Native Collagen Scaffold + PHMB Acts as a Barrier to Bioburden and Supports Progression of Wounds through the Intrinsic Wound Healing Cascade in Two Distinct *In Vivo* Wound Models

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Abstract

Wounds, whether chronic or acute, are prone to bacterial colonization and subsequent biofilm formation resultant from the loss of the body's natural protective barrier, the skin. While planktonic bacteria are often handled by the hosts immune system, the microbes become difficult to eradicate if they develop into a biofilm, resulting in delayed wound healing and a dampened immune response. Utilizing MRSA-infected surgical and dermal wound models, we highlight how a native collagen wound matrix embedded with polyhexamethylene biguanide (PCMP*) provides a barrier to microbial growth and subsequent biofilm reformation.

Two animal models were evaluated: 1) infected bilateral fracture complex surgical wound canine model and 2) infected large dermal wound porcine model. For the surgical model, wounds were generated, infected via implant hardware coated with MRSA for 7 days, then surgically debrided to remove the infection and covered with a single application of PCMP for up to 10 days. For the dermal model, wounds were generated, infected with MRSA for 3 days, then surgically debrided to remove the infection and covered with PCMP, with reapplication every 5 days for a maximum total of 20 days. Outcome measures across both studies included bacterial load, genetic assessment, histological assessment, and wound closure (porcine model only).

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PCMP as an Antimicrobial Barrier to Biofilm Reformation in an Infected Porcine Model

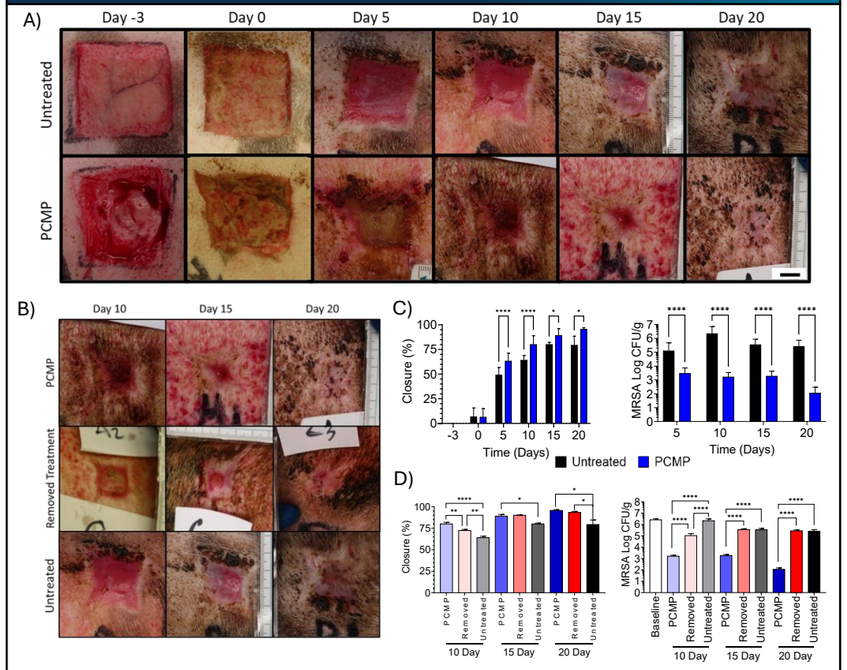

Figure 1. Ongoing coverage with PCMP resulted in wound closure and reduced bioburden, while removal of PCMP prior to closure results in biofilm reformation. A) Representative images of wounds at different timepoints for PCMP and untreated wound groups. Scale bar set to 1cm. **B)** Representative images of wounds on days 10, 15 and 20 from PCMP, removal of treatment (Removed Treatment), and untreated wound groups. **C)** Wound closure calculated as percent area normalized to initial wound size & bioburden assessment. Wounds with PCMP had statistically less MRSA compared to untreated controls. **D)** Percent wound closure assessment & bioburden assessment. Removal of antimicrobial barrier at all timepoints allowed for resurgence of bioburden compared to continued PCMP coverage. Average ± standard deviation reported; * p ≤ 0.05; ** p ≤ 0.01; **** p < 0.0001.

Enhanced Monocyte/Macrophage Gene Expression Observed Following PCMP Barrier Use

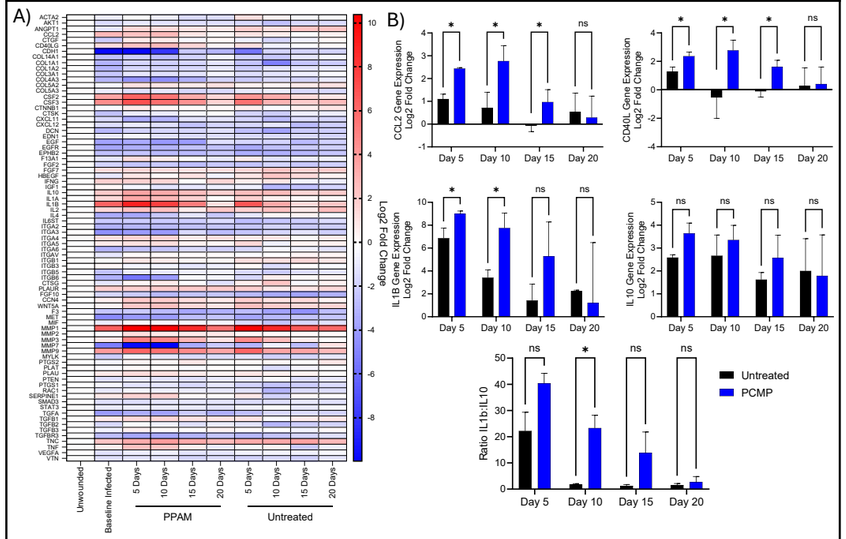

Figure 2. Wound-related gene expression markers evaluation showed increased levels of genes associated with monocyte/macrophage recruitment following use of PCMP as an antimicrobial barrier. A) ΔΔCt analysis performed using unwounded skin as baseline expression. Heatmap expressed as Log2 fold change in expression. **B)** Wounds covered with PCMP resulted in statistically elevated recruitment of monocytes/macrophages through CCL2 and subsequent activation with CD40L compared to untreated controls. Statistically elevated effector inflammatory response (IL-1β) and a ratio of IL-1β:IL-10 indicating a strong M1-like phenotype for PCMP wounds compared to untreated wounds. Average ± standard deviation reported; * p ≤ 0.05; ns = not significant.

PCMP Supported Healing as an Antimicrobial Barrier in a Complex Surgical Model

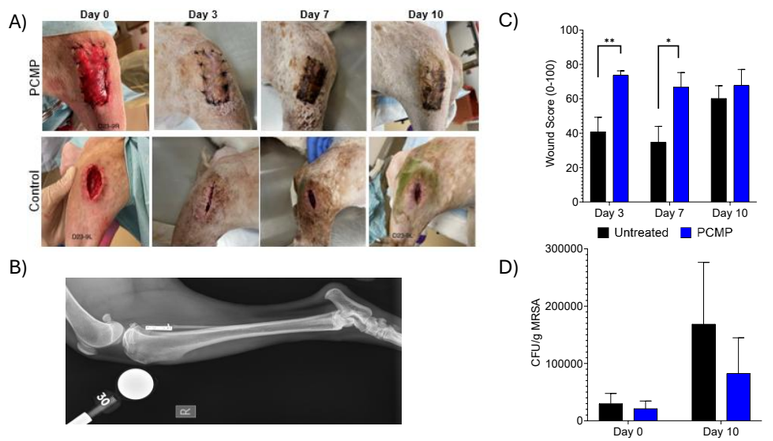

Figure 3. Representative gross wound images and canine surgical model. A) Representative wound images of PCMP and untreated control groups at days 0, 3, 7, and 10 post-treatment. **B)** Representative radiographic image of the repaired fibular defect site is shown in the right lateral view. **C)** Wound assessment scores from 3 blinded assessors for PCMP and untreated control groups at days 3, 7, and 10 days post-treatment. Scores range from 0-100, with 0=extremely poor and 100=excellent appearance of the wound. Mean ± standard deviation reported; *p<0.05, **p<0.01. **D)** Quantitative microbial load results for the difference in microbial load between the control and PCMP groups prior to I&D (day 0) and 10 days post-treatment. Average ± standard error of mean reported.

Histological Assessment of Barrier Properties of PCMP

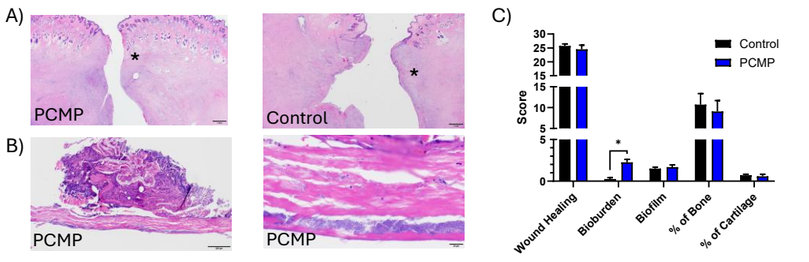

Figure 4. Representative histological assessment at repair sites and histological scoring. A) Representative images showing innate wound healing at the surgical sites (denoted by *) for PCMP and control groups at day 10 post-treatment. Scale bars indicate 1mm. **B)** Representative photomicrographs demonstrating bacterial infiltration in PCMP. Scale bars indicate 200µm and 20µm, respectively. **C)** Graphs presenting the median (range) wound healing, bioburden, and biofilm histologic scores, along with the mean ± standard deviation of fracture site histomorphometry for the PCMP and control groups at 10 days post-treatment. PCMP showed significant coccoid bacterial retention, whereas the control dressing had changes at days 3 and 7. This result is attributed to PCMP being fixed to the wound bed throughout the duration of the study, whereas the non-adherent pad was exchanged multiple times during wound examination. When viewed alongside the pig study, where resurgence of bioburden required product removal, it is likely that this bacteria found in PCMP is not viable and the presence of these retained bacteria and cell debris in the PCMP contributed to disparities in histological outcomes.

Differential Regulation of Immunomodulatory Markers Following PCMP Barrier Use

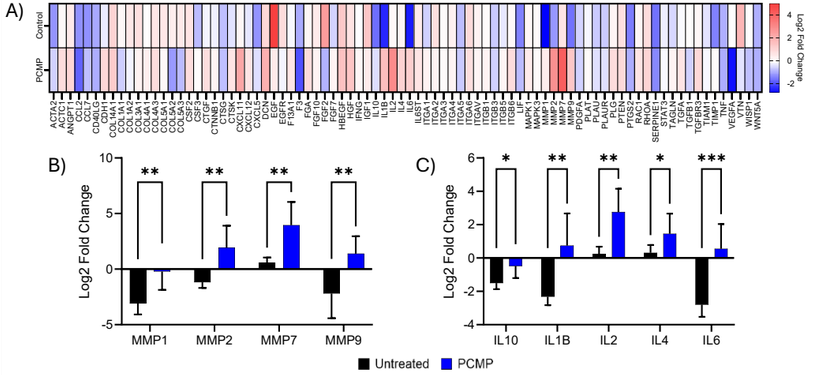

Figure 5. Comparative gene expression showed significant differences in MMP and inflammatory targets following use of PCMP as an antimicrobial barrier. A) Heat map summarizing gene expression changes in PCMP and control wounds. Gene expression changes in **B)** matrix metalloproteinases and **C)** immune-modulating cytokines. Elevated MMPs are required for resolution of infection and proceeding through the innate wound healing response, while elevated levels of IL-2 and IL-4 are associated with T-cell-mediated adaptive immunity. Mean ± standard deviation reported; * p ≤ 0.05** p ≤ 0.01*** p ≤ 0.001.

Conclusions

PCMP acted as an antimicrobial barrier and within the porcine model showed reduced bioburden and nearly complete wound closure by the end of the study. The genetic assessment highlighted the monocyte/macrophage pathway to keep MRSA bioburden minimized. Treatment to closure was critical, as removal prior to closure resulted in resurgence of bioburden. In a canine model, a comparative analysis of bacterial burden between control versus PCMP groups at baseline (pre-I&D) and 10 days post-treatment resulted in PCMP showing lower bioburden levels. PCMP as an antimicrobial barrier resulted in better wound scores on days 3 and 7 compared to controls.

<u>These studies demonstrate the role of PCMP as an antimicrobial barrier that supports progression of innate wound healing and reduces biofilm reformation in complex wounds.</u>

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References: PMID 40251887 & PMID 41009757