Native Type I Collagen Matrix with PHMB Products Provide a Durable Solution to Matrix Metalloproteinase Inhibition

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Introduction

While acute wounds progress through the four phases of wound healing, chronic wounds often stall at the inflammation phase, resulting in an excess of inflammatory cells and elevated matrix metalloproteinase (MMP) levels. MMPs are critical for remodeling the wound environment, but excess expression can result in the breakdown of newly formed extracellular matrix (ECM) and granulation tissue. As such, wound products consisting of native ECM that can inhibit MMPs may help wounds progress through the natural healing process. In this study, we characterized native ECM matrix products and evaluated both durability in an *in vitro* chronic wound model and the ability to inhibit MMPs.

Methods

- Initial characterization of purified native type 1 collagen ECM plus polyhexamethylene biguanide (PCMP) and PCMP-Extra Thick (PCMP-XT) was performed using histological and scanning electron microscopy (SEM) imaging.
- PCMP, PCMP-XT, and Ovine Forestomach Matrix (OFM), were assessed for product degradation using simulated wound fluid (SWF) containing collagenase I and II as an *in vitro* chronic wound model.
- MMP inhibition utilizing a solid-state assay for each product was tested (Enzo MMP kits, Farmingdale, NY).

PCMP and PCMP-XT Maintain Native Collagen Structure

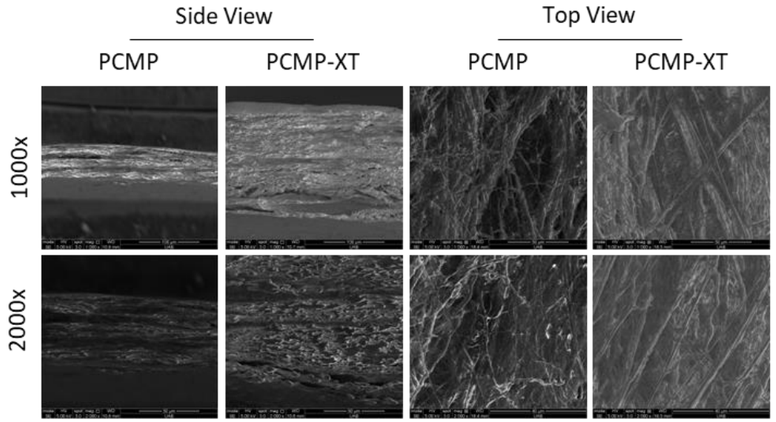

Figure 1. Scanning Electron Microscopic (SEM) characterization of PCMP and PCMP-XT. SEM images were taken from the side-cut (side view) as well as from the top of the product. Native collagen basketweave structure is identifiable from the top view while the differences in thickness are observable from the side.

PCMP-XT is 2.5 times thicker than PCMP

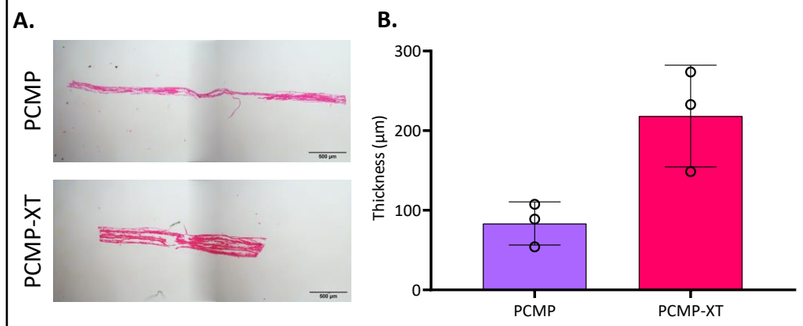

Figure 2. H&E staining of PCMP and PCMP-XT were analyzed for thickness using ImageJ software. **(A)** Representative H&E images used for measuring product thickness. Scale bar set represents 500 µm. **(B)** Average product thickness of 3 lots of PCMP and PCMP-XT. Data presented as Means ± Standard Deviation.

Collagen Cross-linking Allows PCMP Products to Withstand Degradation in *in vitro* Chronic Wound Model

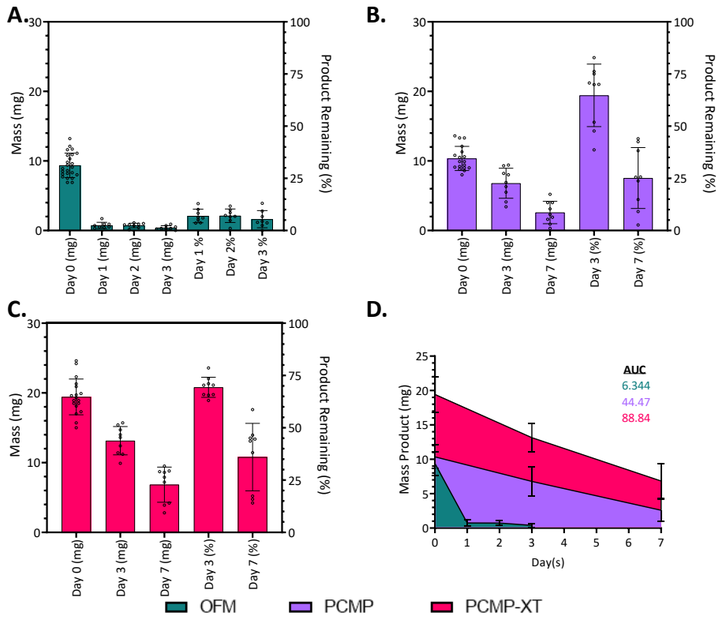

Figure 3. Degradation of PCMP, PCMP-XT, and OFM in SWF + Collagenases. **(A, B, C)** Respective graphs showing mass of 2 cm x 1 cm pieces on days 0, 3, and 7 on the left portion of the graph, and percent product remaining post degradation on the right. **(D)** Area under the curve analysis assessing degradation of products indicated rapid degradation of OFM in comparison to PCMP products.

PCMP Products Inhibit more MMPs than OFM

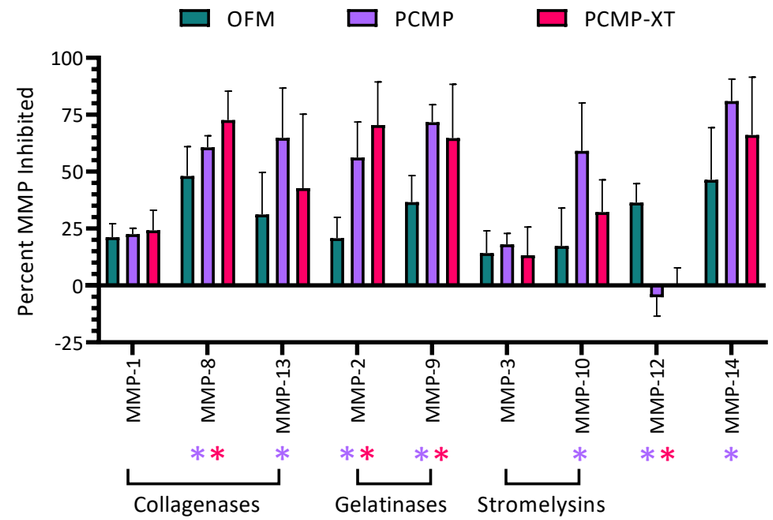

Figure 4. PCMP products inhibit more MMPs than OFM. Unpaired Welch t-test were performed to compare PCMP products to OFM. Colored asterisk correlates with the whether the product was statistically different from OFM ($p < 0.05$).

Conclusions

- Native collagen structure is maintained for both PCMP and PCMP-XT.
- PCMP had an average starting mass of 10.36 mg, with 6.79 mg and 2.59 mg remaining after 3 days and 7 days, respectively, representing a total degradation of roughly 75%.
- PCMP-XT had an average starting mass of 19.43 mg, with 13.14 mg and 6.84 mg remaining after 3 days and 7 days, resulting in 65% degradation.
- OFM was unable to withstand degradation, going from an average mass of 9.36 mg to 0.74 mg after 24 hours.
- While all products were able to inhibit a range of MMPs, PCMP/PCMP-XT were statistically better than OFM at inhibiting a range of collagenases, gelatinases, and stromelysins.

- **<u>EDC crosslinking and native collagen structure of PCMP and PCMP-XT resulted in improved durability and MMP inhibition in *in vitro* models.</u>**

Acknowledgements

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