# Human Keratin Matrices Modulate Inflammatory Crosstalk Between Keratinocytes and Macrophages

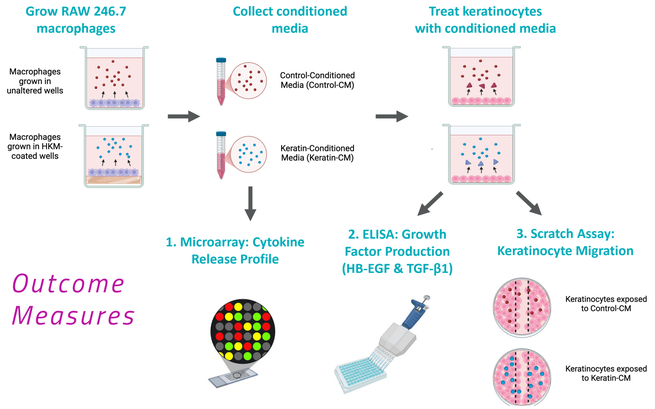
Sidhartha Malla; Allison N. Ramey-Ward, PhD; Shakesia Smith, BS; Thomas H. Barrows, PhD
ProgenaCare Global, LLC
Marietta, GA, USA 30067

## INTRODUCTION

Human keratin matrices (HKMs) have shown great promise as a novel wound care product, with recent studies demonstrating accelerated chronic wound closure with keratin[1,2]. *In vitro* work suggests this is due to the ability of keratin to modulate macrophage polarization [3], to a healing phenotype. However, the inflammatory environment of the wound is quite complex, dependent on crosstalk between immune cells and other cells. In this work, we studied how keratin-driven changes in macrophage biology influence healing downstream through intercellular communication to promote epidermal keratinocyte activation.


***Human Keratin Matrix (HKM)***

## METHODS

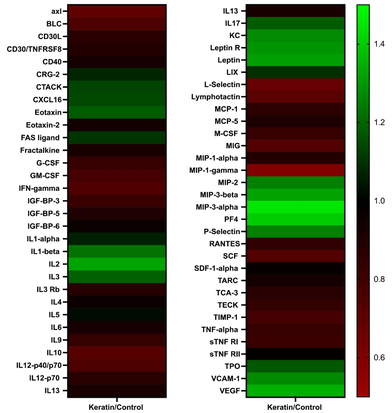



*Outcome Measures*

## SIGNIFICANCE

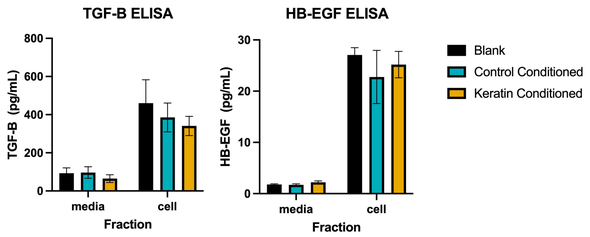
The response of inflammatory cells, such as macrophages, in wounds is a critical component of healing as well as non-healing outcomes. This research shows how human keratin matrices (HKM) may influence the inflammatory response in wounds, and how that then affects wound healing.

## RESULTS



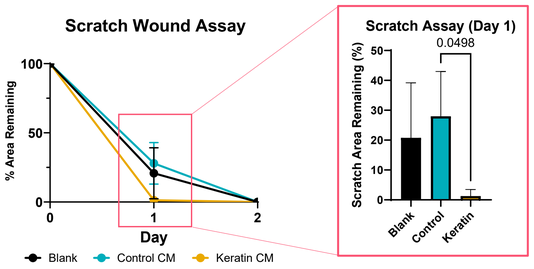

**1. Microarray: Cytokine Release Profile**

*Conditioned media (CM) from macrophages grown on tissue culture plastic (control) or HKM (keratin) was analyzed for cytokine expression. Heatmap shows keratin/control expression values for each cytokine.*




**2. ELISA: Growth Factor Production (HB-EGF & TGF-β1)**

*Cell and media fractions were collected from epidermal keratinocytes treated with control or keratin CM and assayed for key growth factors in wound healing. Intracellular expression was higher than released protein, and there were trends of downregulation of TGF-β1 with keratin CM. Intracellular HB-EGF showed a trend of upregulation when exposed to keratin CM compared to control.*

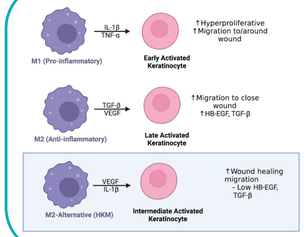



**3. Scratch Assay: Keratinocyte Migration**

In vitro *scratch wound fill time was assessed in epidermal keratinocytes treated with control or keratin CM. Though all scratches closed quickly, keratinocytes in CM from keratin-exposed macrophages showed significantly smaller scratches after 24 hours. By Kruskal-Wallis test with Dunn's multiple comparisons of n=3 culture wells per group.*

## DISCUSSION

The results from this study suggest HKM polarized macrophages to an M2-alternative state, aligning with past literature [3]. Differential expression of both inflammatory and anti-inflammatory cytokines in CM from HKM-grown macrophages (e.g. the downregulation of both TNFα and IL-10) did not follow traditional expression patterns of M2 anti-inflammatory macrophages, but instead indicate a transitional phase requiring additional study to confirm. The increased migration of keratinocytes exposed to HKM-grown macrophage CM without upregulation of classical biochemical markers of activation (TGF-β and HB-EGF) suggest an early-activated keratinocyte phenotype, and further study at longer timepoints may be warranted. This data demonstrates HKM-mediated immunomodulation resulting in differential keratinocyte activity, further supporting the benefit of HKM in wound healing.




*Proposed "M2-alternative" macrophage activation in response to exposure to HKM. Future research could more closely investigate the immune phenotypes present in wounds treated with HKM.*

## REFERENCES

1. Armstrong DA, et al. (2024). *Int Wound J;* 21(9): e70029.
2. Ramey-Ward AN, et al. (2024). *Wound Rep Regen;* 32(3): 257-67.
3. Fearing BV & Van Dyke ME. (2014). *Acta Biomater*; 10(7): 3136-44.