Resolution of Bioburden and Wound Closure in a Porcine Deep Reticular Dermal Wound Model

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Background & Methods

Collagen matrices have been shown to provide a target for aberrant proteolytic activity in a chronic wound environment. Combining these extracellular matrices with antimicrobials such as polyhexamethylene biguanide (PHMB) has demonstrated both *in vitro* and *in vivo* to prevent the reformation of biofilm. We designed a porcine full thickness wound model infected with methicillin-resistant *Staphylococcus aureus* (MRSA) to model the clinical environment of chronic wounds. This study evaluated the impact of an antimicrobial barrier (PCMP^) on reformation of biofilm and wound resolution.

4cm x 4cm x 3mm deep reticular dermal wounds were generated on the backs of pigs (n=3), infected with MRSA (USA300), and allowed to form a biofilm for 72 hours. After biofilm formation, wounds were sharply debrided to model standard of care and subsequently PCMP was applied as an antimicrobial barrier. In order to assess wounds, PCMP was removed every 5 days; Days -3 (unwounded baseline, N=4), 0 (infected and debrided baseline, N=6), 5 (N=2), 10 (N=5), 15 (N=5), and 20 (N=3) post application. Progression of normal wound healing responses was assessed using gene expression (RT^2 Profiler™ PCR Arrays, Pig Wound Healing, Qiagen). Genes that were statistically different from unwounded skin were used for STRING analysis with Markov Cluster Algorithm (MCL) inflation parameter of 3. GO terms from these clusters were then plugged into "reduce and visualize gene ontology" (REVIGO) to determine high level GO terms related to various phases of the wound healing process.

Compared to unwounded skin, wounds with an antimicrobial barrier applied resulted in expected progression through the wound healing phases including: macrophage recruitment/activation and ECM remodeling. We observed statistically increased expression of genes associated with monocyte/macrophages recruitment (CCL2), activation (CD40LG), and subsequent response (IL-1α, IL-1β, IL-10, and TNF), which resolved as expected as the wounds progressed towards healing. We also observed an expected upregulation and subsequent downregulation of collagenase (MMP1), gelatinase (MMP9), and stromelysin (MMP3) as the wound progressed towards healed. REVIGO analysis found GO terms associated with normal wound repair, starting with inflammation that progressed towards cellular proliferation and migration and ECM remodeling as wounds closed.

Together, these findings highlight that sharp debridement followed by application of an antimicrobial barrier (PCMP) helped prevent biofilm reformation resulting in normal wound progression through the phases of wound healing.

^PuraPly® AM, Organogenesis, Canton, MA

Experimental Model

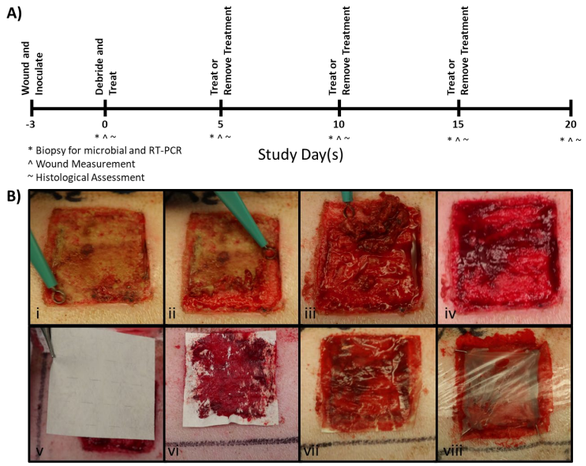

Figure 1: Experimental timeline, product application, and biopsies. **A)** Study design timeline. 4cm x 4cm x 3mm deep reticular dermal wounds were generated and inoculated with MRSA for 72 hours to allow for biofilm formation. After inoculation, wounds were debrided and randomly assigned to groups that either maintained PCMP throughout the study or had removal of PCMP during the course of closure. Histological, bacteriological, and wound closure assessment was performed at all dressing changes. **B)** Representative images of product application. Wounds were sharply debrided (**i-iv**), product was applied to the fresh wound bed (**v-vi**), wetted with saline to ensure contact of antimicrobial barrier with wound bed (**vii**), then stapled and covered with secondary dressing (**viii**).

Antimicrobial Barrier to Biofilm Reformation

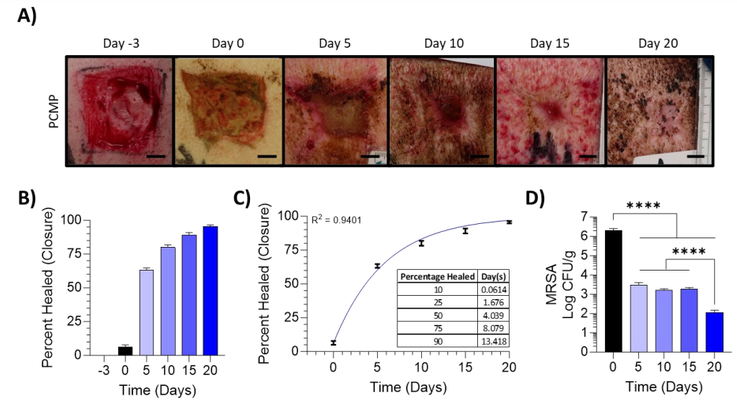

Figure 2: Closure and MRSA level assessment. **A)** Representative images of wounds at dressing changes. Scale bar set to 1cm. **B)** Wound closure calculated as percent area normalized to initial wound size. **C)** Exponential plateau fit modeling predicted wound healing. **D)** Impact of PCMP barrier on reformation measured by MRSA levels at all timepoints compared to baseline-debrided wounds. Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test. **** P < 0.0001.

Progression Through Normal Phases of Wound Resolution

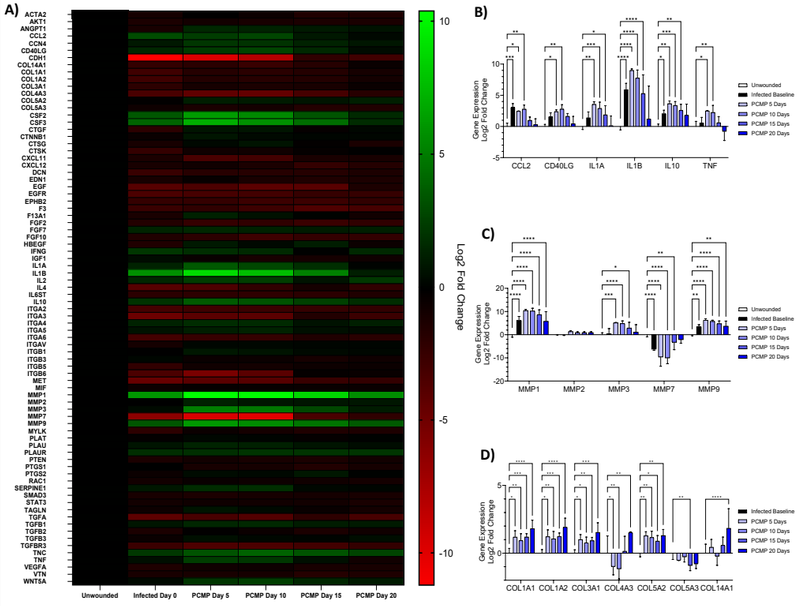

Figure 3: PCMP barrier supporting normal progression through phases of wound healing. A) $2^{-\Delta\Delta Ct}$ performed using unwounded skin as baseline expression. Heatmap expressed as Log_2 fold change in expression. **B)** Recruitment and activation of monocytes/macrophages. Statistically elevated levels of chemoattractant (CCL2), activation markers (CD40LG), and effector molecules (IL1A, IL1B, IL10, and TNF) observed early and returning to baseline over time. **C)** MMPs are statistically elevated with expression tapering down over time as remodeling phase occurs. **D)** Changes in collagen expression throughout normal phases of healing. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p < 0.0001.

Protection with PCMP Supports Progression Through the Wound Healing Cascade

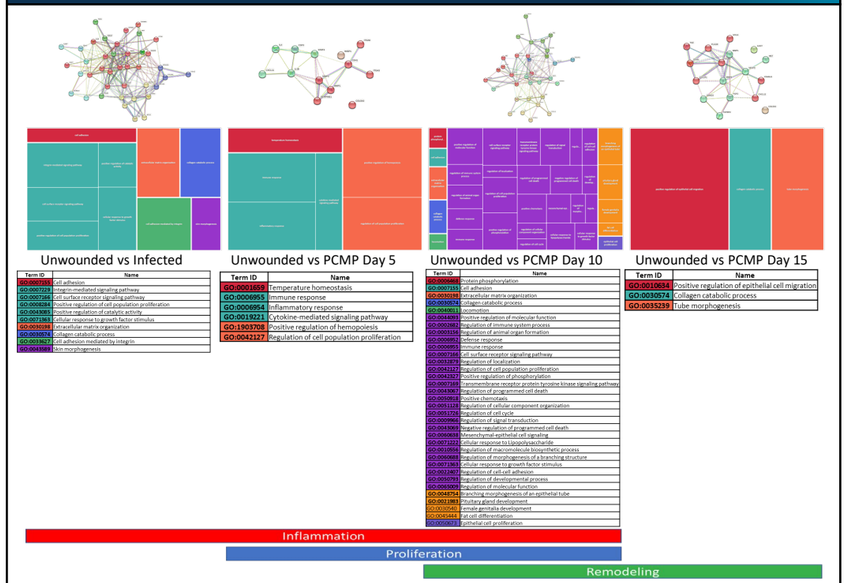

Figure 4: Gene Ontology (GO) terms give a snapshot of wounds status and normal progression from inflammation to proliferation/remodeling phases. STRING assessment of statistically up/down regulated genes from Infected baseline Day 0, PCMP Day 5, Day 10, and Day 15. Extracted GO terms were reduced with REVIGO to remove redundant terms. Terms highlight the impact of an antimicrobial barrier protecting the wound and allowing normal progression through the wound healing cascade.

Impact of Removal of Antimicrobial Barrier to Biofilm Reformation

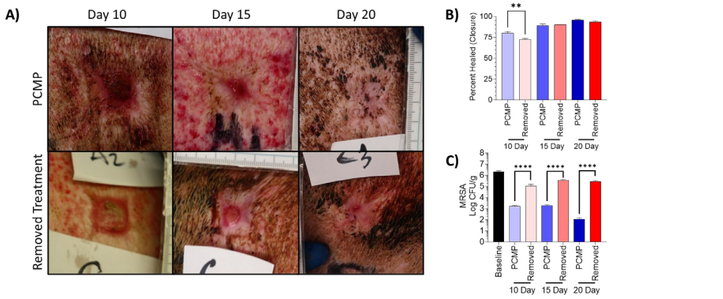

Figure 5: Removal of PCMP antimicrobial barrier prior to closure results in MRSA biofilm reformation. A) Representative images of wounds on days 10, 15, and 20 days. **B)** Percent wound closure. Welch's T=test. ** P < 0.01. **C)** Bioburden assessment at all timepoints removal of PCMP resulted in resurgence of bioburden compared to continual protection of the wound bed with PCMP. Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test. **** P < 0.0001.

Conclusions

1. Sharp debridement + PCMP antimicrobial barrier resulted in protection of the wound bed from resurgence of MRSA biofilm resulting in nearly full wound closure by end of study.
2. Sharp debridement + PCMP antimicrobial barrier protected wounds from biofilm reformation as evidenced by bioburden levels >4 log fold reduced compared to baseline by the end of the study.
3. Protection of the wound bed with PCMP resulted in wound progression through the normal wound healing cascade as highlighted by genetic assessment.
4. Removal of PCMP barrier resulted in resurgence of MRSA levels.

ORGANOGENESIS