GelATA Wound Care Dressing to Manage Biofilm Caused by Persister Bacteria

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Background

Biofilm infection commonly complicates wound healing. Persister bacteria are a subpopulation of transiently antibiotic-tolerant bacterial cells that are able to resume growth after a lethal stress. Mechanisms underlying biofilm formation of such subpopulation of bacteria are different than those understood for the general bacterial population[1]. Extracellular DNA (eDNA) is a principal constituent within the biofilm matrix. While DNase treatments have demonstrated efficacy in eradicating standard biofilms, they are ineffective against persister biofilms[1]. (**Fig1**).

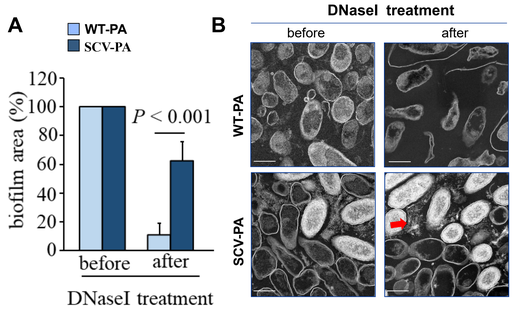

Figure 1. SCV (small colony variant) -PA biofilm are resistant to DNaseI digestion.

(**A**) Quantification of wild-type (WT-PA) and persistent (SCV-PA) biofilm before and after DNaseI treatment.(n=6).

(**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.

- 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[1]. We reported that a DNase resistant biofilm of PAO1ΔwspF can be disrupted by aurine tricarboxylic acid (ATA), a chemical inhibitor of covalent binding between eDNA and protein.

Objective

To test the efficacy of GelATA wound care dressing (ATA incorporated into a polymer-based gel[2]) 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 Yorkshire white pigs (n=5).
- Polymicrobial persister biofilm infection was established with clinical isolates of *Pseudomonas Aeruginosa* (PAO1 ΔwspF) and *Staphylococcus Aureus* (S. aureus rexB) at 10^8 colony forming units (CFU)/ml.
- Wounds were treated with either standard of care dressing (Acticoat™) or GelATA once weekly. At day 28 postburn, the treatment of GelATA treated wounds 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) on weekly bases.
- Tissue biopsy for histology and Scanning Electron Microscopy (SEM) were done at day 56.

Results

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

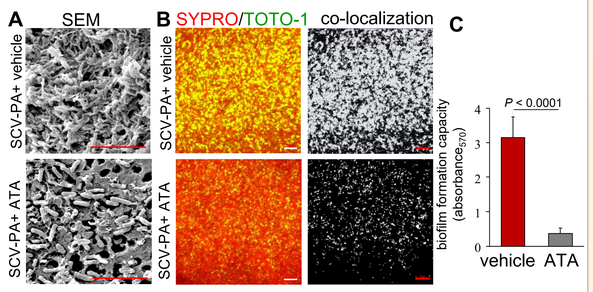

Figure 2. Inhibition of DNA–protein interaction compromised *in vitro* SCV-PA biofilm formation. (A) SEM images of SCV-PA biofilm at 24h treated with buffer and ATA. Scale bar, 5µm. (**B**) Confocal microscopic images showing **SYPRO® Ruby** and **TOTO-1** staining of SCV-PA biofilm at 24h treated with 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

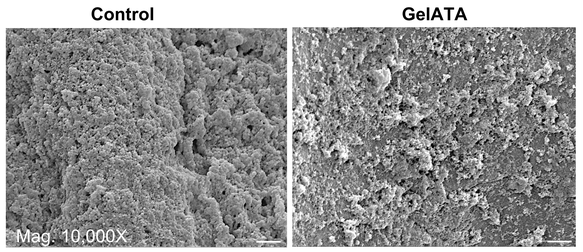

Figure 3. Effect of GelATA on P-biofilm infection. Representative SEM images at day 56. GelATA treated wounds showed visibly less biofilm-like structures on wound tissue in a well-established *Pseudomonas* wspF and *Staphylococcus* rexB biofilm compared to control (Acticoat) wounds.

GelATA improved functional burn wound closure

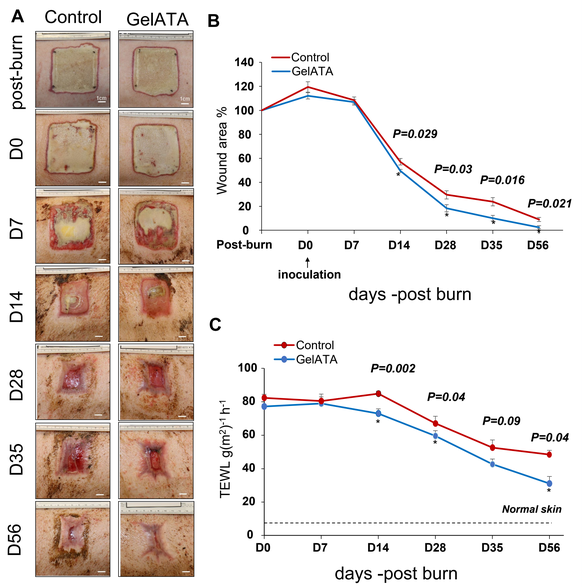

Figure 4. Effect of GelATA on burn wound closure. Burn wounds were induced to the dorsum of pigs and treated with silver dressing (Acticoat) or GelATA. (**A)** Digital images of wound closure over the timeline of study are shown(scale=1cm). (**B)** Quantitation of wound closure shows a significant improvement in visual and. (**C**) Functional wound closure (low TEWL) in GelATA treated wounds. Data are shown mean ± SD; n=5.

GelATA accelerated wound re-epithelialization

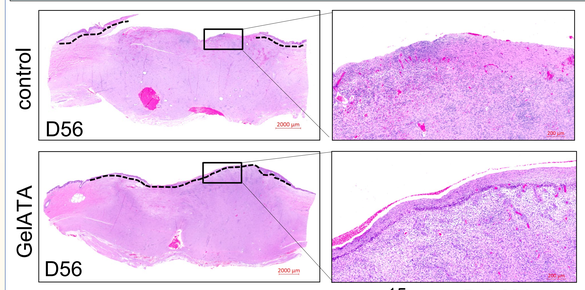

Figure 5. Effect of GelATA on wound re-epithelialization. Representative H&E images showing significant complete epithelialization of GelATA treated wounds that was evident by day 56 compared to control wounds. Scale=2000um and the inset =200um. (n=4 pigs) .

Conclusion

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

Future direction

GelATA is currently at Technology Readiness Level (TRL 4) and the porcine studies will concurrently test the safety in the animal model system while addressing the anti-biofilm and wound healing efficacy of the GelATA dressing, Pre-IND and IND will then follow, seeking FDA approval before beginning clinical studies.

References

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