EVALUATION OF COLLAGEN WOUND MATRIX-MICRONIZED: BIOCHEMICAL, PHYSICAL, FUNCTIONAL, AND *IN VITRO* PROPERTIES AS A WOUND MANAGEMENT MATRIX

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INTRODUCTION

The extracellular matrix (ECM) is a 3D network comprised of structural proteins, with collagen being the most abundant. Fibroblasts and their interaction with ECM proteins are known to be crucial in the wound healing cascade. Matrix metalloproteinases (MMPs) are responsible for ECM degradation and play an important role in remodeling. Often, MMP levels are increased and dysregulated within chronic wounds. Collagen Wound Matrix-Micronized (CWM-MZ*) is an acellular, ECM-based biomaterial with a large surface area that increases contact with wounds. In this study, we characterized the biochemical and structural properties of CWM-MZ, inhibition of MMPs and proteases, along with fibroblast attachment, proliferation, and migration using *in vitro* models.

*PuraPly® MZ, Organogenesis, Inc., Canton, MA

METHODS

Collagen content was quantified using hydroxyproline and Sircol (Sirius Red) assays. Physical characteristics were assessed utilizing scanning electron microscopy (SEM) imaging. For the *in vitro* assays, surfaces were either coated with CWM-MZ or purified calf-skin collagen or left uncoated (negative control). Fibroblast attachment was evaluated at 90 minutes using DAPI staining. Cell proliferation was assessed using a NucleoCounter NC-200 automated cell counter, with shorter times to doubling indicating more robust proliferation. Cell migration was assessed using a standard wound scratch assay, in which a scratch was created using a P200 tip on a confluent cell monolayer. Total protease and collagenase/gelatinase proteolytic activity were evaluated using gelatin or casein fluorescein-labeled substrates. We also evaluated the inhibitory effect of MMPs using a MMP inhibition solid state assay from Enzo Life Sciences.

CWM-MZ BIOCHEMICAL AND PHYSICAL CHARACTERISTICS

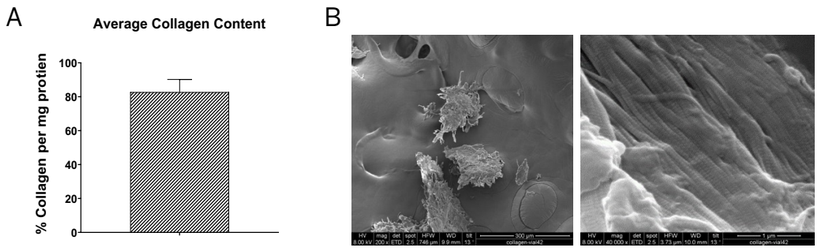
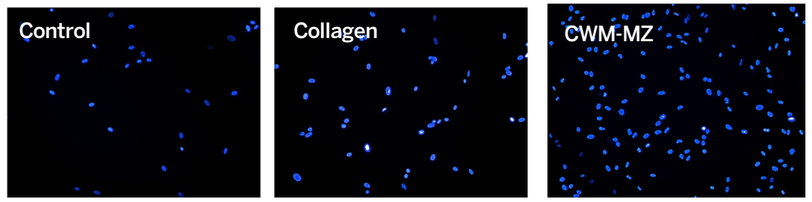


Figure 1. (A) CWM-MZ collagen content; (B) representative SEM images of CWM-MZ. Quantification and qualitative assessment highlight native collagen content.

EVALUATION OF CELL GROWTH AND ATTACHMENT



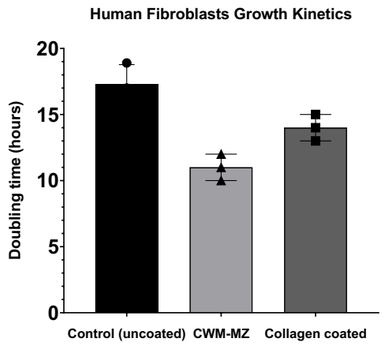


Figure 2. Representative images of cell attachment at 90 minutes post seeding using DAPI staining were imaged at (10X) magnification. Quantification of doubling time of fibroblasts seeded onto collagen, CWM-MZ or uncoated surface. Average ± standard deviation. More fibroblasts adhered when cultured on CWM-MZ-coated surfaces compared to other groups, and cells proliferated more robustly, with a doubling time of 11 hours on CWM-MZ compared to purified collagen (14 hours) and uncoated surface (17.3 hours).

CELL MIGRATION USING WOUND SCRATCH ASSAY

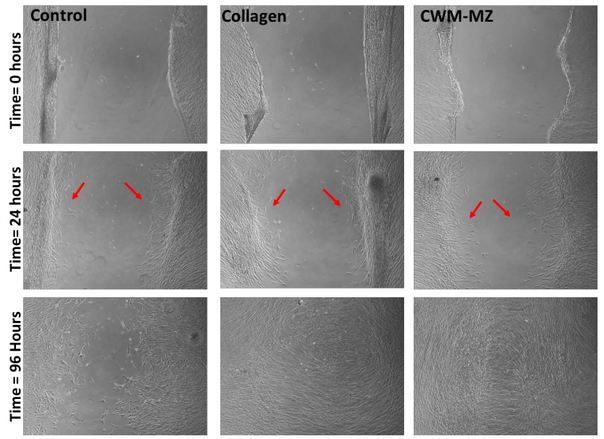
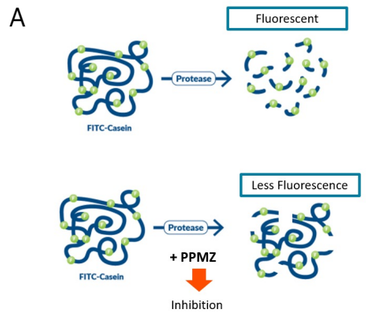
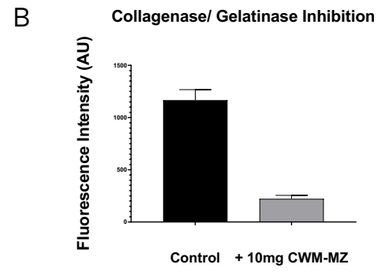
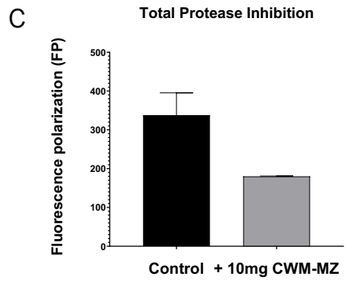


Figure 3. Representative images of primary fibroblasts cultured with on glass surfaces coated with CWM-MZ, purified collagen, or untreated. Red arrows indicate margins of wound scratch. Cells migrated at a higher capacity when cultured on CWM-MZ compared to collagen and control groups.

EVALUATION OF TOTAL PROTEASES AND MMP ACTIVITY







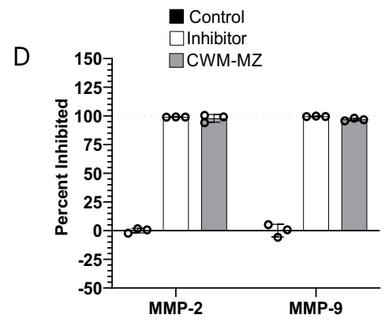


Figure 4. (A) Protease fluorescent detection assays were used to examine the inhibition of protease activity by CWM-MZ. Evaluation of (B) collagenase/gelatinase and (C) total protease activity using CWM-MZ extract, and (D) MMP-2 and MMP-9 inhibition using a solid-state assay. Protease inhibition assays showed CWM-MZ of reduced total protease activity by 52% and collagenase/gelatinase activity by 82%. Additionally, CWM-MZ inhibited the activity of matrix metalloproteinases (MMPs)-1, -2, -8, and –9.

CONCLUSIONS

CWM-MZ is comprised of collagen that retains the complex architecture and composition of native tissue. *In vitro*, we found CWM-MZ supported enhanced fibroblast attachment, proliferation, and migration. CWM-MZ resulted in robust inhibition of proteases including total proteases and collagenase/gelatinases. Additionally, it inhibits the activity of MMPs, which are often dysregulated in chronic wounds.

In summary, these findings highlight CWM-MZ properties and how they are relevant to common wound management processes.

ORGANOGENESIS