# 5-Layer Native Collagen Matrix with PHMB Inhibits Matrix Metalloproteinases and Resists *In Vitro* Degradation

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## INTRODUCTION

While acute wounds readily progress through the wound healing cascade, chronic wounds often stall in the inflammatory phase, resulting in increased pro-inflammatory factors and elevated matrix metalloproteinases (MMPs). Extracellular matrix (ECM)-based products have emerged as a promising treatment for chronic wounds due to their ability to function as a scaffolding to support wound healing. In this study, a 5-layer crosslinked native ECM with polyhexamethlyene biguanide (PCMP-XT†) was evaluated for composition, MMP inhibition capacity, and scaffold durability and functionality using a *in vitro* chronic wound model.

†PuraPly®AM-XT, Organogenesis, Canton, MA

## METHODS

Structural characterization of PCMP-XT was conducted using histological staining and scanning electron microscopy (SEM). MMP inhibition was evaluated using a solid-state, fluorometric MMP Drug Discovery Kit (Enzo). PCMP-XT was subjected to degradation with either collagenase type I or collagenase type II in PBS, or alternatively in an *in vitro* chronic wound model consisting of simulated wound fluid (SWF) with both collagenases type I and II (SWF+). Throughout degradation, scaffold durability and functionality was evaluated by ECM loss, changes in mechanical properties, *in vitro* zone of inhibition assays, and fibroblast attachment and growth.

## CHARACTERIZATION OF 5-LAYER PCMP-XT

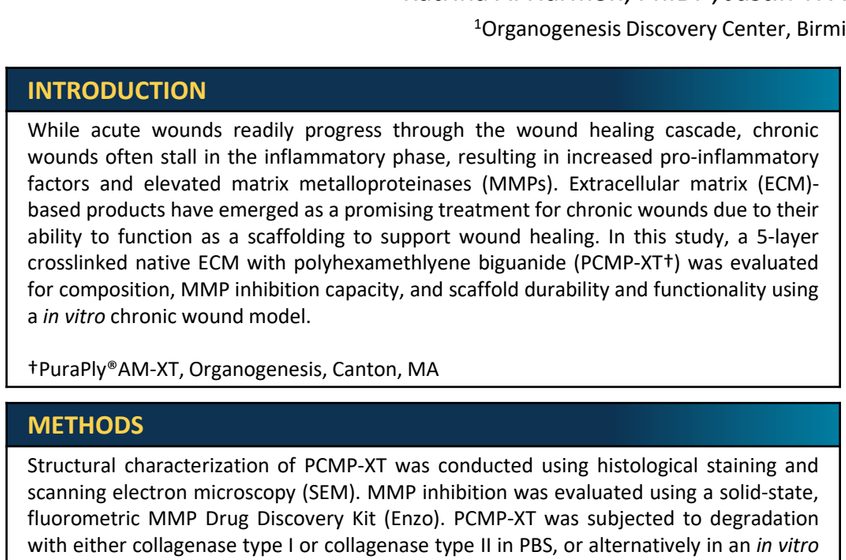


**Figure 1: Characterization of PCMP-XT.** Representative SEM images (2000x) of (A) top-down and (B) cross-sectional view. (C) Representative cross-sectional H&E-stained PCMP-XT (20x). (D) Inhibition of MMP activity. Scale bar indicates 50µm for all images. Bars represent average ± standard deviation.

## PCMP-XT WITHSTANDS RAPID DEGRADATION *IN VITRO*

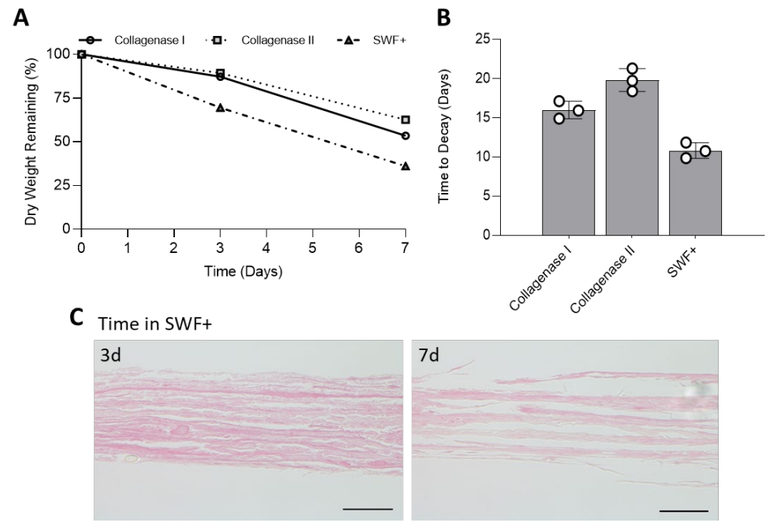


**Figure 2: Degradation of PCMP-XT.** (A) Time-course *in vitro* degradation in collagenase type I, collagenase type II, and simulated chronic wound model with collagenases types I and II (SWF+). (B) Predicted time to decay based on best fit linear regression. Bars represent average ± standard deviation. (C) Histological assessment after 3 and 7 days in SWF+ (20x); scale bar indicates 50µm.

## PROPERTIES OF PCMP-XT FOLLOWING EXPOSURE TO SWF+

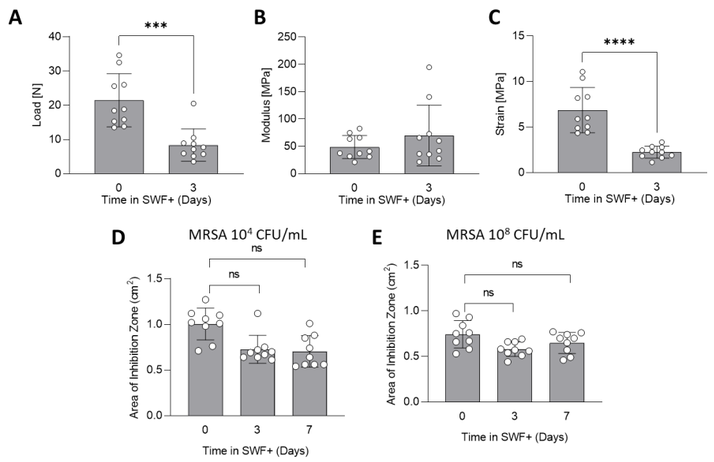


**Figure 3: Characterization of PCMP-XT after exposure in an *in vitro* chronic wound model.** Mechanical properties of intact and 3-day degraded PCMP-XT: (A) load, (B) modulus, and (C) strain. (D-E) *In vitro* zone of inhibition against MRSA of intact, 3-day, and 7-day degraded PCMP-XT. Bars represent average ± standard deviation. *** denotes p<0.001; **** denotes p<0.0001.

## SCAFFOLD PROPERITES OF INTACT PCMP-XT

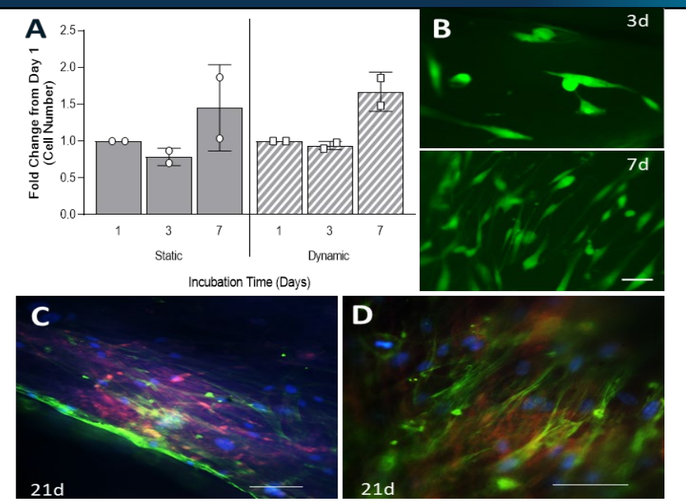


**Figure 4. Fibroblast attachment on intact PCMP-XT.** (A) Cell proliferation represented by cell number over time after static and dynamic seeding of fibroblasts on PCMP-XT. (B) Representative Calcein AM-stained (green) images of fibroblasts attached to scaffolds 3 and 7 days after seeding (20x). (C-D) Representative immunofluorescence images of fibroblast culture on PCMP XT scaffolds for 21 days, highlighting growth, migration and collagen deposition (blue, nuclei; green, f-actin, red; col 1). Scale bar indicates 50 µm.

## PCMP-XT SUPPORTS CELL ATTACHMENT AFTER SWF+ DEGRADATION

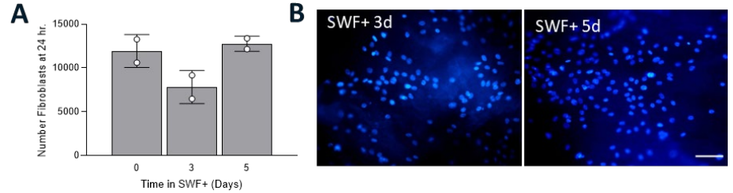


**Figure 5: Scaffold characterization after exposure to an *in vitro* chronic wound model.** (A) Fibroblast attachment (24h) onto intact, 3-day, and 5-day degraded samples and (B) representative DAPI-stained (blue) images (20x; 50µm scale bar). Bars represent average ± standard deviation; points represent average of three replicates from two independent cell lots.

## CONCLUSIONS

PCMP-XT inhibited a wide range of MMPs, while *in vitro* degradation assays revealed PCMP-XT was resistant to rapid degradation. Exposure in an *in vitro* chronic wound model (SWF+) resulted in the most aggressive degradation with a predicted time to decay >7 days. While mechanical testing was impacted by SWF+ degradation, zone of inhibition against MRSA remained consistent. PCMP-XT served as a scaffold for fibroblast attachment and proliferation, and additionally scaffold functionality was maintained through degradation process. Overall, these results demonstrate the ability of PCMP-XT to inhibit proteases, withstand rapid degradation, and function as a scaffold for cell growth, migration and collagen deposition.

ORGANOGENESIS