A HUMAN SKIN EQUIVALENT (HSE) FOR IN VITRO PERMEATION TESTING OF FORMULATIONS

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OBJECTIVE:

- To develop a bioengineered Human Skin Equivalent as a suitable model for in vitro permeability testing.
- To evaluate the model for its efficacy for permeability testing of agents and multicomponent formulations.

INTRODUCTION:

The skin serves as both an absorptive organ and a barrier against the transport of toxic compounds. Estimation of permeation of compounds through the skin requires animal experiments or in vitro testing using animal or human skin. While animal skin does not reflect the same barrier properties as human skin, human skin poses problems of procurement, high cost and large variations in results from donor to donor. Skin equivalents, such as the Human Skin Equivalent (HSE) developed in our laboratory, are in vitro cultured skin models that can be used for testing the skin permeation of compounds. The HSE is a full thickness reconstructed skin that has been cultured in modified conditions to develop a permeability barrier closer to that of human skin in vivo. We have characterized the HSE for its morphology, lipid composition and permeability and compared it to human skin and the commercially available model Epiderm FT®.

METHODOLOGY:

- **Development of the HSE (Fig. 1)**
  - **Dermal matrix:** Dermal tissue was made by contraction of bovine Type I collagen by dermal fibroblasts.
  - **Formation of epidermis:** Neonatal Keratinocytes (~700,000 per layer) were seeded on top of the dermal layers, allowed to attach and grown submerged in media for 7 days.
  - **Formation of full thickness HSE:** The skin cultures were exposed to the ALI (Air Liquid Interface) to allow differentiation of the basal epidermal layer into a multilayered epidermis, including the stratum corneum. During culture at the ALI for 21 days, the skin was fed with modified media consisting of external fatty acids, ascorbic acid and a PPAR-α agonist.

- **Characterization**
  - **Morphology:** Sectioning, paraffin embedding and Hematoxylin and Eosin staining
  - **Lipid composition:** Thin Layer Chromatography

- **Permeability testing:** Vertical Franz Diffusion cells
  - **Skin models:** HSE (modified), Epiderm FT® and human skin
  - **Agents:** Caffeine, Malathion and DEET

- **Formulation:** A retardant cream, SERPACWA (Skin Exposure Reduction Paste Against Chemical Warfare Agents)
- **Replicates:** n = 3-8.

RESULTS:

- **Fig. 1:** Preparation of the Human Skin Equivalent (HSE)
- **Fig. 2:** A cross section of a full thickness HSE (Modified).
- **Fig. 3:** A horizontal section of the epidermis (bottom up) from the HSE (Modified) showing the flattened corneocytes forming the stratum corneum.
- **Table 1:** Lipid profiles of the HSE (modified and control) and human skin. The data is expressed as mean percent of total lipids ± SD.
  
<table>
<thead>
<tr>
<th></th>
<th>HSE Modified</th>
<th>HSE control</th>
<th>Human skin</th>
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</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>23.8 ± 2.1</td>
<td>19.5 ± 3.8</td>
<td>39.2 ± 2.1</td>
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<tr>
<td>Ceramides</td>
<td>14.1 ± 0.5</td>
<td>8.6 ± 1.4</td>
<td>12.2 ± 2.2</td>
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<tr>
<td>Cholesterol</td>
<td>25.3 ± 3.1</td>
<td>25.9 ± 3.6</td>
<td>19.4 ± 2.9</td>
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<tr>
<td>Fatty acids</td>
<td>10.1 ± 0.5</td>
<td>6.7 ± 0.6</td>
<td>8.1 ± 0.6</td>
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CONCLUSIONS:

Culture of the modified HSE with external fatty acids, ascorbic acid and the PPAR-α agonist enabled normalization of epidermal differentiation, leading to production of essential barrier lipids and a stronger permeability barrier. Permeability parameters predicted were similar to Epiderm FT® for agents tested. The HSE also effectively predicted the retardant properties of SERPACWA and overall demonstrated lower variability than human skin. With further validation, the HSE can serve as a reliable in vitro model for permeability screening of formulations.