Impact of a Micronized Collagen Wound Matrix on Protease Inhibition and Fibroblast Responses *In Vitro*

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INTRODUCTION

Wound healing is an intricate biological process partially regulated by proteases, including matrix metalloproteinases (MMPs) and serine proteases. Dysregulation of this process results in the disruption of the balance in ECM degradation and remodeling, impeding normal wound healing. In this study, we investigated the interaction of Collagen Wound Matrix-Micronized (CWM-MZ*) on common proteases typically found in the wound environment. We also developed an *in vitro* simulated wound fluid (SWF) model to characterize the impact of CWM-MZ on fibroblasts stressed by SWF. We evaluated the ability of CWM-MZ to support normal fibroblast activity, including maintenance of cell viability and normal angiogenic responses to fibroblast eluates.

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METHODS

- To assess the impact of CWM-MZ, we evaluated the inhibitory effect of CWM-MZ on total protease, collagenase, elastase, and gelatinase activity using gelatin or casein-fluorescein-labeled substrate assays.
- An *in vitro* model was developed using transwell inserts with SWF containing a protein-rich salt solution in culture with fibroblasts.
- Using this model, Collagenase II (50 or 25 CDU/mL) was evaluated with and without CWM-MZ to assess its impact on cell viability using CellTiter-Glo® after 4 hours of culture at 5% CO_2 and 37°C.
- For tube formation assays, human umbilical vein endothelial (HUVEC) cells were seeded on basement-membrane extract and treated with fibroblast eluate derived either from cells cultured in SWF alone or SWF + CWM-MZ for 12 hours. Subsequently, HUVECs were stained with Calcein AM and imaged.

IN VITRO SWF MODEL FOR CWM-MZ IMPACT ON FIBROBLASTS

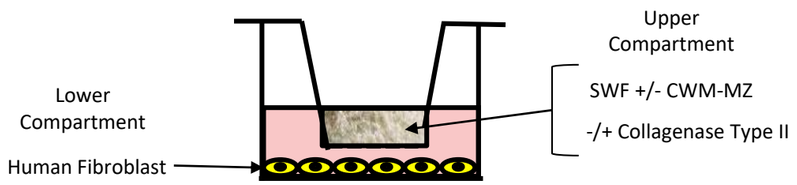


Figure 1: We developed an *in vitro* SWF model to assess CWM-MZ's impact on stressed fibroblasts. The model, using transwell chambers and permeable membranes, explored the wound fluid environmental effects on fibroblasts and relevant cells.

INHIBITION OF COLLAGENASE WITH CWM-MZ PRESERVES CELL VIABILITY

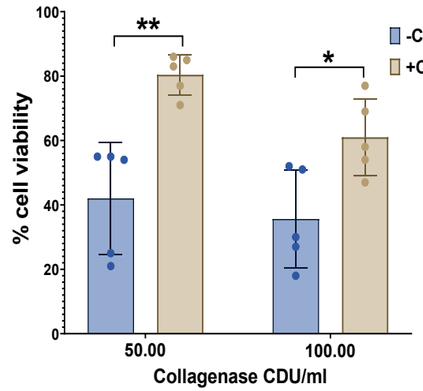


Figure 2: In the *in vitro* SWF model, the addition of CWM-MZ resulted in maintaining cell viability, with 80.4% viable cells in the 50 CDU/ml group compared to 42% viability in the group without CWM-MZ. Similarly, in the 100 CDU/ml group, cell viability was maintained at 61% in the CWM-MZ group versus 35% without. We hypothesize the result to be related to the reduction of protease activity.

IN VITRO ANGIOGENESIS ASSAY

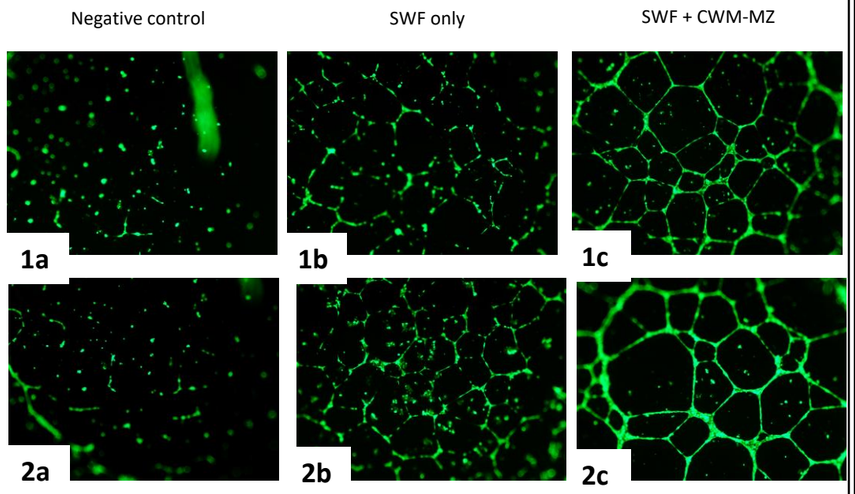
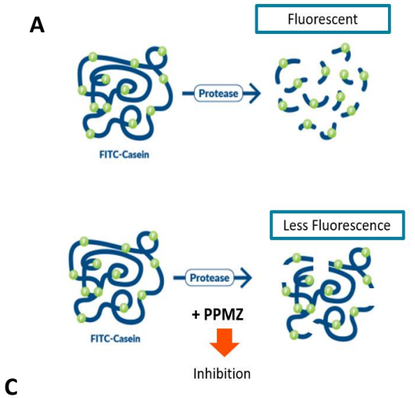


Figure 3: Visual assessment of tube formation was conducted using gelled basement membrane extract (Cultrex) and HUVEC cells cultured with media conditioned with SWF only VS. SWF + CWM-MZ. HUVEC cells, stained green with Calcein AM, were observed 12 hours post-incubation with fibroblast eluates at 5X magnification. When CWM-MZ was added to SWF (c), fibroblast eluates resulted in robust tube and branch formation of HUVECs compared to eluates from fibroblasts cultured with SWF alone (b).

PROTEASE REDUCTION WITH CWM-MZ



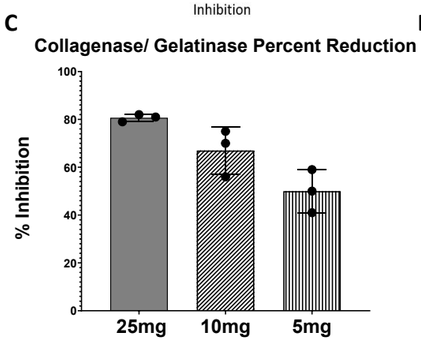


Figure 4. (A) Protease fluorescent detection assays with FITC-Casein were employed to assess the inhibitory effect of CWM-MZ on protease activity. **(B)** Dose-dependent reduction of protease activity by CWM-MZ was demonstrated after 30 minutes of incubation at concentrations of 25, 10, and 5 mg/mL. Specifically, at these concentrations, CWM-MZ reduced total protease activity by 66%, 45%, and 24%, respectively. **(C)** Collagenase/gelatinase activity showed dose-dependent reduction after 30 minutes of incubation at CWM-MZ concentrations of 25, 10, and 5 mg/mL of 80%, 67%, and 50%, respectively. **(D)** Elastase activity exhibited dose-dependent reduction with CWM-MZ at concentrations of 25, 10, and 5 mg/mL of 80%, 70%, and 43%, respectively.

CONCLUSIONS

CWM-MZ is comprised of collagen that retains the complex architecture and composition of native tissue. This study demonstrated CWM-MZ's dose-dependent reduction of proteases commonly found in chronic wounds. Additionally, fibroblast exposure to CWM-MZ in the context of SWF resulted in improvements to the environment. In summary, these findings highlight key properties of CWM-MZ and how they are relevant to wound management processes.