3D HUMAN EPIDERMIS EQUIVALENT (HEE) MODEL AND ITS APPLICATIONS

Tricia Conti, MS; Elizabeth J. Bevels; and Nina Bionda, PhD iFyber LLC, Ithaca, NY

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iFyber's human epidermis equivalent model provides a versatile *in vitro* testing platform for a variety of applications. Interactions between the skin and topically applied substances or materials can be evaluated. The models can also be employed for the assessment of bacterial colonization and testing of antibacterial products. Additionally, both infected and non-infected wounds can be incorporated, making this model useful in the assessment of wound care products.

Three-dimensional, organotypic human skin models have emerged as important *in vitro* tools for a variety of uses, including dermatology and skin biology research, industry testing of consumer and prescription products (e.g., cosmetics and pharmaceuticals), and clinical applications such as skin grafting (1-3). These human skin equivalent (HSE) models closely mimic the structure and functionality of native skin and are thus more relevant for these applications as compared with two-dimensional cell monolayers. Moreover, HSE models provide attractive alternatives to *ex vivo* models utilizing human skin samples, which are limited in availability and suffer from lack of reproducibility due to the diversity of donors (2). HSE models also provide benefits over *in vivo* animal models, which are not always comparable to human skin and which are being abandoned, especially in the cosmetics industry, in favor of *in vitro* alternatives due to ethical and legal considerations (3). A wide variety of studies have employed HSE models, including skin irritation, corrosion, and toxicity; dermal drug delivery and drug permeation; wound healing; and bacterial infection mechanism and antimicrobial testing (1-3).

The HSE model consists of two parts: a dermal component of fibroblasts embedded in a collagen matrix and an epidermal component of differentiated keratinocytes. Described here is a simplified version of the HSE model – human epidermis equivalent (HEE) which encompasses only the epidermal layer of human skin. In this model, keratinocytes are seeded onto cell culture inserts and cultured for 2-4 days, submerged in culture medium, to allow the keratinocytes to proliferate. The constructs are then brought to the air-liquid interface, which encourages the keratinocytes to differentiate and form the stratified layers observed in native skin (Figure 1). After 7-17 days of culture at the air-liquid interface, the constructs are ready for experimentation.

The HEE model provides a good approximation of human skin not only in terms of replicating the structure, but also in mimicking the barrier function and producing cytokines and growth factors normally present in human skin. iFyber has demonstrated the use of this model for such applications, as well as in modeling bacterial infection. A few applications of the HEE model are detailed in the following sections.

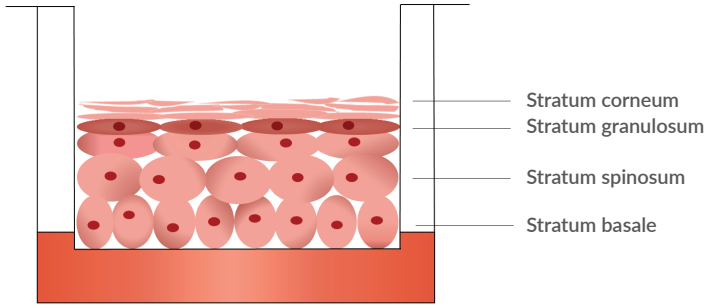

Figure 1: Diagram of the HEE model. This model consists of keratinocytes differentiated to form the stratified layers characteristic of human epidermis. (Adapted from Haisma, et al. (4))

APPLICATION: EVALUATION OF BARRIER FUNCTION

To demonstrate the barrier function, we applied Lucifer Yellow, a fluorescent dye that is not taken up by cells, to the apical side of HEEs in order to observe its ability to penetrate the epidermal layers. Following a 2-hour incubation, a sample of the medium from the basolateral side was taken and the fluorescence was measured. An empty culture insert was used as a positive control. As shown in Figure 2, the dye was not detectable for the HEE samples, indicating the presence of a functional barrier.

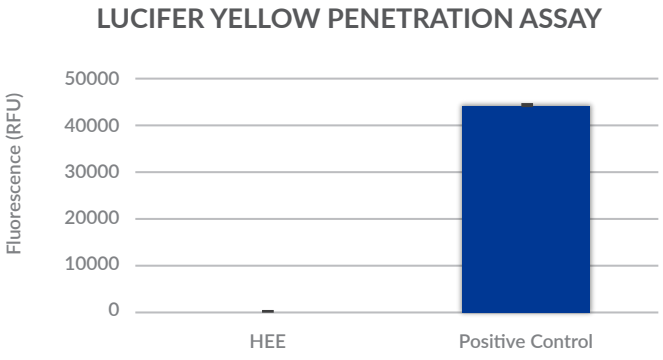

Figure 2: Lucifer yellow penetration assay performed on HEEs cultured at the air-liquid interface for 15 days.

APPLICATION: ASSESSMENT OF PROTEIN SECRETION

iFyber has analyzed the secretome of the HEEs as well as that of a monolayer of human keratinocytes (Ker-CT, ATCC CRL-4048). To this end, the Human XL Cytokine Array (R&D Systems ARY022B) was employed, following the manufacturer's protocol. The use of this array has been described in a separate white paper. Figure 3 shows the most notable signals observed in a

comparison of the secretome of HEEs cultured at the air-liquid interface for 15 days versus that of a monolayer of keratinocytes cultured for 24 hours on a tissue culture plate. Several proteins related to the growth and differentiation of keratinocytes, i.e., EGF, GDF-15, GRO-α, IGFBP-3, were detected in the HEE sample but not in the Ker-CT monolayer sample. Additionally, angiogenesis factors (angiogenin, VEGF, etc.) and proteins involved in tissue remodeling (MMP-9) and wound healing (PDGF-AA) were expressed at higher amounts in the HEE than the Ker-CT monolayer. These results demonstrate that the HEE provides a more physiologically relevant model of human skin in terms of protein secretion as compared with a monolayer of human keratinocytes.

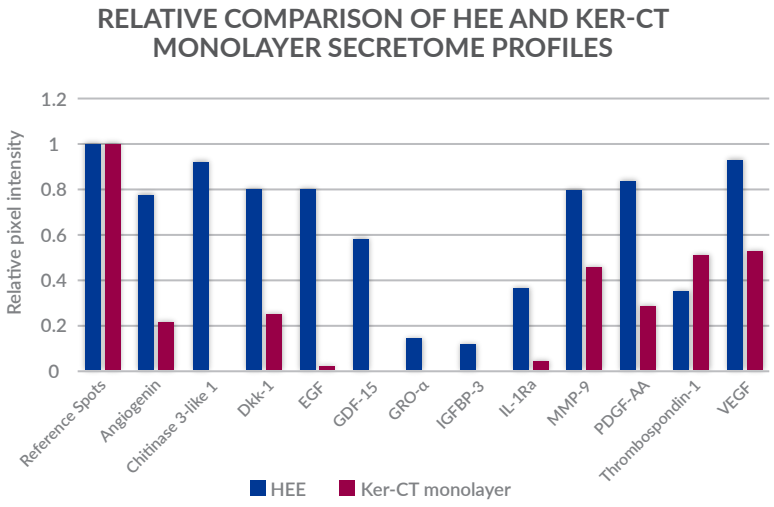

Figure 3: Comparison of cytokines and growth factors secreted by HEEs and a monolayer of human keratinocytes (Ker-CT).

APPLICATION: UTILIZING HEE MODELS TO STUDY BACTERIAL COLONIZATION

To demonstrate the capacity of these models to be used in bacterial infection and antimicrobial studies, we inoculated HEEs with *Pseudomonas aeruginosa* (ATCC 27312). HEEs cultured at the air-liquid interface for 15 days were inoculated with 5 µL of 5 x 10^8 CFU/mL *P. aeruginosa* and incubated for 24 hours under standard culture conditions. To recover bacteria attached to the HEEs, the tissues were first washed three times with PBS to remove non-adherent bacteria. Next, the membranes were cut away from the culture inserts, submerged in a buffer, and put through a series of sonication cycles. Samples from the buffer were serially diluted and plated for enumeration. The results showed that approximately 7 logs of bacteria were recovered after a 24 h incubation on HEEs (Figure 4). Tissue culture plate set up with inserts for HEE growth are shown in Figure 5.

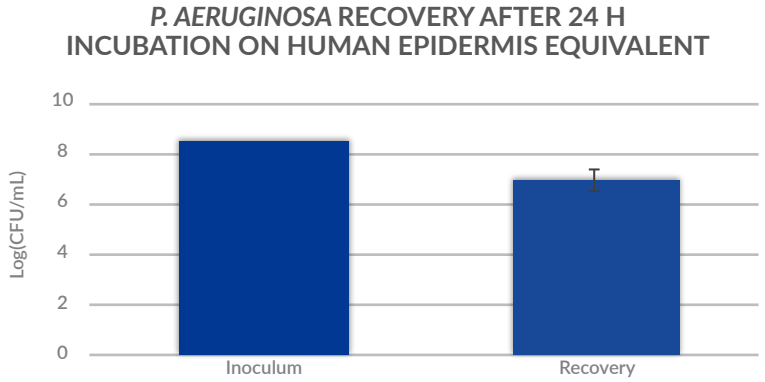

Figure 4: Survival of *Pseudomonas aeruginosa* 27312 after 24 hours incubation on HEEs.

In conclusion, iFyber has successfully implemented a Human Epidermis Equivalent (HEE) model, which mimics the structure, barrier function, and secretome of native human skin, and is more physiologically relevant than a monolayer culture of human keratinocytes. Shown here are data demonstrating the ability of HEEs to approximate native skin and the amenability of these models to a variety of biological assays. These models are a valuable tool for use in a variety of applications, including the development of wound care products, dermal drug delivery systems, and testing of topically applied products.

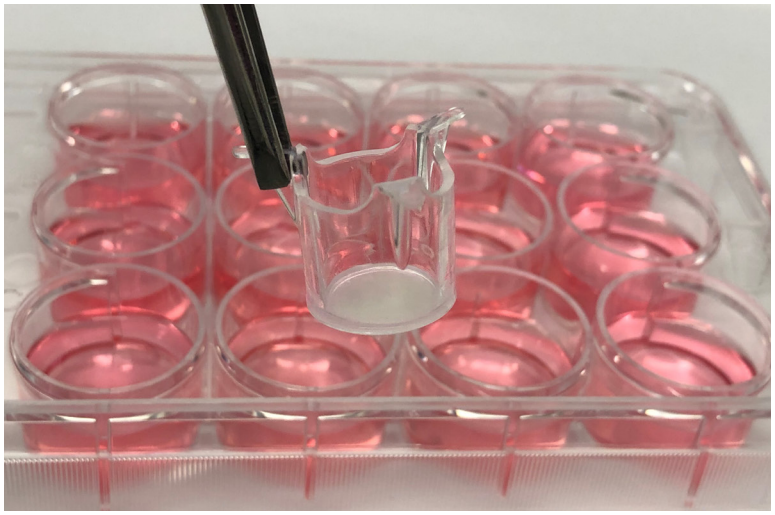

Figure 5: Tissue culture plate and insert with established HEE tissue.

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2. Ali, N., et al. British Journal of Dermatology. 2015; 173: 391-403.
3. El Ghalbzouri, A., et al. Toxicology In Vitro. 2008; 22: 1311-1320.
4. Haisma E.M., et al. Antimicrobial Agents and Chemotherapy. 2016; 60(7): 4063-4072.