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Antimicrobial Effects of a Collagen Wound Matrix with a Broad-Spectrum Antimicrobial Using a Porcine Deep Dermal Model

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Abstract:

In 2019, biofilms were estimated to result in a global cost of $281 billion by delaying the rate of wound healing.[1] Biofilms have been shown to increase expression of various matrix metalloproteinases; treatment of these wounds with native collagen matrices could provide an additional target for this increased proteolytic activity. Polyhexamethylene biguanide (PHMB), a broad-spectrum antimicrobial, has been previously shown to reduce wound bioburden; recent studies performed by our lab have further confirmed the antimicrobial efficacy of PHMB to reduce Methicillin Resistant Staphylococcus aureus USA300 (MRSA USA300).[2-4] The aim of this study was to examine the ability of a novel crosslinked collagen matrix with PHMB (PCMP*)[5] to prevent biofilm reformation and support healing in wounds with established MRSA-biofilm.

Ten deep reticular dermal wounds measuring 4cm x 4cm x 3mm deep were made with a specialized electrokeratome in the paravertebral and thoracic area in three specific pathogen free (SPF) pigs (Looper Farms, NC). All wounds were inoculated with 100 μL of MRSA USA300 at 10^4 CFU/mL and covered with polyurethane film dressing (Tegaderm; 3M USA) for 72 hours to allow for biofilm formation. Wounds then underwent standard sharp debridement, with two wounds per pig recovered for baseline microbial counts. The remaining wounds were treated with PCMP for a minimum of 5 days with reapplication every 5 days for up to 20 days. Select wounds had PCMP treatment removed after 5, 10, or 15 days to assess whether continuation of treatment was necessary for sustained bioburden reduction and wound closure.

Wounds were assessed for MRSA counts on days 10, 15 and 20. At all assessment time points, wounds treated with PCMP exhibited a lower MRSA count compared to baseline wounds. Furthermore, removal of treatment resulted in resurgence of bioburden, whereas continued PCMP treatment reduced bacterial counts reduced by more than 99%, supporting continual treatment through wound closure with PCMP to prevent microbial proliferation. These studies may have important clinical implications in the use of tissue matrices with antimicrobials to reduce bioburden and potential costly wound infections.

*PuraPly AM™ Antimicrobial Wound Matrix (Organogenesis Inc., Canton, MA)

Introduction:

Several studies using polyhexamethylene biguanide (PHMB) have demonstrated efficacy against several gram-positive and gram-negative bacteria as well as yeast and fungi.[6,7] The chemical mechanism behind this polymeric biguanide occurs when this compound reacts with acidic membrane lipids, which then increases membrane permeability and abates the invasive microorganism.[8] The implementation of purified Type I porcine collagen into a matrix containing PHMB (Puraply™ AM) could provide protection against microbes while supporting the wound healing process. This study evaluated the efficacy of the PHMB in combination with a novel crosslinked collagen matrix.

References

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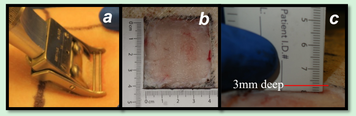
Materials and Methods:

1. Experimental Animals:

Swine (3) were used as our experimental animal due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.[9]

2. Wounding Technique:

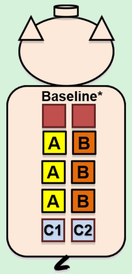
A specialized electrokeratome (photo a) was used to create ten (10) deep reticular dermal wounds measured 40mm x 40mm x 3mm deep (photo b and c) on the paravertebral and thoracic area of each animal.



3. Inoculation:

- After creation of wounds, 100μl of Methicillin Resistant *Staphylococcus aureus* (MRSA USA300) was used to inoculate each wound by scrubbing (10^4 CFU/ml) inoculums into each wound with a teflon spatula (30 seconds).
- Three (3) wounds were assigned to each experimental group (2 groups total). Two (2) for Baseline and two (2) more for PCMP Control.
- All wounds were then covered with a polyurethane film for 72 hours (to allow biofilm formation).[10]

4. Experimental Design:



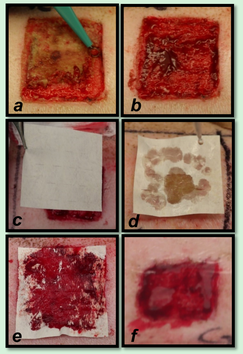
Treatments Groups
A. PCMP^ Multiple Applications
B. PCMP^ Single Application
C1-C2. PCMP^ Control after 5, 10 or 15 days

^Crosslinked Collagen Matrix with PHMB [PuraPly™ AM]

***Baseline wounds were recovered 72 hours after inoculation and debridement.**

5. Product Application Regimen:

a. After 72 hours, all wounds were debrided with a 4mm curette on Day 0.
b. Upon removing the necrotic tissue from debridement, wounds were ready for product application.
c. Sterile forceps were used to distribute each individual dressing matrix to cover the entire wound bed area.
d. PCMP matrices were hydrated with 200μL of sterile saline which was delivered with a micropipette.
e. Saturated PCMP dressing to ensure contact to wound bed.
f. All treated wounds were covered with Tegaderm dressings.



- Animals after 5, 10 or 15 days of PCMP application, the dressings removed and then reapplied and hydrated with 400μL of sterile saline. On those days, animals followed its specific treatment regimen with either PCMP or Tegaderm dressings (see Table 1 for each animal's timeline).

Table 1: Timeline for the Switch Study

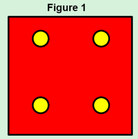
	Days after wounding																						
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Pig 1	Wound/ Inoculate			PCMP					PCMP or Tegaderm					FINAL ASSESSMENT										
Pig 2	Wound/ Inoculate			PCMP					PCMP					PCMP or Tegaderm					FINAL ASSESSMENT					
Pig 3	Wound/ Inoculate			PCMP					PCMP					PCMP					PCMP or Tegaderm					FINAL ASSESSMENT

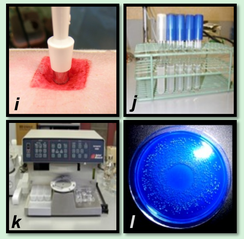
6. Wound Recovery:

Microbiology Analysis:

- Baseline wounds were recovered after debridement.
- On days 10, 15 or 20 post treatment, three wounds per group were recovered from 4 different areas in wound bed (see figure 1) by using a 6mm punch biopsy (photo i) for microbiology analysis.
- Biopsies were homogenized and combined with a scrub solution.
- Serial dilutions were made (photo j) and quantified using the Spiral Plater System (which deposits a defined amount (50μL) of suspension over the surface of a rotating agar plate: photo k)
- MRSA USA300 was isolated on ORSAB (Oxacillin Resistance Screening Agar Base) incubated at 37±2°C for 36-48 hours (photo l).
- The colony forming units per g (CFU/g) were calculated and comparison of the means was analyzed.

Figure 1





Results:

- Baseline debrided wounds had a bacterial count of 6.31±0.46 Log CFU/g, demonstrating the highest bacterial count for the entire study (Figure 2).
- **On day 10**, PCMP single application in wounds had the highest bacterial wounds at 5.02±0.58 Log CFU/g, compared to the other groups, with a bacterial reduction of 97.98% when compared against baseline wounds treated with PCMP.
  - Wounds where PCMP was reapplied showed a count of 3.13±0.28 Log CFU/g, (98.71% reduction) when compared to PCMP single application.
  - When compared against baseline wounds recovered after debridement, PCMP wounds exhibited bacterial reductions of 99.93%.
- **On day 15**, those wounds with a single application of PCMP reached the highest bacterial count at 5.54±0.27 Log CFU/g. These wounds were substantially higher than baseline wounds after day 5, by having bacterial reductions of at least 99.16%. Additionally, these results on day 15 reached bacterial reductions of 99.40% and 99.28% when compared against baselines from days 10 and 15, respectively.
  - Wounds where PCMP was reapplied had a bacterial count of 3.18±0.29 Log CFU/g. When this result was compared against baseline after debridement and PCMP single application, there were bacterial reductions of at least 99.57%
  - PCMP results on day 15 were also compared against baseline wounds had a bacterial reductions of 99.40 and 99.28%, respectively.
- **On day 20**, PCMP single application had a MRSA counts of 5.44±0.23 Log CFU/g.
  - Wounds where PCMP was reapplied reached bacterial counts of 2.05±0.43 Log CFU/g (99.96% bacterial reduction when comparted against PCMP single application), having the lowest MRSA presence throughout the entire study. When compared against baseline wounds from days 5, 10 and 15, there were bacterial reductions of at least 94.70% but when compared against baseline wounds after debridement there was a bacterial reduction of 99.99%.
  - Baseline wounds after debridement had substantially higher bacterial counts than any other group, while those baseline wounds from days 5, 10 and 15 showed slightly similar counts ranging from 3.33 to 3.47 Log CFU/g (Figure 2). Those wounds treated with PCMP had similar counts on days 10 and 15 but each group substantially lowered their respective MRSA counts by day 20.

Figure 2: MRSA counts for each group over time (day)

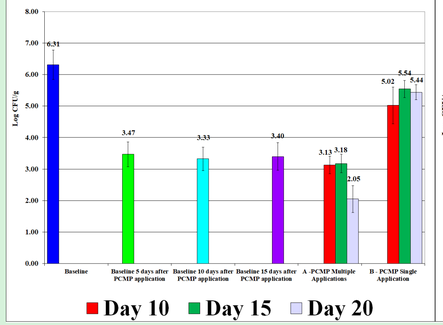
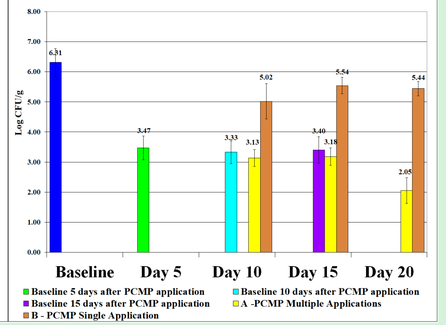


Figure 3: MRSA counts per day for each group



Conclusions

These results suggest that a purified native collagen matrix with PHMB (PCMP) reduced MRSA counts at all assessment times. Multiple applications of PCMP after 20 days showed a significant reduction in MRSA counts than a single application on days 5, 10 or 15. Studies on the effects of the matrix containing PHMB against other pathogenic microorganisms and its effects on supporting healing should be warranted.

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