Impact of Extracellular Matrix Graft Composition on Degradation Dynamics and Scaffold Functionality

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INTRODUCTION

Extracellular matrix (ECM) grafts are emerging as a promising option for chronic wounds due to their scaffold properties. Here, two porcine small intestinal submucosa-derived cross-linked collagen matrices with PHMB (PCMP*, 2 layers; PCMP-XTº, 5 layers) were compared to ovine forestomach matrix (OFM^, 1 layer) derived from propria submucosa. These grafts were assessed for their durability in a simulated wound environment and functionality as a scaffold by their ability to inhibit proteases and support cell attachment and proliferation.

*Puraply® AM, Organogenesis, Canton, MA
ºPuraply® AM-XT, Organogenesis, Canton, MA
^Endoform, Aroa Biosurgery, San Diego, CA

METHODS

Matrices were evaluated for structure using scanning electron microscopy (SEM) and histology, ability to modulate matrix metalloproteinases (MMPs) using fluorometric assays, and graft durability using an *in vitro* degradation model comprised of simulated wound fluid plus collagenases type I and II (SWF+). Throughout the degradation process, scaffold functionality was assessed using an *in vitro* assessment of primary human dermal fibroblast attachment and proliferation.

BIOPHYSICAL STRUCTURE OF EXTRACELLULAR MATRIX SCAFFOLDS

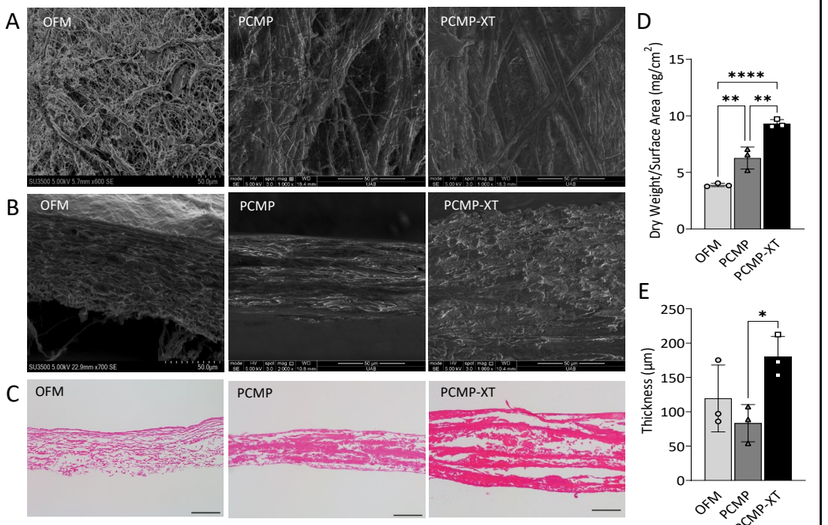

Figure 1. Characterization of extracellular matrix (ECM) scaffolds. Representative scanning electron microscopy images (2000x) of **(A)** top-down and **(B)** cross-sectional views. **(C)** Representative cross-sectional images of H&E-stained matrices (20x). **(D)** Average dry weight per surface area and **(E)** scaffold thickness. Average ± standard deviation reported. Significance denoted as *p<0.05, **p< 0.01, ****p<0.0001. Scale bar indicates 50 µm for all images.

ECM SCAFFOLDS MODULATE MATRIX METALLOPROTEINASES

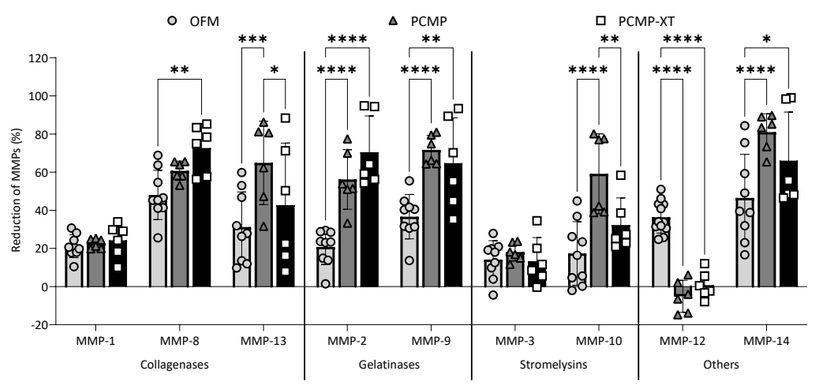

Figure 2. Reduction of matrix metalloproteinase activity by ECM scaffolds. Reduction of collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10), and others (MMP-12, -14) by ECM scaffolds. Average ± standard deviation reported. Significance denoted as *p<0.05, **p< 0.01, *** p<0.001, ****p<0.0001.

PCMP AND PCMP-XT WITHSTAND RAPID DEGRADATION *IN VITRO*

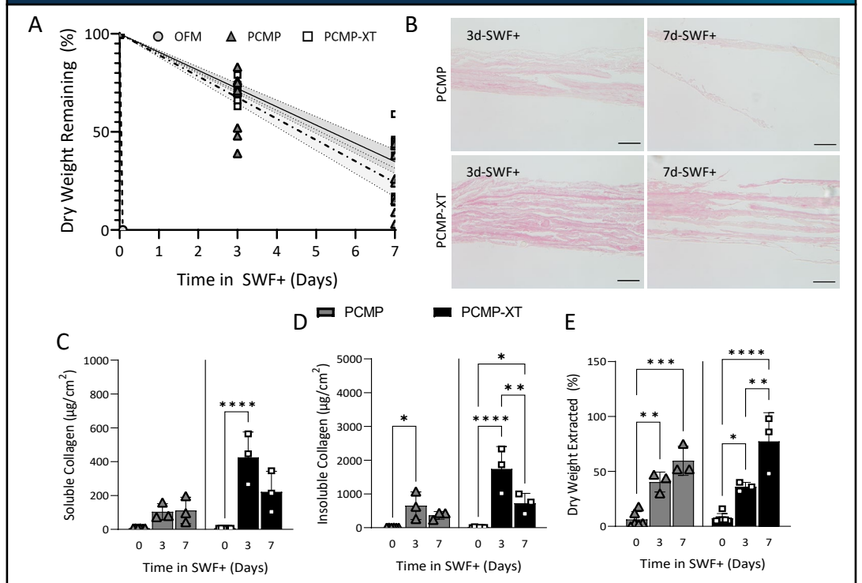

Figure 3. *In vitro* degradation of ECM scaffolds. (A) Time-course *in vitro* degradation in a simulated chronic wound model consisting of simulated wound fluid (SWF) with collagenases I and II (SWF+). **(B)** H&E assessment after 3 (3d) and 7 days (7d) in SWF+ (20x); scale bar 50µm. **(C)** Soluble and **(D)** insoluble collagen content and **(E)** extraction efficiency of PCMP and PCMP-XT. Average ± standard deviation reported. Significance denoted as *p<0.05, **p< 0.01, ***p<0.001, ****p<0.0001.

SCAFFOLD PROPERTIES OF INTACT AND SWF+ DEGRADED MATRICES

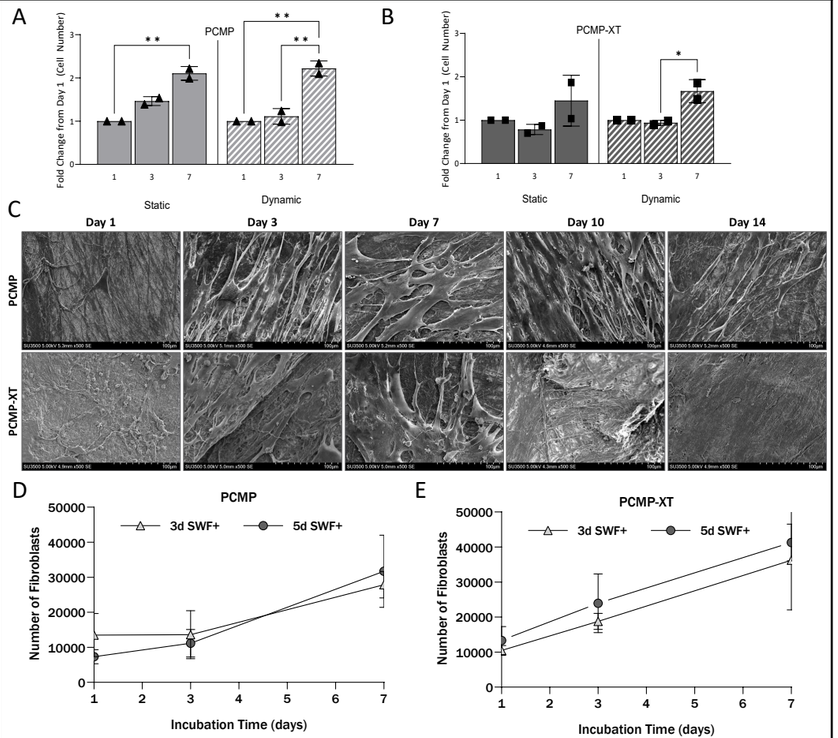

Figure 4. Fibroblast attachment on intact and partially degraded PCMP and PCMP-XT. Fibroblast cell attachment and proliferation over time after static and dynamic seeding on intact **(A)** PCMP and **(B)** PCMP-XT. **(C)** Representative SEM images of cell attachment and culture on intact ECM scaffolds (500x); scale bar 100 µm. Fibroblast attachment on 3 and 5 day degraded **(D)** PCMP and **(E)** PCMP-XT. Average ± standard deviation reported for all graphs. Significance denoted as *p<0.05, **p< 0.01.

CONCLUSIONS

- ECM grafts of varying compositions differ in matrix structure and thickness.
- All matrices reduced matrix metalloproteinases; however, PCMP and PCMP-XT were overall more inhibitory compared to OFM.
- Both PCMP and PCMP-XT withstood rapid degradation (>7 days) in a SWF+ model compared to OFM, which rapidly degraded.
- PCMP and PCMP-XT served a scaffold for fibroblast attachment and proliferation over 14 days
- Partial degradation resulted in more robust cell attachment, hypothesize due to degradation revealing additional sites for cell attachment.

<u>These results demonstrate that differing compositions of ECM result in varying MMP reduction and overall durability in an *in vitro* degradation model; furthermore, scaffold properties of PCMP and PCMP-XT were maintained throughout degradation.</u>

ORGANOGENESIS