Human Keratin Matrices Support Intercellular Communication In Vitro

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INTRODUCTION

Wound healing involves complex and highly regulated signaling between various cell types in the wound area. Among these are growth factors, such as epidermal growth factor (EGF) and transforming growth factor beta (TGFβ). Growth factors can promote cell activation and replication through mitogen activated protein kinases (MAPKs)1, and the p38-MAPK pathway has been implicated in wound healing2. Recent work has shown that human keratin matrices (HKMs) cause wound bed cells such as epidermal keratinocytes and dermal fibroblasts to vary their expression of signaling molecules, including EGF and TGFβ3. However, a true wound environment sees these cell types communicating with each other via these and other paracrine factors. Because HKM treatment has been reported to support healing in chronic wounds4, we investigated various conditions of human epidermal keratinocyte (HEK) and human dermal fibroblast (HDF) coculture, as well as the subsequent kinase activity due to growth factor signaling.

METHODS

3 Days

Direct Coculture

Indirect Coculture

3 Days

Indirect Coculture

ELISA

Supernatant

Cell Lysate

RESULTS

HKM Increases Intracellular HB-EGF in Direct Epidermal-Dermal Coculture

Direct coculture of HEKs and HDFs on HKM resulted in a significant increase in intracellular heparin-binding EGF (HB-EGF) expression in these cells. However, EGF expression was not changed. *p<0.01 by two-way ANOVA with Sidal’s multiple comparisons.

HKM Increases TGFβ1 Expression in Indirect Epidermal-Dermal Coculture

EGF expression was also not changed with indirect coculture of HEKs and HDFs on HKM. TGFβ1 release tended to increase with HKM contact, and intracellular TGFβ1 was significantly increased. *p<0.05 by two-way ANOVA with Sidal’s multiple comparisons.

HKM Increases p38-MAPK Signaling in Coculture

Cells grown on HKM did not upregulate p38-MAPK signaling in monoculture; indirect cocultures with fibroblasts contacting HKM showed an increase in p38-MAPK phosphorylation. **p<0.01 by Welch’s ANOVA with Dunnett’s multiple comparisons.

SIGNIFICANCE

Recent clinical results have shown treatment with HKM supports healing of chronic wounds, including diabetic lower extremity ulcers, even when the wound did not respond to previous treatment with other advanced biomaterial products. This work highlights unique pathways involved in HKM signaling that give insight into the varying responses of wounds to wound care products.

DISCUSSION

Contact with HKM had a differential effect on intracellular growth factor signaling, particularly relating to dermal fibroblasts. Of note, intracellular HB-EGF and TGFβ1 were upregulated due to contact with HKM, and release of factors was not significantly affected, as shown previously5. Differential growth factor expression in cocultures was reflected in intracellular signaling, as p38-MAPK activation was upregulated in coculture models where fibroblasts directly contacted HKM. This fibroblast-centric response reflects recent clinical data showing increased granulation tissue formation in chronic wounds treated with HKMs6. TGFβ1 is an important factor in wound inflammation, granulation, angiogenesis, and re-epithelialization, as well as fibroblast activation7. Our data suggests the primary benefit of human keratin in chronic wounds may be related to the fibroblast response. The coculture model in this work is limited, as the wound milieu is highly complex and dynamic. Though more study is needed, this is the first evidence of differential cellular signaling with exposure to HKM.

REFERENCES

4. Ellis S et al. ePlasty. 2023; In press.
5. Images created with BioRender.com