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POSTER BOARD: P384

DEVELOPMENT AND VALIDATION OF A RHE MODEL WITH IMPAIRED BARRIER FUNCTION TO ASSESS CLASS IIB MEDICAL DEVICE BIOCOMPATIBILITY

S. Balzaretti, L. Ceriotti, F. Carriero and M. Meloni
VitroScreen, Milan (Italy)

INTRODUCTION

Substance based Medical Devices (MD) belong to a large heterogeneity of products intended by the manufacturer to be used, alone or in combination, for specific human medical purposes and which do not achieve their principal intended action by pharmacological, immunological or metabolic means. The EU Medical Device Regulation n. 2017/745 (MDR) indicates that the physical and functional properties of the skin barrier are discriminating factors for MD classification: MD applied to intact skin are classified as IIa while MD that come into contact with injured skin or mucous membranes are IIb. According to the new MDR "Injured skin or mucous membrane' means an area of skin or a mucous membrane presenting a pathological change or change following disease or a wound" and furthermore according to MEDDEV 2. 4/1 Rev. 9 on classification of medical devices "A skin might be considered as injured either because of pathological (e.g. diabetic ulcers) or external factors (e.g. burns)". Therefore, substance based devices applied to injured skin or mucous membrane have an increased potential to exert local toxicity and a higher potential to be absorbed or locally dispersed in the human body.

In order to comply with the new MDR an impaired barrier function seems to be a new and fundamental requirement to be addressed when conducting biocompatibility, as well as percutaneous absorption studies, for product to be classified as Class IIb.

AIM OF THE STUDY

The aim of the study is to perform a preliminary validation of a 3D human reconstituted human epidermis (RHE) model with epidermal barrier impairment. An experimental approach previously applied to perform hazard identification of chemicals on a RHE with impaired barrier function (1) has been optimized by applying a Multiple Endpoint Analysis (MEA) approach which included the endpoints readout schematically reported in **Figure 1**.

References [1] Puginier M. et al., Skin irritation Potential on RHE with Impaired Barrier Poster at Eurotox 2017 Sawada N. et al., 2003 Tight junctions and human disease Medical Electron Microscopy 36(3):147-56 | [2] Schäfer-Korting M. et al., 2008, The Use of Reconstructed Human Epidermis for Skin Absorption Testing: Results of the Validation Study ATLA | [3] Casiraghi A. et al., 2017 In vitro method to evaluate the barrier properties of medical devices for cutaneous use Regulatory Toxicology and Pharmacology 90:42e50 | [4] De Jong W.H. et al., Round robin study to evaluate the reconstructed human epidermis (RhE) T model as an in vitro skin irritation test for detection of irritant activity in medical device extracts Poster at SOT 2017, Toxicology in Vitro 50 (2018) 439–449

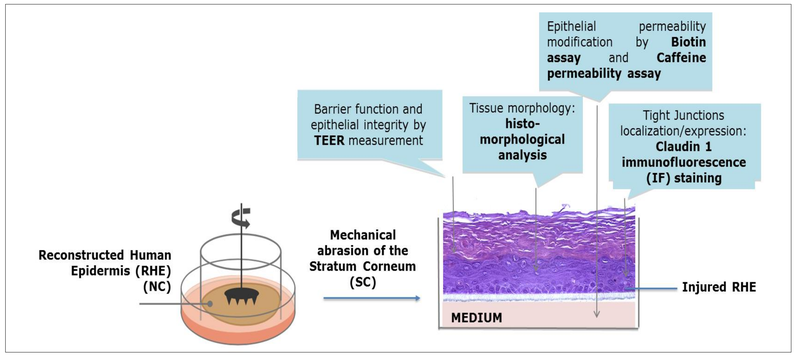

Figure 1. MEA approach adopted to characterize epidermal barrier impairment up to 24h from abrasion.

ESTABLISHMENT OF RHE MODEL WITH IMPAIRED BARRIER FUNCTION

A commercially available 3D reconstructed human epidermis model (RHE/S/17, EpiSkin Laboratories, Lyon, France) has been used to characterize the injured RHE model on seven different batches : 16-RHE-052; 17-RHE-035; 17-RHE-085; 17-RHE-096; 18-RHE-037; 18-RHE-112; 18-RHE-118. The injured model has been induced by a mechanical abrasion targeting the epidermal physical barrier, the stratum corneum (SC) and the Tight Junctions (TJs) associated to granular layer without involving deeper epidermal structures. It was considered important to avoid the induction of an inflammatory process in the viable epidermis: for that reason a limited number of SC layers has been removed.

The Poster P 384 reports the most representative results of the whole research project performed to explore the interest and relevance of different analytical approaches to validate the barrier impairment and the performances of the injured RHE.

MONITORING BARRIER FUNCTION MODIFICATIONS

TEER is an indirect assessment of TJs stability and consequently is a direct measure of the functionality of barrier function in epithelial tissues: it reflects the global resistance of the barrier linked both to the TJs structure and tissue thickness (Figure 2).TEER is the testing parameter of the OECD TG 430 (skin corrosivity on RAT skin) in order to classify ingredients for their capacity to severely modify the barrier: substances are classified as corrosive to skin if TEER values are at least 2 times lower (≤ 5 kΩ) compared to the negative control (10-25 kΩ). This approach that includes a functional measure of the barrier (TEER) coupled with the quantification of a probe (sulforhodamine B) passage through the tissue seems interesting and it has been adopted as a robust readout parameter to validate the RHE with barrier impairment.

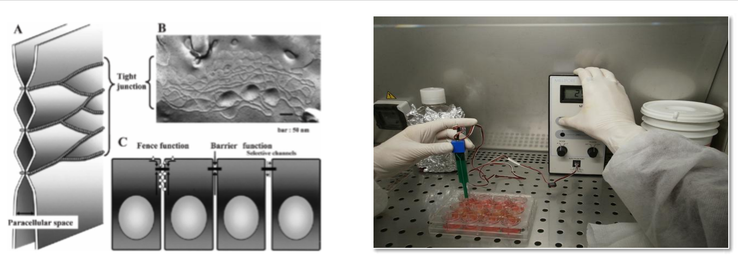

Figure 2. Tight Junctions (left). Millicell-ERF instrument for TEER measurement (right).

In **Figure 3** are reported normalized TEER measurements after abrasion and during 24h. The mechanical injury has induced a significant reduction of TEER values (about 50%) and barrier function modification has been found stable up to 24h. No recovery of the TEER values was observed.

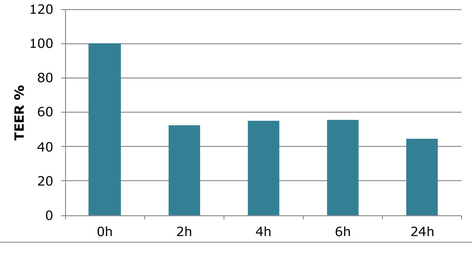

Figure 3. Normalized TEER measurements after abrasion up to 24h.

CAFFEINE PERMEABILITY ASSAY

The impact of the abrasion in the caffeine 0,5% kinetics through the injured model (3) has been quantified to characterize model permeability 1h after injury. Caffeine in the receptor compartment (1 mL) has been quantified using an Agilent 1290 infinity UPLC system (Santa Clara, USA) equipped with a reverse phase ACQUITY UPLC BEH C18 column and the Empower 3 software (Waters, USA) for data acquisition and processing.

Compared to the Negative Control (intact skin) the caffeine kinetics in the injured RHE has been modified at 1h: caffeine permeates much more the injured model (**Table I**). The assay when coupled with the TEER measure has demonstrated that 1h after abrasion the RHE barrier function was modified showing a reduced TEER value and the caffeine passage at this time has been modified: increase of 96% after 1h and 69% after 2h.

TABLE I	30 min	1h	2h	3h
Negative Control Intact RHE	0,35%	1,11%	2,16%	2,42%
Injured	0,67%	31,94%	7,13%	3,58%

MORPHOLOGY AND TJs MODIFICATIONS: H&E, CLAUDIN-1, BIOTIN PERMEATION

In the **Figure 4** the H&E staining shows a reduction of SC thickness immediately after 1h 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. The Claudin -1 IF shows a significant reduction (arrows) of protein expression in the granular layer 1h after the abrasion. Biotin is a relatively small (244.3 Daltons) vitamin used as tracker in order to evaluate the integrity of skin barrier in an inside-out permeation model: impairment of cellular junctions increases the para-cellular transit of biotin towards upper layers as shown in the RHE injured at 24h.

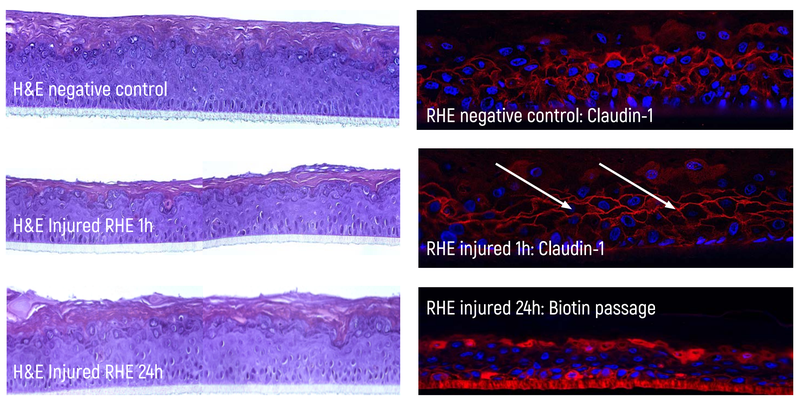

Figure 4. Morphological study on injured RHE compared to negative control (intact RHE).

CONCLUSION

Seven different batches of RHE have been used to characterize the RHE model with impaired barrier function, the so called 'injured model', using a MEA approach.

The results of the injured RHE model characterization are summarized below:

- TEER values reduction was of 50% 1h after abrasion and no recovery to the basal values has been observed up to 24h after injury
- Caffeine permeation kinetics has been modified and it has been demonstrated to be significantly increased after 1h (+96% at 1h and +69% after 2h)
- Morphological modifications (H&E, Claudin 1 and Biotin permeation) have been observed and maintained up to 24h indicating the persistency of an increased epidermal permeability
- The involvement of the viable epidermis was avoided: no cytotoxicity was measured by MTT on the RHE with impaired barrier neither release of pro-inflammatory cytokines (TNF-α) or LDH release during the 24h after injury (data not shown)

The results have confirmed the reproducibility of the measurements, suggesting the interest of the use of this injured model to assess the irritation potential and local absorption of Medical Device products intended to be applied on fragile skin with impaired barrier function.

The RHE model used in this project has been proposed and validated in an international round robin validation study for biocompatibility assessment of MD extracts (4).

The newly established injured RHE model seems a robust and reproducible candidate to assess the biocompatibility taking into account the classification requirements of the MDR for Class IIb MD.