Human Keratin Matrices Suppress Matrix Metalloproteinase Activity *In Vitro* via Zn^{2+} Binding

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INTRODUCTION

Enzyme activity, such as that of matrix metalloproteinases (MMPs) is an important part of wound healing[1]. However, elevated protease activity (EPA) has been attributed to wound chronicity in multiple wound types[2,3]. The ability to modulate enzyme activity is therefore desirable in the treatment of chronic wounds.

Control of protease activity is also important in the use of skin substitute products, which are often made of the same extracellular matrix proteins these enzymes can degrade. Keratin, however, is resistant to degradation by many common enzymes[4], including MMPs, and may even suppress MMP activity[5].

In this work, we investigated the ability of a human keratin matrix (HKM) to suppress MMP activity. We then probed the mechanism by which HKM may reduce EPA in a chronic wound environment. Finally, we investigated the effect of HKM on ECM deposition by fibroblasts *in vitro*.

Human Keratin Matrix (HKM)

METHODS

Protease Concentration and Activity

Samples of 1 cm^2 HKM were incubated for 1 hour with MMP-1 and MMP-9. Supernatants were assayed for enzyme activity and total protein levels.

Zinc Chelation

Samples were incubated in 8 µM $ZnCl_2$ solutions for one hour, and supernatants assayed with a Zn^{2+} detection kit.

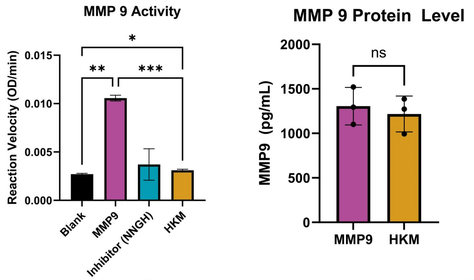
Collagen Deposition

Human dermal fibroblasts (HDF's) were grown for 14 days on HKM-coated or control surfaces, then stained with aniline blue to visualize collagen.

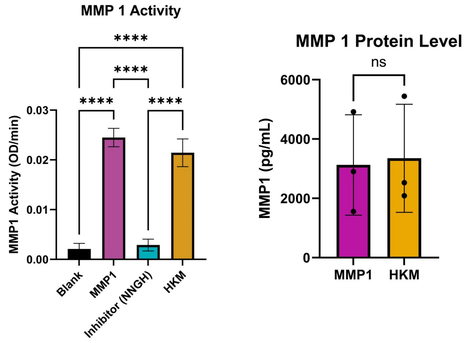
SIGNIFICANCE

Elevated protease activity, a hallmark of chronic wounds, can prematurely damage or degrade protein-based advanced wound care products. Understanding how different materials interact with the wound environment can help providers select the appropriate wound care solutions for their patients. This can improve outcomes, reduce treatment time, and control wound care costs.

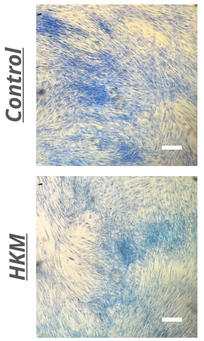
RESULTS



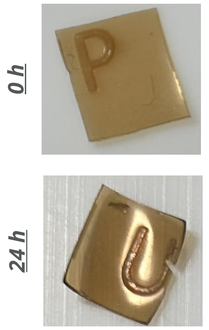
*Incubation of MMP-9 with HKM reduces the activity of MMP-9 without reducing enzyme protein levels. *p<0.05, **p<0.01, ***p<0.001 by Brown-Forsyth ANOVA with Welch's post test (activity) and Welch's t-test (protein). Activity data presented previously[6] , added here for context.*



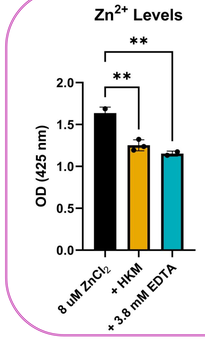
*Incubation of MMP-1 with HKM did not reduce enzyme activity or protein levels. ****p<0.0001 by Kruskal Wallis ANOVA with Dunn's post test (activity) and Welch's t-test (protein).*



Aniline blue staining of HDFs. Scale bar = 200 µm.



Samples before (top) and after (bottom) incubation in MMP-1 for 24 hours. Results for MMP-9 were comparable.



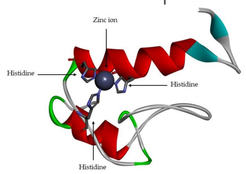
*Incubation of a $ZnCl_2$ solution with HKM showed significant reduction in Zn^{2+} ions. Chelation of zinc ions in solution by HKM was not statistically different from EDTA. *p<0.05, **p<0.01, ***p<0.001 by one-way ANOVA with Tukey's post-test.*

DISCUSSION

In this work, we studied if HKM could reduce enzymatic activity as a potential benefit to wound healing. HKM reduced MMP-9 activity, but not MMP-1 activity as previously reported with synthetic keratin peptides[5].

This reduction in MMP-9 activity came without any significant change in supernatant enzyme levels. Some skin substitute products are thought to provide a "sacrificial" protein source for enzymes, preferentially removing them from the wound environment. The present results do not reflect this for HKM.

MMP activation occurs by Zn^{2+} binding to thiol-containing histidine amino acids. Because keratin contains thiol-rich cysteine residues, we assayed for Zn^{2+} chelation by HKM and found reduced Zn^{2+} ion levels in solution, suggesting how it may reduce MMP-9 activity.



MMP bound to Zn^{2+}. Reprinted from[7] under Creative Commons CC BY 4.0

MMPs are important for wound healing, though EPA is detrimental. Future work may investigate MMP levels in wounds treated with HKM to further understand the role of this zinc-chelation property in wound healing.

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