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DEVELOPMENT AND VALIDATION OF A RHE MODEL WITH IMPAIRED BARRIER FUNCTION: APPLICATION TO MEDICAL DEVICES BIOCOMPATIBILITY ASSESSMENT

Marisa Meloni, Francesco Ranzini, Silvia Balzaretti and Laura Ceriotti - VitroScreen, In Vitro Research Laboratory, Milan (Italy)

MEDICAL DEVICE DEFINITION AND CLASSIFICATION

Medical devices (MD) are defined as any instrument, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for **specific medical purposes** (e.g. prevention, treatment or alleviation of disease) **without involving pharmacological, immunological or metabolic means** but which may be assisted in their function by such means.
Substance based MD are a heterogeneous class of products for which the Medical Device Regulation **EU n. 2017/745 (MDR)** has introduced new classification rules to obtain a suitable risk-based classification based on application site, site of action and presence/absence of absorption and metabolism. Substance based MD which are applied to external body barriers and intended to act locally are classified according to Rule 21: the physical and functional properties of the skin barrier are the discriminating factor as described in the following scheme:

Substance based MD acting at local barrier level

- Class IIa: **MD applied to intact skin**
- Class IIa: MD applied to nasal or oral cavity as far as the pharynx
- **Class IIb: MD applied on injured skin** or mucous membranes

The application site is important for:
- Product classification based on mechanism of action
- MD classification based on risks
- Substance based MD biocompatibility assessment

MEDICAL DEVICE BIOCOMPATIBILITY ASSESSMENT

MD biocompatibility is accessed according to ISO 10993 (Biological evaluation of medical devices) which reports the use of cell monolayers for MD cytotoxicity and animal testing for dermal irritation potential. These approaches have poor predictivity *vs* humans and give no information on mechanism of action at the barrier level. Furthermore, they are not sustainable with respect to Directive EU 2010/63 which promotes the replacement of animal testing for scientific purposes.

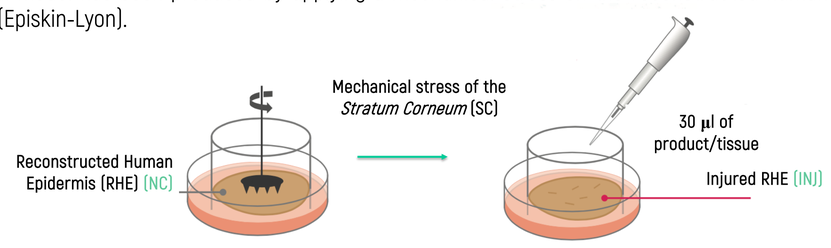
The biocompatibility of substance based MD that are intended to come into contact with injured skin should be assessed on skin models which mimic the biological target of the MD: human injured skin with modified properties at the *stratum corneum* (SC) level. Such model should present compromised barrier function at the moment of MD application even so in a context of tissue homeostasis.

***In vitro* reconstructed human tissue models** are recognized as being sensitive and reliable models to replace or reduce laboratory animal use in preclinical studies (1). They have been recently introduced in the MD sector through a successful round robin study (2) which justifies their use in the biocompatibility assessment of MD extracts according to an ISO standard under development (3). Due to their commercial availability and standardized tissue features, they can be suitable systems to produce models with controlled skin barrier functions targets of various MD.

AIM OF THE STUDY

The objective was to develop a reproducible epidermal injured model with impaired barrier function to assess the biocompatibility of products intended to be applied on injured skin and to be classified as Class IIb (4).

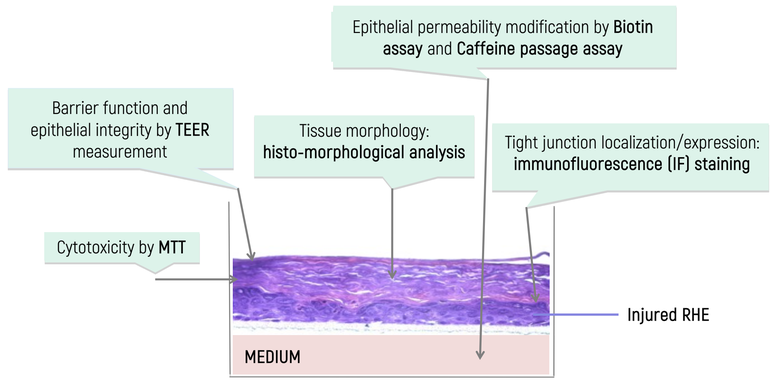
The lesional model has been produced by applying a mechanical stress on the reconstructed human epidermis (RHE, J17) model (Episkin-Lyon).



MULTIPLE ENDPOINT ANALYSIS (MEA) APPROACH

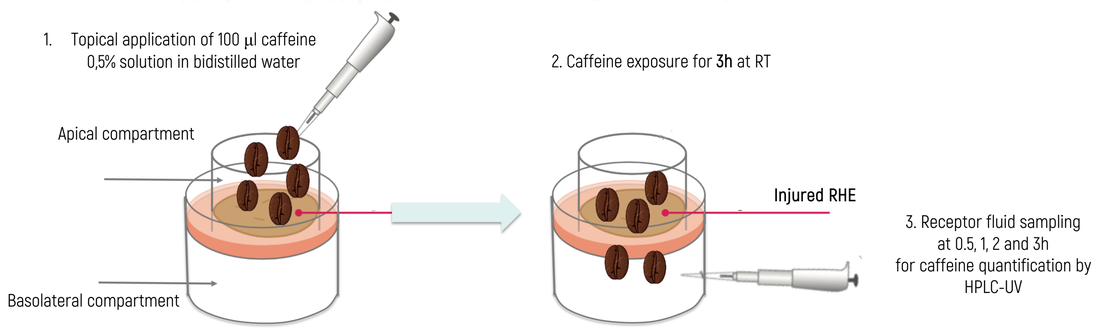
A MEA approach (5) has been adopted to characterized the jnjured skin model as reported in the **results.**

The MEA approach on additional parameters is proposed to assess the biocompatibility of substance based MD to be applied on skin and to investigate their mechanism of action.



CAFFEINE PASSAGE ASSAY

Scheme of the caffeine passage assay on injured RHE models.



RESULTS

Cell viability

The results after 24h from injury suggest non cytotoxic effects of the mechanical injury (cytotoxicity predicted for viability < 50%).

The injury targets mainly the SC without involving the viable layers. The tissue is viable and remains suitable for biocompatibility studies.

	Cell viability
NC	100%
INJ	79%

TEER measurements

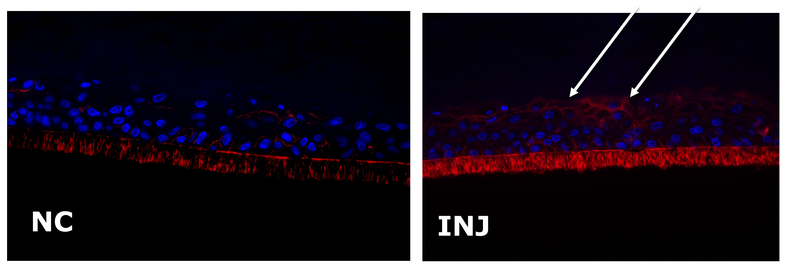
TEER values were found to be decreased *vs* time t=0h (before injury).

The injury has induced a modification to the paracellular flux which was not completely rescued after 24h.

	OHM*cm²	
	T=0h (before injury)	After 24h
NC	4121 ± 1166	3649 ± 1209
INJ	2975 ± 558	1890 ± 441

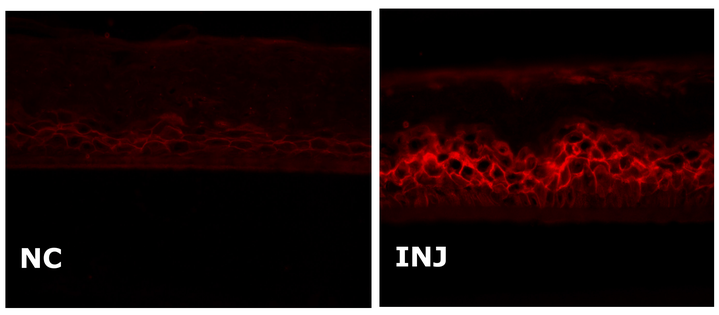
Biotin assay

Biotin staining in the granular layer of INJ is higher compared to the Negative Control (NC).
The injury has induced a modification to the Tight Junctions still visible after 24h from injury.



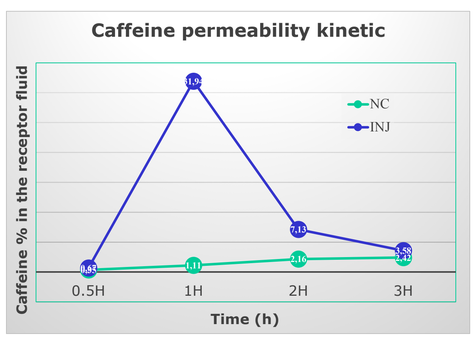
Tight Junctions protein IF: Claudin 1

24h after injury, Claudin 1 expression was found to be increased as compared to the Negative Control (NC), which may indicate that the tissue is up-regulating Claudin 1 to recover from injury.



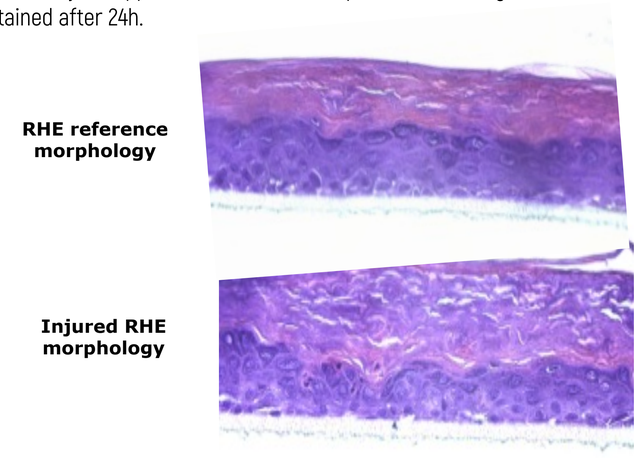
Caffeine passage assay

The results reported a transient increase of caffeine passage compared to the Negative Control (NC): the caffeine permeated more compared to intact skin with a peak after 1h. After 3h, caffeine permeation values come back to the ones recorded for the NC.
These results suggest that the injured tissues activate repair mechanisms very quickly after mechanical stress to recover the fence and barrier function.



Hematoxylin and Eosin (H&E) staining

Injured tissue shows a modified SC lamellar structure where the cohesion within the layers appears as reduced compared to the Negative Control and maintained after 24h.



References
1. *Gordon S. et al., 2015 Non-Animal Models of Epithelial Barriers (Skin, Intestine and Lung) in Research, Industrial Applications and Regulatory Toxicology - Altex 32(4), 327-378*
2. *W.H. De Jong et al. Toxicology in Vitro 50 (2018) 439-449*
3. *ISO/CD 10993-23 Biological evaluation of medical devices - Part 23: Determination of skin irritation of medical device extracts using Reconstructed human Epidermis (RhE) under development*
4. *Puginier M. et al., Skin Irritation Potential on RHE with Impaired Barrier Poster at 53rd Congress of the European Society of Toxicology (EUROTOX 2017), 10-13 September, Bratislava*
5. *Meloni, M. et al., 2002. The importance of multiple endpoint analysis (MEA) using reconstituted human tissue models for irritation and biocompatibility assay. Invitox Congress Proceedings 4, 7*

CONCLUSIONS

A model of RHE with impaired barrier function has been developped and characterized using a MEA approach. Globally all the results have confirmed that during the first 3h and up to 24h the RHE fence and barrier properties have been impaired: between 1h and 3h a faster penetraton and modified kinetics of caffeine was observed. The modified properties of injured RHE models have been demonstrated up to 24h by a stable impairment of TJs as measured by TEER (significant reduction of values), biotin assay (increase biotine permeation) and Claudine-1 immunofluorescence staining (increase protein expression) and mainteined morphological tissue modifications as assessed by H&E.