible. The need of the hour is to close the time gap between burn injury and burn treatment in the field setting to decrease morbidity and mortality. Under these settings, infection, particularly with biofilm forming bacteria, present a significant challenge due to their recalcitrance to treatment with standard-of-care interventions.

Persister bacterial phenotypes such as small colony variants (SCV) are a subpopulation of antibiotic-tolerant bacterial cells that are often hyperbiofilm forming in nature[1].

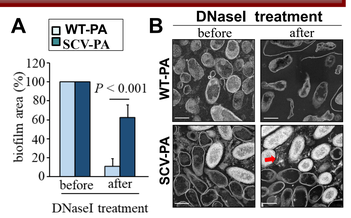


Figure 1. SCV-PA biofilm are resistant to DNaseI digestion. A. Quantification of wild-type (WT-PA) and persistent (SCV-PA) biofilm before and after DNaseI treatment. (**B**) STEM images compared ultrastructure of WT and SCV-PA biofilm with no treatment and following DNaseI treatment. Red arrow highlighted unaffected thread-like eDNA at the center of clumps of vesicle-like structure and cell debris (right image). Scale bar, 1µm.

The key to managing such hostile biofilms of persister bacteria is complete eradication and one approach is to dismantle the structural framework of these biofilms. Extracellular DNA is a major component of the biofilm. DNase treatments can eradicate standard biofilms but not persister biofilms[2] (Fig1).

Our work showed that, fragmented extracellular DNA (eDNA) released from a persister strain of Pseudomonas aeruginosa (PAO1ΔwspF) biofilm was responsible for resistance to disruption by DNase[2]. We reported that a DNase resistant SCV biofilm of *Pseudomonas aeruginosa* (PAO1ΔwspF strain) can be disrupted by Aurintricarboxylic acid (ATA), a chemical inhibitor of covalent binding between eDNA and protein[2].

Objective

To test the efficacy of GelATA™ wound care dressing (ATA incorporated into a polymer-based gel[3]) against polymicrobial persister biofilm infection in a preclinical porcine burn wound model.

Methods

- In vivo testing : Eight 2"x2" full thickness burn wounds were made on the dorsum of (70-80lbs) female domestic white pigs (n=12 wounds in 3 pigs).
- Polymicrobial SCV biofilm infection was established with *Pseudomonas Aeruginosa* (PAO1 ΔwspF) and *Staphylococcus Aureus* (S. aureus rexB) at 10^8 colony forming units (CFU)/ml both which are clinical isolates.
- Wounds were treated with either placebo dressing (Acticoat™) or GelATA™ once weekly, at day 28 GelATA™ was switched to Elastogel™ alone until day 56.
- Progression of burn wound healing was followed using noninvasive imaging (1) digital photography (2)Trans Epidermal Water Loss (TEWL).
- Tissue biopsy for histology and Scanning Electron Microscopy (SEM).

Results

Inhibition of DNA–protein interaction compromised in vitro SCV-PA biofilm formation

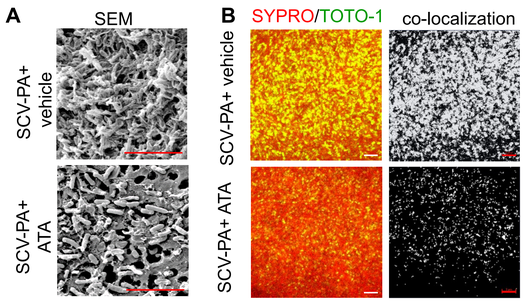


Figure 2. Inhibition of DNA–protein interaction compromised *in vitro* SCV-PA biofilm formation. (A) SEM images of SCV-PA biofilm at 24h treated with buffer and ATA. Scale bar, 5µm. (**B**) Confocal microscopic images showing **SYPRO® Ruby** and **TOTO-1** staining of SCV-PA biofilm at 24h treated with buffer (vehicle control) or ATA. The co-localization of EPS protein (red) and eDNA (green) are shown as white dots. Scale bar, 5µm (**C**) Crystal violet assay of PAO1 biofilm at 12h treated with buffer, 500ng of intact genomic DNA and digested genomic DNA (n=8). Inhibition of DNA-protein interaction compromised in vitro PAO1DwspF biofilm formation. Data are shown mean ± SD.

GelATA™ disrupted wound biofilm

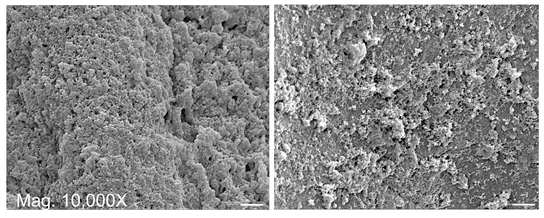
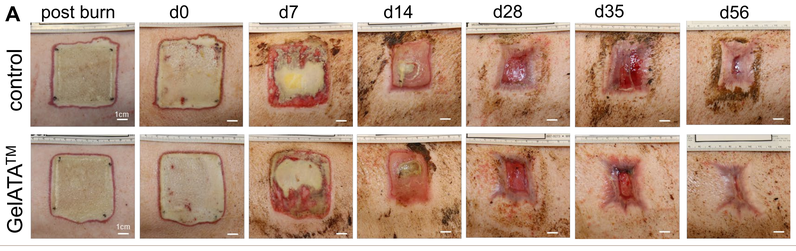


Figure 3. Effect of GelATA™ on Persister-biofilm infection. Representative SEM images at d56. GelATA™ treated wounds showed visibly less biofilm-like structures on wound tissue in a well-established *Pseudomonas* wspF and *Staphylococcus* rexB biofilm in GelATA™ treated wounds compared to control.

GelATA™ improved functional burn wound closure



Results

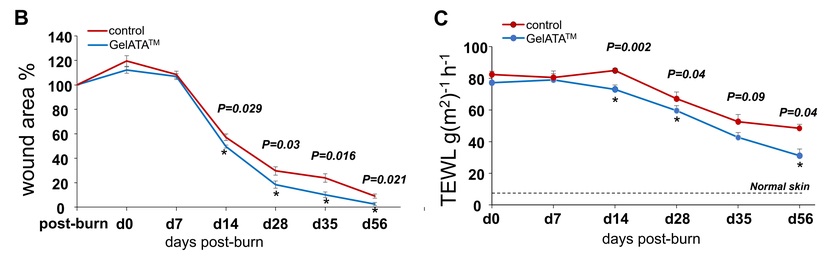


Figure 4. Effect of GelATA™ on burn wound closure. Burn wounds were induced to the dorsum of pigs and treated with silver dressing (Acticoat) or GelATA™. (**A)** Digital images of wound closure over the timeline of study are shown(scale=1cm). (**B)** Quantitation of wound closure shows a significant improvement in visual and. (**C**) Functional wound closure (low TEWL) in GelATA™ treated wounds. Data are shown mean ± SD; (n=12 wounds in 3 pigs).

GelATA™ accelerated wound re-epithelialization

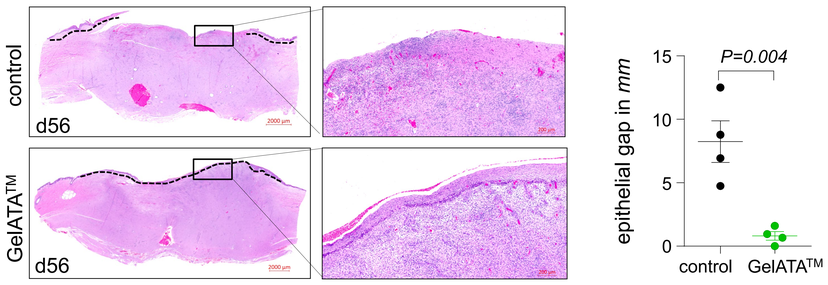


Figure 5. Effect of GelATA™ on wound re-epithelialization . Representative H&E images showing significant complete epithelialization of GelATA™ treated wounds that was evident by d56 compared to control Acticoat. Scale=2000um inset =200um.(n=12 wounds in 3 pigs).

Conclusion

This work presents first in vivo evidence for the efficacy of GelATA™ in disrupting persister biofilm and promoting functional wound closure in a pre-clinical porcine burn wound model.

Acknowledgment

We thank Southwest Technologies for providing the dressing.

References

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