PCMP for Complex Wounds: Impact on Gene Expression and Wound Healing in a Preclinical Canine Model

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Introduction: Infected surgical wounds often require ongoing irrigation and debridement (I&D) every 3-4 days until infection is resolved to allow for progression through the wound healing cascade. Repeated I&D is a burden for patients, resource demanding, and financially costly to the hospital system and payors. We hypothesize that utilizing PCMP, a cross-linked native porcine collagen matrix scaffold embedded with antimicrobial polyhexamethylene biguanide (PHMB) would protect these wounds and support wound healing. To model a common complex wound healing scenario in orthopedics, we utilized a canine model mimicking clinical complications associated with implant-infected fractures and accompanying draining wounds[1-2]. Gene expression analysis and wound healing assessments were used to compare coverage with a non-adherent wound dressing alone or with PCMP as a barrier. This study aimed to evaluate whether PCMP might protect a complex wound environment for 7 -10 days, potentially reducing or eliminating the need for ongoing irrigation and debridement.

Methods: All in-life animal research was conducted under an IACUC-approved protocol (#16680) at the University of Missouri. In 8 dogs, bilateral 1 cm fibular defects (n=16) were created and stabilized using plate and screw fixation. Each plate was incubated for 48 hours with a biofilm-producing suspension of *Staphylococcus aureus* at a concentration of $1x10^5$ CFU/mL prior to use. Plates were washed to remove free-floating bacteria and utilized to stabilize fibular defects and surgical incisions closed routinely. At day 7 post-op, all surgical wounds were clinically infected and draining, and underwent standard-of-care open irrigation and debridement, followed by placement of a non-adherent dressing alone or PCMP (n=8/group). Each dog received both treatments, one in each hindlimb, alternating sides to minimize bias. The degree of wound healing was assessed at days 3, 7, and 10 days post-treatment using both radiography and wound assessment scoring by three blinded independent assessors. Quantitative RNA expression of 84 genes using a multiplex panel of wound targets was performed using RT^2 Profiler PCR Arrays (Qiagen, Cat# 330231). Standard ΔC_T and fold-change ($2^{-\Delta\Delta CT}$) of normalized gene expression were calculated using GraphPad Prism software. Data are presented as mean ± standard deviation. Statistical significance was determined using t-tests and denoted where * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p<0.001$.

Results: On days 3 and 7, treatment with PCMP was associated with significantly higher wound assessment scores (better wound healing) than the control group (73.8 ± 7 versus 40.8 ± 24; *p=0.0012*) and (66.8 ± 24 versus 34.8 ± 26; *p=0.024*), respectively. By Day 10, although treatment with PCMP scores trended higher compared to the control group (67.9 ± 26 versus 60.2 ± 21), the improvement was not statistically significant (*p=0.53*). Quantitative RNA expression at 10 days revealed differences in several targets related to ECM proteins, matrix metalloproteinases, and inflammation pathways. Treatment with PCMP resulted in significantly greater expression of ECM genes, including COL1A1, COL1A2, COL5A1, COL5A2, and COL5A3, and significantly reduced expression of genes associated with proteases and tissue breakdown including matrix metalloproteinases (MMP; MMP-1, -2, -7, and -9). The PCMP group overall also showed lower expression of inflammation-related cytokines, including CXCL11, IL-2, IL-4, and IL-6, suggesting a reduction in inflammation in the PCMP treatment group.

Discussion: In this study, PCMP was successful in supporting progression of wound healing as evidenced by higher wound scores and significant changes to key pathways of gene expression suggesting progression through the healing cascade.

Significance: In this preclinical model, we found that PCMP effectively supported healing in a complex wound scenario commonly seen in orthopaedics. Based on these findings, PCMP has the potential to decrease the need for multiple trips to the operating room for open I&D reducing the resource and financial burden on hospitals and payors. While additional work is needed to translate these findings to clinical use, these results support the potential for PCMP to improve outcomes for patients.

References: 1) Reizner W, Hunter JG, O'Malley NT, Southgate RD, Schwarz EM, Kates SL. A systematic review of animal models for *Staphylococcus aureus* osteomyelitis. Eur Cell Mater. 2014 Mar 25;27:196-212. 2) Fitzgerald RH. Experimental osteomyelitis: description of a canine model and the role of depot administration of antibiotics in the prevention and treatment of sepsis. J Bone Jt Surg Am. 1983; 65: 371- 380.

Figure 1: Heat Map Illustrating Gene Expression Changes in Treatment Groups for Specific Pathways of Interest in the Gene Array

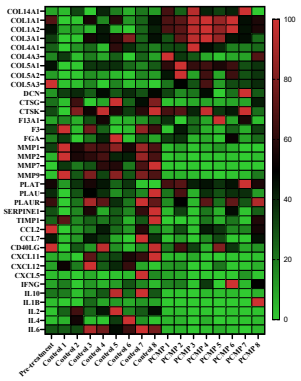

Figure 2: Relative Fold Change with qRT-PCR Illustrating Differences in ECM Composition, Remodeling, and Inflammatory Cytokines