A Human Keratin Hydrogel Matrix Modulates Cytokine Production in Keratinocytes and Fibroblasts

Allison N. Ramey-Ward PhD, Kesia Smith, Thomas H. Barrows PhD
ProgenaCare Global, LLC. Marietta, GA, USA 30067

Inflammation in Wound Healing

When the skin is wounded, keratinocytes become activated to produce growth factors, cytokines, and keratins[1]. These signaling factors trigger other cells - immune cells, fibroblasts, and neighboring keratinocytes - to become activated in the wound area and promote healing[1]. Dysregulation specifically in the immune responses involved in healing is thought to contribute to wound chronicity[2-3], and is attributed to comorbidities such as diabetes and peripheral artery disease[4].

Recently, a novel human keratin hydrogel matrix (HKHM) has been shown to promote healing of chronic wounds. Here, we investigate how the HKHM affects the production of growth factors and cytokines in epidermal keratinocytes and dermal fibroblasts.

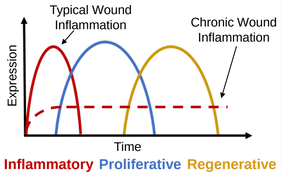
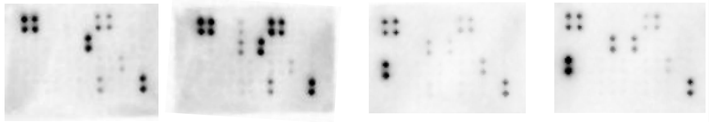


Figure adapted from Berger, Chou, and Hammond (2021)[5]

Results

Epidermal Keratinocyte | Dermal Fibroblast

Control | HKHM | Control | HKHM



Chemiluminescent images of human cytokine microarray show clear differences in expression patterns of many factors between cell types, as expected, and as a result of exposure to HKHM-coated surfaces.

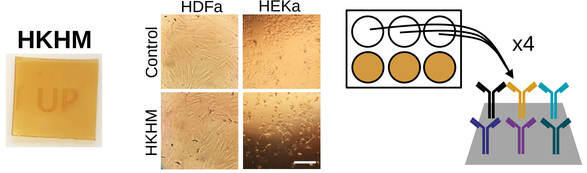
Clinical Significance

Some conditions, such as diabetes and vascular insufficiencies, dysregulate the healing process. This is due in part to a chronic inflammatory response in these wounds instead of a transition from acute inflammation to regenerative processes[5]. Human keratin products assist in the closure of such wounds, but our results suggest they are not broadly anti-inflammatory. Instead, **HKHMs upregulated acute, not chronic, inflammation,** a novel insight into the HKHM mechanism of action. We also observed **significant upregulation of some pro-angiogenic factors**. Improved vascularization in chronic wounds have been reported anecdotally with human keratin wound care products, and further investigation is needed to fully understand this effect.

Chronic wounds are complex environments not fully modeled by the lone responses of keratinocytes and fibroblasts here. Future work may study the interaction between wound cell types, or the effect of HKHMs on immune cell recruitment and polarization.

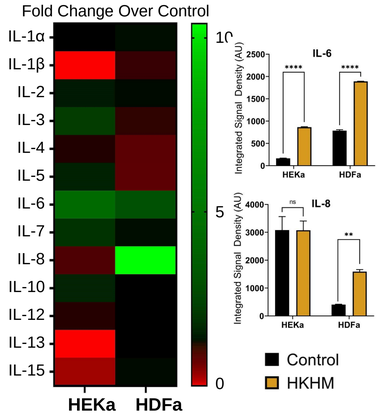
Methods

Adult human epidermal keratinocytes (HEKa) or adult human dermal fibroblasts (HDFa) were cultured on surfaces coated with HKHM for 5 days. Media collected on day 5 was analyzed by a human cytokine antibody microarray (Ray Biotech). Data represent the combination of n=3 bioreplicates.



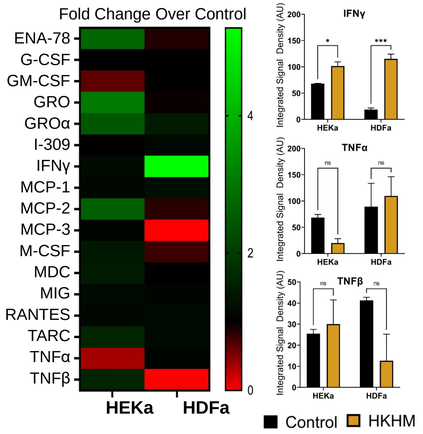
Left: Example of an HKHM product. Middle: Representative brightfield images of cell attachment in various culture conditions after 24 hours. Scale bar = 500 µm; contrast adjusted for visualization. Right: Schematic diagram of experiment.

Interleukins (ILs)



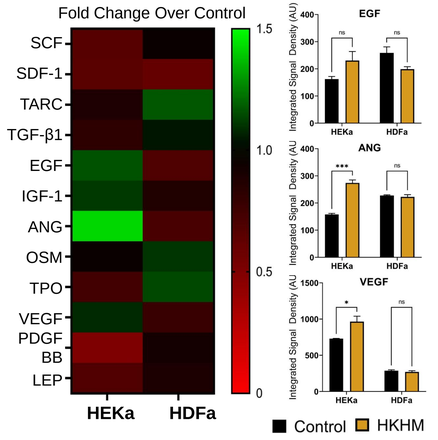
Cell expression of IL-6, associated with inflammatory regulation in wounds[6], was upregulated. Pro-angiogenic IL-8[7], also linked to keratinocyte migration[8], was also upregulated. Pro-inflammatory IL-1 and IL15 were downregulated. **p<0.01, ****p<0.0001 by 2-way ANOVA with Tukey's post-test.

Inflammatory Factors



HKHM induced both pro- and anti-inflammatory expression changes in cells, upregulating Interferon γ (IFNγ) while downregulating Tumor Necrosis Factors (TNFα, TNFβ) indifferent cell types. *p<0.5, ***p<0.001 by 2-way ANOVA with Tukey's post-test.

Other Factors



HKHM did not broadly upregulate growth factor expression. Further evidence was seen of an angiogenic effect through upregulation of Angiogenin (ANG) and Vascular Endothelial Growth Factor (VEGF). *p<0.5, ***p<0.001 by 2-way ANOVA with Tukey's post-test.

References

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a.ramey-ward@progenacare.com
linkedin.com/in/allison-ramey

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