# In Vitro Epidermis Model with Impaired Barrier Function to Assess Skin Irritation Potential

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## Introduction

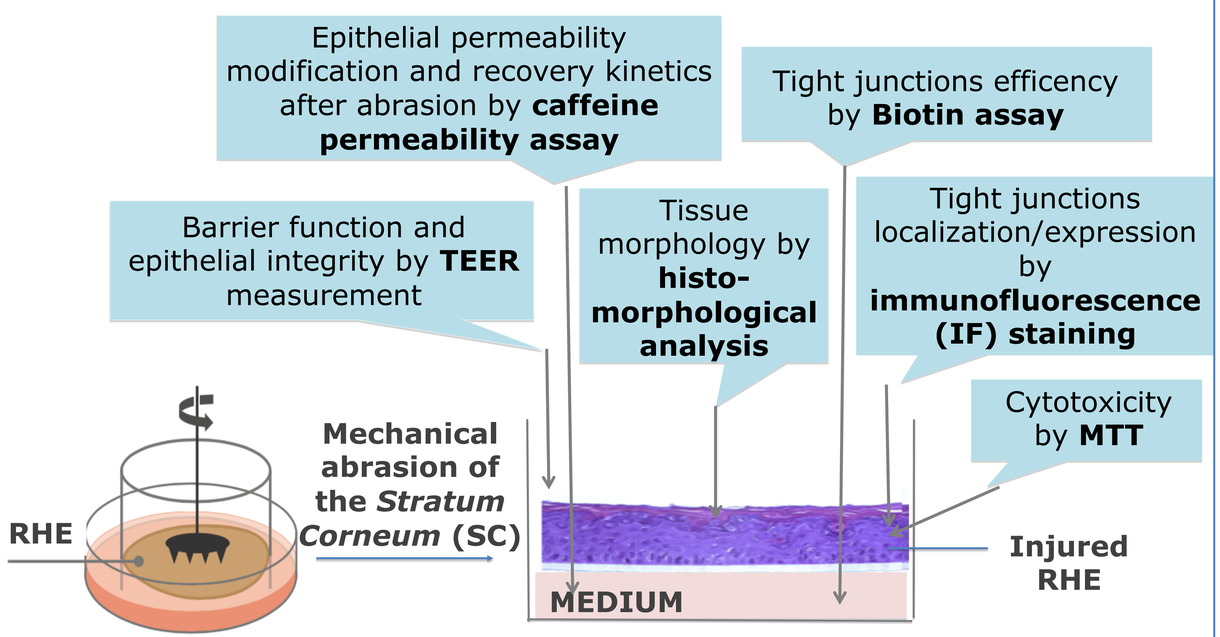
The skin is the largest organ of the body, providing a protective barrier against bacteria, chemicals and physical insults while maintaining homeostasis in the internal environment (1). The main epidermal permeability barrier is localized to the *stratum corneum (SC)* while a second line of protection is provided by the sealing effect of tight junctions (TJs) situated in the granular layer (2). Changes in lipid composition in the *SC* or injuries can lead to disturbances in the skin barrier leading to "fragile skin". Skin that is temporarily weakened by environmental aggression, local or systemic treatments or by skin disease (3-4) requires appropriate management, including the use of skin care products that allow the skin to strengthen its natural protective qualities.

## Objective

The aim of the study is to develop and characterize an *in vitro* skin model with impaired barrier function to assess the irritation potential of dermo-pharmaceutical products designed specifically for "fragile skin" (5).

## Materials & Methods

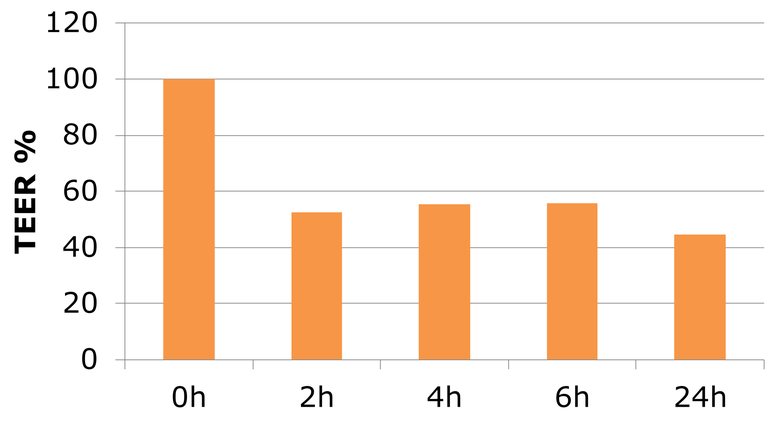
The surface of a Reconstructed Human Epidermis (RHE) model (0.5 $cm^2$, 17 days differentiation) was mechanically abraded targeting the *SC* without involving deeper epidermal structures. After injury a Multiple Endpoint Analysis (MEA) approach has been adopted to characterized the injured model in term of viability, barrier function and morphology.




MEA approach adopted to characterize a RHE model with epidermal barrier function up to 24h from abrasion.

## TRANSEPITHELIAL ELECTRICAL RESISTANCE (TEER) MEASUREMENTS

As TEER values reflects the global resistance of the barrier linked both to the TJs structure and tissue thickness, TEER measurements can be used monitor barrier function modifications. The analysis of normalized TEER values at different time-points after abrasion (from t=0 to t=24h) suggest that the mechanical injury has induced a significant reduction of TEER values (about 50%) and barrier function modification has been maintained stable up to 24h without recovery.




Normalized TEER measurements up to 24h from abrasion.

## CAFFEINE PERMEABILITY ASSAY

Caffeine penetration through the injured model has been quantified by HPLC to characterize model permeability feature after abrasion**.** Caffeine penetration kinetics has been found modified: caffeine permeation through the injured model increased of 96% after 1h and 69% after 2h compared to the Negative Control - intact RHE (**Table I**).

**Table I.** Caffeine permeation through Negative Control (intact RHE) and injured RHE up to 3h from abrasion.

| | 30 min | 1h | 2h | 3h |
|---|---|---|---|---|
| **Negative Control - Intact RHE** | 0.35% | 1.11% | 2.16% | 2.42% |
| **Injured RHE** | 0.67% | 31.94% | 7.13% | 3.58% |

## CELL VIABILITY

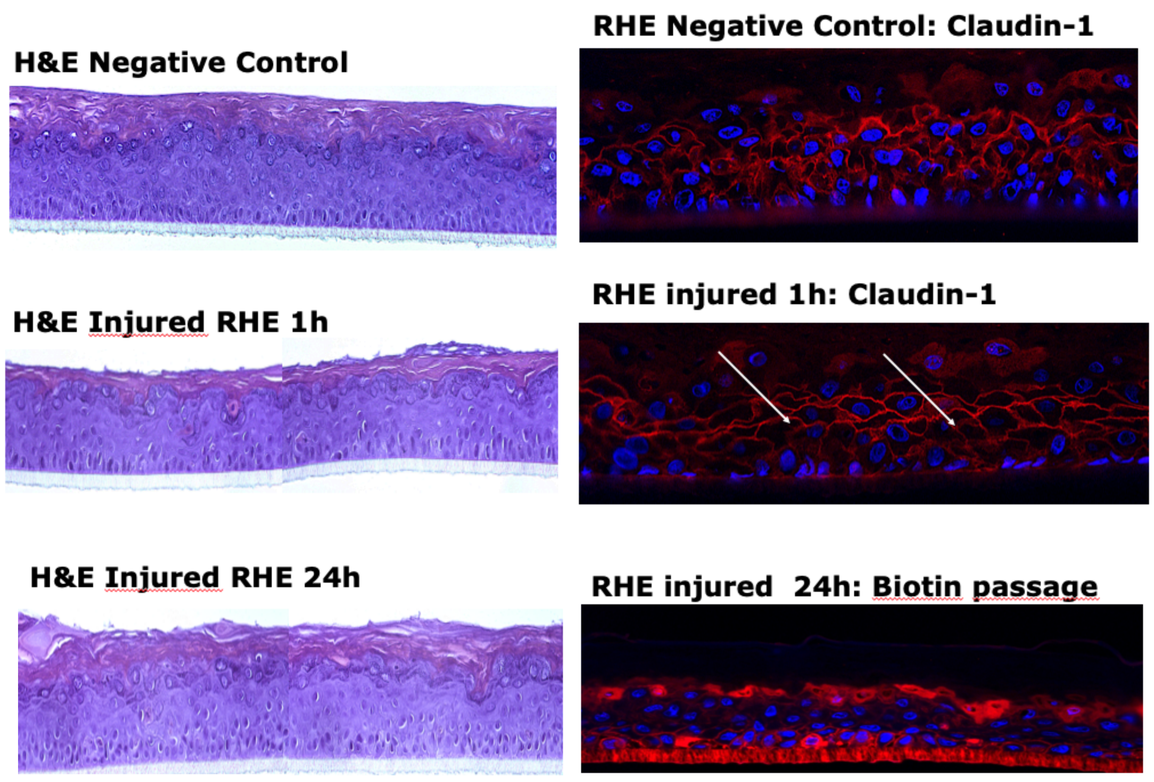
In **Table II** are reported cell viability data of intact RHE (NC) and injured RHE after 24h from abrasion. The data suggest non cytotoxic effects of the mechanical injury (cytotoxicity predicted for viability < 50%) indicating that the abrasion has targeted mainly the *SC* without involving the viable layers. Therefore, the injured RHE model is viable and remains suitable for biocompatibility studies.

**Table II.** Viability of intact RHE and injured RHE at 24h from abrasion

| | Cell viability |
|---|---|
| Negative Control - Intact RHE | 100% |
| Injured RHE | 79% |

## MORPHOLOGY and TJs MODIFICATIONS

After 1h from abrasion **H&E staining** shows a reduction of *SC* thickness that appears partially recovered at 24h. However, RHE shows a modified *SC* lamellar structure where the cohesion within the layers appears as reduced compared to the Negative Control (NC). This observation is supported by **Claudin-1 IF** images that show a significant reduction (arrows) of protein expression in the granular layer 1h after the abrasion compared to the NC.
Biotin is a relatively small (244.3 Daltons) vitamin used as tracker to evaluate the integrity of skin barrier in an inside-out permeation model. In the injured RHE after 24h from abrasion the **biotin para-cellular permeation** towards upper layers has been found increased suggesting fence properties impairment.




Morphological study on injured RHE compared to NC (intact RHE).

## Conclusion

The results have shown that a reproducible abrasion of RHE model is is possible and it is characterized by an increased permeability to caffeine during the first 3h, impairment of fence properties at TJs level, as measured by Biotin assay, Claudin-1 protein expression and TEER values while cell viability and tissue morphology have not been significantly modified.

The injured RHE model represents a sensitive and predictive tool to:

- assess skin tolerance of topically applied ingredients and products intended for "fragile skin"
- early identification of toxicity mechanisms that correlate with infra-clinical reactions.

**References**

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