# Generation of a Full Thickness Human Skin Equivalent for In Vitro Functional Studies

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## INTRODUCTION

Skin is the largest organ in the human body and serves as a critical barrier between the internal and external environment. Due to it's multi-layered structure and its cutaneous innervation which connects it to the central nervous system, the skin is also regarded as one of the most complex organs. Skin damage (i.e. burn, chronic wound, disease) can lead to permanent disability or even death without proper therapeutic intervention. Despite previous efforts, there is an outstanding need to further optimize robust human skin equivalent (HSE) models for pre-clinical research. HSEs are bio-engineered constructs that are composed of a mixture of primary human cells (i.e. dermal fibroblasts and keratinocytes) and components of the extracellular matrix. Full thickness HSE models have a number of commercial and pre-clinical applications (**Figure 1**). To further highlight this need, the tissue engineered skin substitute market is projected to be worth US $2.4 billion in 2024, and is anticipated to reach US $3.5 billion by 2034. Here, we described an optimized protocol for reproducible construction of full-thickness HSE constructs by co-culturing primary human dermal fibroblasts and primary human epidermal keratinocytes at the air-liquid-interface.

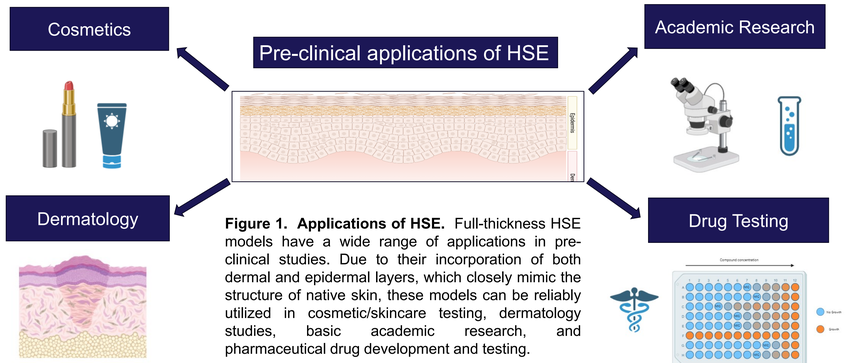


**Figure 1. Applications of HSE.** Full-thickness HSE models have a wide range of applications in pre-clinical studies. Due to their incorporation of both dermal and epidermal layers, which closely mimic the structure of native skin, these models can be reliably utilized in cosmetic/skincare testing, dermatology studies, basic academic research, and pharmaceutical drug development and testing.

## METHODS

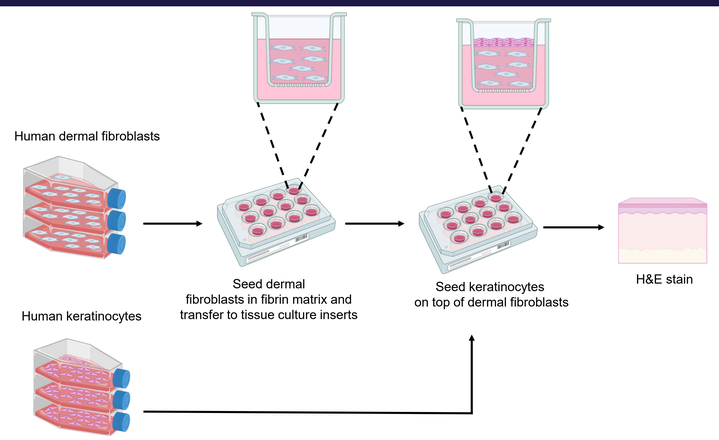


**Figure 2. Process flow for HSE production.** Primary cultures of human dermal fibroblasts and keratinocytes are initiated. Human dermal fibroblasts are then seeded within a fibrin matrix and the solution is transferred into tissue culture insert(s) within a multi well plate. Primary keratinocytes are seeded directly on top of the fibroblasts in the tissue culture inserts to establish the HSE. Cultures are then lifted to the air-liquid-interface and are fed with differentiation medium for up to two weeks. H&E staining is performed to examine histological and structural morphology of the HSE constructs.

## RESULTS

**Figure 3. Appearance of HSE constructs.** HSE constructs were generated and allowed to differentiate for a period of two weeks. After the differentiation period, the HSE constructs were fixed in methanol prior to preparation for histological analysis. Representative images show the appearance of the fixed constructs after removal from the tissue culture inserts.

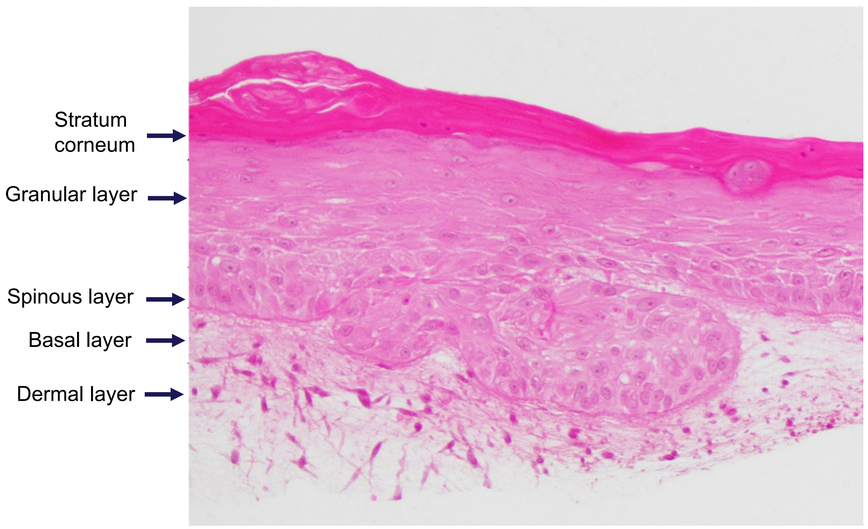


**Figure 4. Histological staining of HSE constructs.** Fully differentiated HSE constructs were fixed in methanol prior to processing for histological analysis. Hematoxylin and eosin (H&E) staining was performed and microscopic images were acquired. Representative image shows the appearance of HSE constructs after approximately 21 days in culture, displaying a well-differentiated epidermis with four distinct layers: an outmost layer of stratum corneum, a thin layer of stratum granulosum characterized by flattened keratinocytes, a stratum spinosum characterized by polyhedral-shaped keratinocytes, and an innermost basal layer containing basal cells. The basal layer sits atop the dermal layer which contains dermal fibroblasts embedded in an extracellular matrix. n=6 constructs (data only shown for 1 replicate).

## RESULTS

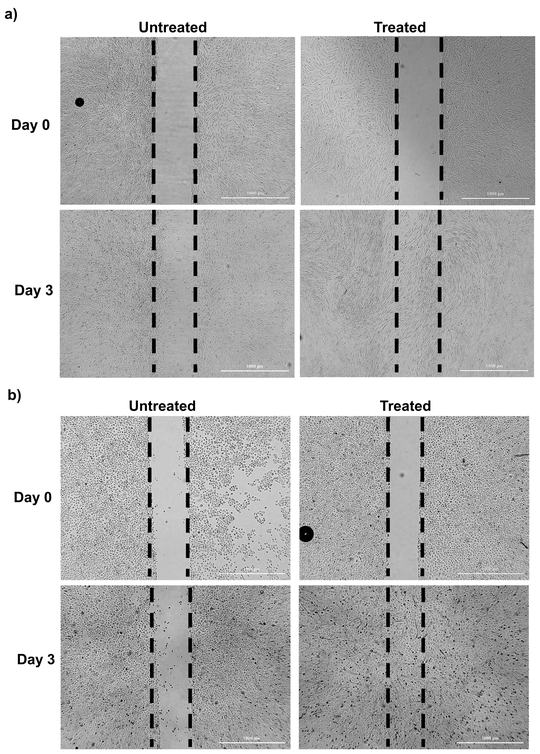


**Figure 4. In vitro cell migration assay using 2D cultures of HSE cell types.** Primary human dermal fibroblasts and primary keratinocytes were seeded into cell culture inserts and grown to confluence. Upon removal of inserts cells were treated with either basal media (untreated) or basal media + extracellular vesicles (EVs) from stem cells. Cells were imaged daily to monitor cell growth. Representative phase contrast images are shown after three days in culture. Scale bar = 1000 µM. n = 2.

## CONCLUSION

- ATCC has recently generated protocols which demonstrate the capability to reproducibly manufacture full-thickness HSE constructs from primary human dermal fibroblasts and primary human keratinocytes.
- Characterization of HSE constructs by H&E staining revealed a well-differentiated epidermis on top of a dermal layer. Our future studies will include more thorough immunostaining to evaluate expression of key markers that are expressed in native skin, including epidermal markers (keratin 10, keratin 14, fillagrin, loricrin) and dermal markers (collagen IV, collagen I, fibronectin).
- Functionality of the individual cell types used to construct HSE demonstrates that both cell types are functional in vitro. Future studies will test functionality of the fully-differentiated HSE constructs to demonstrate their relevance for more advanced assays that can be used to model skin development and/or repair in vitro.

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