Human Keratin Hydrogel Matrices are Degradation Resistant and Maintain Efficacy in Chronic Wound Models

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INTRODUCTION

When inflammation becomes dysregulated in the wound healing process, excess proteases can accumulate that may slow wound healing[1], and significant research has been conducted to characterize the levels of human neutrophil elastase (HNE) and matrix metalloproteases (MMPs) that contribute to wound chronicity[2].

Many biomaterial advanced wound care products are based in extracellular matrix proteins and are susceptible to protease degradation[3]. Some such products are reapplied weekly or even more frequently to ensure they maintain efficacy.

Keratin has been shown to be both resistant to enzyme degradation[4] and suppressive to enzyme activity[5]. In this work, we tested if the previously demonstrated wound healing efficacy a human keratin hydrogel matrix (HKHM) if applied for multiple weeks at a time. Additionally, we investigated the ability of HKHM to reduce the activity of HNE and MMP-9 to better understand its role in wound healing.

METHODS

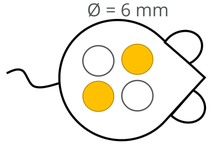
In Vitro

HKHM

13.6 U/mL MMP9 OR 23.6 mU/mL HNE 37°C, 1 hr

Enzyme Activity Assay

In Vivo



Control

HKHM

Debride, measure, and reapply HKHM or control product to wounds

Weekly

OR

Bi-weekly

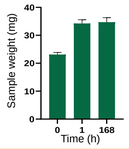
BKS.Cg-Dock7m +/+ Leprdb/J mice

SIGNIFICANCE

Skin substitute advanced wound care products are often reapplied at weekly intervals. If such a product can maintain efficacy for longer, fewer applications would be required to achieve the same healing outcomes. Given the high cost of wound care, this could have a significant impact not only on patient quality of life but also their healthcare costs.

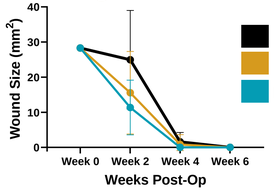
RESULTS

Simulated Wound Fluid

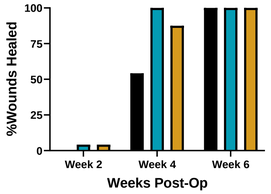


The human keratin hydrogel matrix (HKHM) does not degrade in an artificial wound fluid[6] that mimics the biochemical composition of wound exudate. Increase in sample weight may suggest absorption or adsorption of wound fluid components

a) Average Wound Size



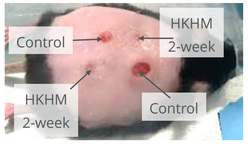
b) % of Wounds Healed



c)

Two-Way ANOVA: Wound Size	
Factor	**p-value**
Treatment	<0.0001
Time	0.004
Interaction	<0.0001

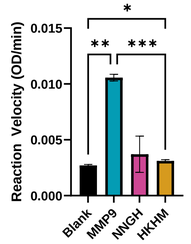
d)



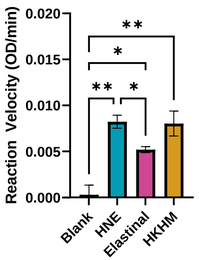
Representative Week 4 Post-op Mouse

Results of the *in vivo* treatment of db/db mouse wounds with standard of care, HKHM reapplied weekly, and HKHM reapplied every two weeks. a) Average wound size measured at two-week intervals. b) Percentage of wounds healed at each wound measurement interval. c) Two-way ANOVA results showing the effect of each treatment on average wound size. d) Representative photo of a mouse at 4 weeks after wounding, when 100% of biweekly HKHM-treated wounds were healed.

MMP-9



HNE



HKHM suppressed the activity of MMP-9 in vitro, to the level of the MMP inhibitor NNGH. HNE activity was not significantly changed. *p<0.05, **p<0.01, ***p<0.001 for n=3 samples by Brown-Forsyth ANOVA with Dunnett's multiple comparisons.

DISCUSSION

Many skin substitute products are hypothesized to work by interaction with wound enzymes. Here, we show that HKHM can suppress MMP-9 activity *in vitro*, but has little efficacy against elastase. The role of neutrophils and their products in wound healing is debated, with both excessive and reduced levels delaying wound healing[7]. Future study is needed to clarify how keratin and neutrophils interact in wound healing.

We also demonstrate that leaving HKHM in place on wounds for up to two weeks does not impact its healing efficacy in a chronic wound model. This is likely due to the physical nature of HKHM's proposed action in the wound, relying on contact with wound bed cells rather than releasing materials or degrading quickly into the wound bed.

HKHM

Suppresses MMP Activity

Persists in the Wound Environment

Maintains Efficacy for At Least 2 Weeks

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