Designing an Attachment Assay for Keratinocytes Stimulated by Fibroblast Conditioned Media

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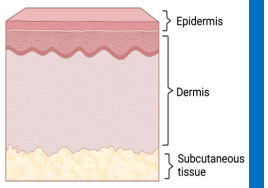
Scan to Connect

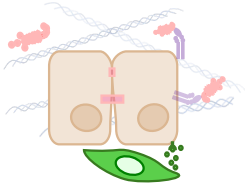
The Epidermis and Keratinocytes

The wound healing process in cutaneous injury and skin grafting is regulated by the back-and-forth communication between different layers of the skin.

The outermost layer of the skin is the epidermis, which acts as a protective layer and composed mostly of keratinocytes.

Underneath this layer is fibroblasts that reside in the dermis, providing structure and support to the skin.

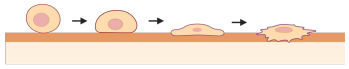




Several studies found that keratinocytes communicate with fibroblasts in a bilateral paracrine model, involving cytokines growth factors such as FGF, KGF, IL-6.

Cell Attachment

Attachment occurs when cells change their physiology or chemical structure to form anchors to the neighboring cells or to the extracellular matrix.



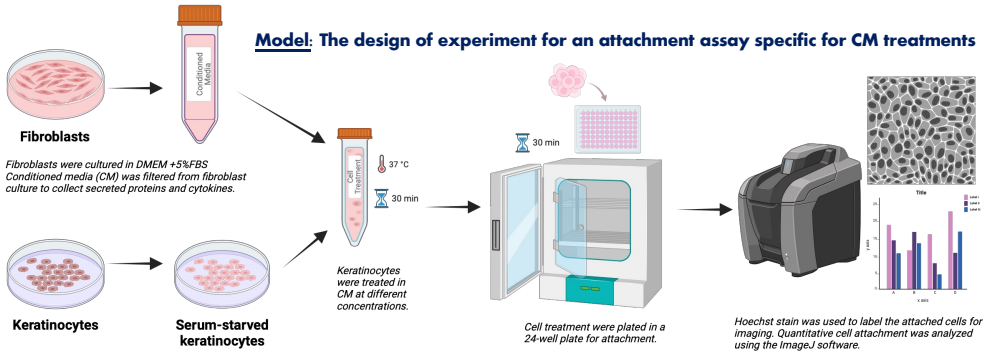
Goals

Identify research focus and optimize experimental variables

Develop a simple and reproducible methodology

Refine and finalize a developed protocol for publication

Experimental Design



Model: The design of experiment for an attachment assay specific for CM treatments

Keratinocytes were cultured in DMEM + 10%FBS. After reaching the desired confluency at ~70%, the culture were changed to serum-free media to starve cells and prepare for treatment.

Table 1. Cell treatments at different CM concentrations

CM 1%	CM 1%	CM 1%	FBS 1%	FBS 1%	FBS 1%
CM 5%	CM 5%	CM 5%	FBS 5%	FBS 5%	FBS 5%
CM 10%	CM 10%	CM 10%	FBS 10%	FBS 10%	FBS 10%
SF	SF	SF	SF	SF	SF

• FBS was used as positive control and SF as negative control

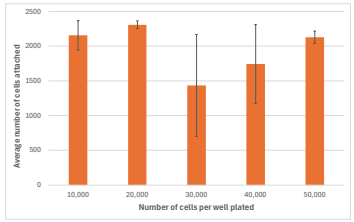


Figure 1. The number of cells is determined to be optimal at 10,000 cells/well for the assay.

• This number assured that there were enough cells to quantify and analyze attachments

CM Results in a Dose-Dependent Increase in Cell Attachment

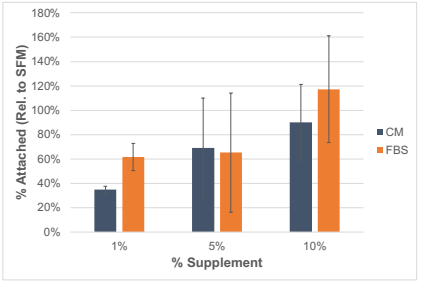


Figure 2. The percent of cell attachments. As the concentration of supplement increased, the number of attachment also followed an upward trend.

• Data were normalized to attachment observed in cells treated with serum-free media (SFM). FBS was used as positive control

CM Leads to Changes in Cell-to-Cell Adhesion and Attachment

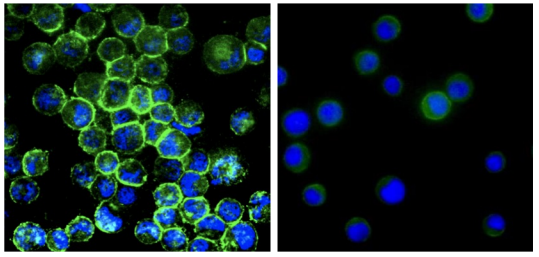
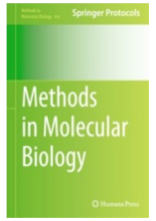


Figure 3. (Left) CM treatment. (Right) Untreated. More cell attachments were observed in CM treatments, with more clusters and cell-to-cell adhesions. There were less cells attached for untreated, cells are spaced and independent of each other.

• For immunofluorescence imaging, Hoechst stain was used to label the nucleus (blue) and 1X Phalloidin-iFluor488 was used to label actin filaments in the membrane (green)

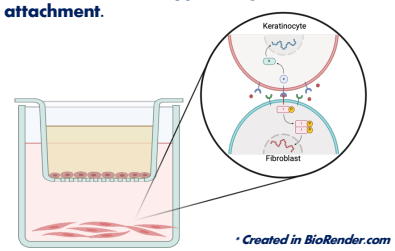
Outcomes

- Produced a low-cost, effective and versatile tool for further investigation into cell attachment.
- Proposed and tested the fibroblast-keratinocyte interactions via attachment.



Future Directions

- Examine how keratinocytes and fibroblasts regulate each other, hypothesizing that their interaction stimulates processes that drive their behaviors beyond the proteins and cytokines they individually secrete.
- Examine the effect of co-stimulated conditioned media on each cell type's migration and attachment.



• Created in BioRender.com

References

Amiri, N., Golin, A.P., Jalili, R.B., Ghahary, A., 2022. Roles of cutaneous cell-cell communication in wound healing outcome: An emphasis on keratinocyte-fibroblast crosstalk. Exp. Dermatol. 31, 475–484. https://doi.org/10.1111/exd.14516

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