Use of the StrataTest® Full-Thickness Human Skin Model for Irritancy and Corrosivity Testing

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Abstract

There is a substantial and growing need for accurate, reproducible in vitro test systems that are capable of replacing animal models in the evaluation and classification of chemicals. StrataTest® is a novel, full-thickness human skin model that can be used in a range of in vitro assays for consumer care products and cosmetics testing. The StrataTest® skin model contains both epidermal and dermal components and exhibits the biological characteristics of normal human skin. This model directly addresses many of the limitations of the currently-validated systems, which contain only an epidermal component. The inclusion of a dermal layer not only provides more consistent surface coverage, but enables paracrine signaling between the tissue compartments, a critical mediator of the in vivo response to chemical exposure. The model is generated using NIKS® keratinocytes, a clinically-tested and consistent source of non-tumorigenic, pathogen-free human keratinocyte progenitors. Use of NIKS® keratinocytes eliminates the problems associated with continuous cell sourcing, as required by primary cells, and improves batch-to-batch consistency. In the current study, the StrataTest® model was evaluated for acute dermal toxicity testing applications using procedures based on protocols validated for other in vitro skin models. StrataTest® tissues were packaged and stored at 4°C for either 2 or 7 days. Following storage, skin tissues were removed from packaging, returned to culture, and allowed to re-equilibrate. StrataTest® skin tissues were used to screen a panel of chemicals and classify them as corrosives or noncorrosives. Similarly, the model was also validated using a series of compounds as irritants or nonirritants. The StrataTest® model accurately characterized a range of chemicals as irritants and corrosives. Importantly, the model provided consistent results at both storage timepoints. These results demonstrate that StrataTest® skin tissues provide a consistent, reliable model for the evaluation and classification of chemical agents.

1. Terminal Differentiation of NIKS® Keratinocytes Generates StrataTest®, a Fully-Stratified, Multi-layered Human Skin Tissue

Historical analysis (hematoxylin and eosin staining) confirmed appropriate tissue architecture formation with distinct basal, spinous, granular, and cornified layers characteristic of stratified squamous epithelia. bar = 50 µm

2. Viability and Barrier Function Analysis of StrataTest® Skin Tissue Demonstrates Lot-to-Lot Consistency

To establish the quality of StrataTest® skin tissues, each lot is evaluated for appearance, tissue morphology, viability and barrier function. This table presents the trended data from nine production lots of the StrataTest® skin model for viability, as determined by MIT (A500), and cutaneous barrier function, as measured by skin surface electrical impedance using a Nova Demoderm®Fluorometer (DPM). Initial DPM values are read at time 0, and the magnitude of DPM change over a defined time period (10 seconds) correlates to the degree of barrier function. Data represents the mean ± SD.

<table>
<thead>
<tr>
<th>Test Chemical</th>
<th>CAS No.</th>
<th>EU Label</th>
<th>DPM change:</th>
<th>StrataTest®</th>
<th>Native Skin</th>
<th>Impaired Barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>A500</td>
<td>0.737 ± 0.056</td>
<td></td>
<td></td>
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3. StrataTest® Skin Tissues Accurately Classify Dermal Corrosives

To determine the ability of the StrataTest® model to correctly characterize the corrosivity potential of compounds was determined. A total of 16 chemicals were screened using established test protocols, including 10 of the 12 OECD reference chemicals as well as an additional 2 chemicals (shaded in gray) where the predictions of validated recalcitrant human epidermis (RHE) model disagreed with in vivo classifications. StrataTest® tissues, stored for either 2 or 7 days, were pre-incubated for 1 hr and test chemicals (30 mL) in 30 mL of media were applied to the surfaces of stratified tissues for either 3 or 11 days. Tissue viability was measured by MIT assay. Tissue viabilities were normalized to the negative control treatment (H2O). Chemicals were classified using the predictive model employed by several-validated RHE models, which defines corrosives as treatments resulting in tissue viability of < 50% after 3 or 9 days.

4. StrataTest® Skin Tissues Accurately Classify Dermal Irritants

To determine the ability of the StrataTest® model to correctly differentiate between irritants and nonirritants, a total of 13 chemicals, displaying a wide range of in vivo irritancy potentials, were screened using established test protocols. StrataTest® tissues, stored for either 2 or 7 days, were pre-incubated overnight and test chemicals were applied to the surfaces of stratified tissues for either 15 or 42 min. Tissues were rinsed thoroughly with PBS and incubated at 37°C for 42 hr. Tissue viability was determined by MIT assay and normalized to negative controls (H2O). Chemicals were classified using a previously-validated predictive model, defined irritants as treatments that reduce tissue viabilities to < 50% after the 42 hr incubation. StrataTest® tissues correctly characterized 12/13 tested chemicals (w.r.t. the in vivo classification). The classification of hexyl salicylate (shaded in gray) as a nonirritant disagreed with previously-studied systems.

References

2. Schurr et al., J of Trauma, 2009; 66; 866-874
3. OECD, 2004, Test Guidelines No. 431
5. Liebsch et al., Toxicol in Vito, 2000; 28:371-401
6. Kandarova et al., Toxicol in Vito, 2006; 20:547-559
8. Baskett et al., Contact Dermatitis, 2005; 51:1-4

Conclusions

- The StrataTest® human skin model displays the tissue architecture of normal human skin.
- Low lot-to-lot variability between independent batches of StrataTest® skin tissues ensures a consistent product for our customers’ research and screening applications.
- The StrataTest® human skin model is stable during storage, exhibiting a consistent response to chemicals over storage for up to 1 week.
- The StrataTest® human skin model is capable of correctly classifying chemicals as irritants or corrosives using previously-established test protocols and predictive models.
- The StrataTest® human skin model responds similarly to validated systems when substituted into the current protocols, and shows the potential for improved performance through optimization of test parameters and predictive models specific to the system.

StrataTest® Full-thickness human skin tissues are a reproducible and reliable model for toxicological screening applications.

For additional information on the StrataTest® model and its research applications, visit Booth 545