Adding the immune component in reconstructed human skin and eye epithelia models

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Introduction:

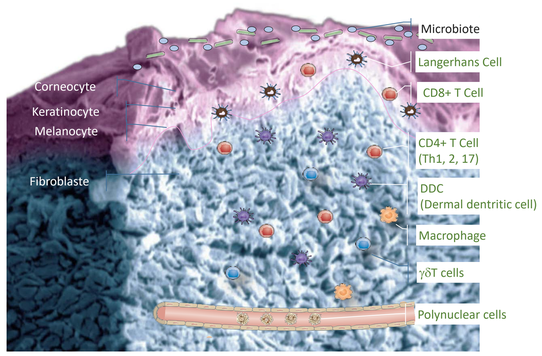
Reconstructed human epidermis (SkinEthic™ RHE) reproduce several physiological properties of *in vivo* human target organs such as barrier function or xenobiotic metabolisms and are widely used for safety and efficacy assessments of ingredients and finished products in chemical, cosmetic, drug and medical devices industries. Several validated alternative methods to animal testing are based on this model (OECD TG431, TG439).

In vivo, residents cells of the immune system are also involved in skin homeostasis regulation by interacting with epithelial cells. The microbiota is also an important player to be integrated into some experimental models. Perturbation of the complex interactions between these different players are associated to several skin disease such as psoriasis or atopic dermatitis.

To enlarge applicability domain of human reconstructed tissues, EPISKIN introduced the immune component in its models either by adding Langerhans cells (LC) inside the epidermis (**SkinEthic™ RHE-LC model**) or by using a new technology, called **cell migration models (CMM)**, which allow colonization of 3D models by different immune effector cells.

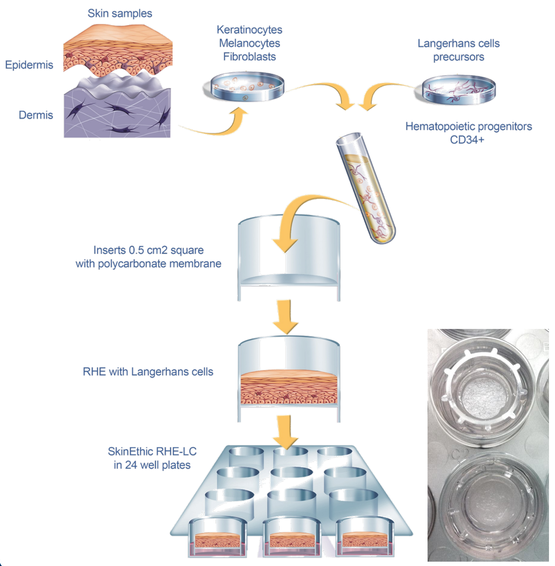
The new generation of EPISKIN's models allows to build modular experimental systems integrating different levels of complexity. This poster presents characterization of these models and two examples of applications.

Cells of the skin immune system



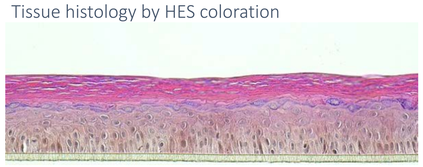
SkinEthic™ RHE-LC: A reconstructed epidermis with Langerhans like cells

The SkinEthic™ RHE-LC model is reconstructed on a polycarbonate filter membrane (0.5 cm^2). The keractinocytes (from foreskin) are seeded with CD34+ derived cells (cord blood) in a (10:1) ratio and cultivated during 17 days.

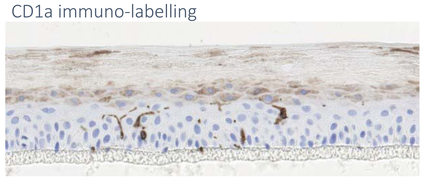


Immunolabelling of histological slices with CD1a and CD207 antibodies show regular repartition of Langerhans cells in the basal and suprabasal layers of the reconstructed epidermis.

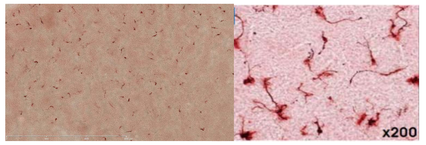
Tissue histology by HES coloration



Localization of Langerhans cells in the reconstructed epidermis by:
CD1a immuno-labelling



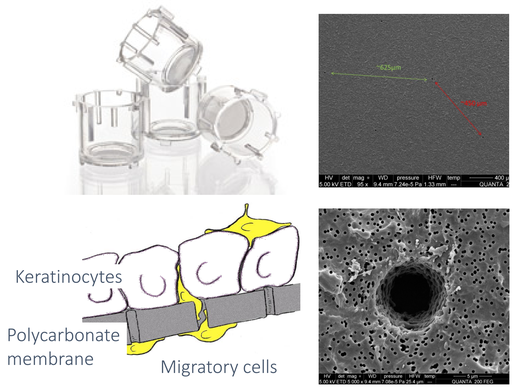
CD207 immuno-labelling on epidermal sheet



CMM technology: Cell migration models for immune cells circulation in reconstructed epithelia

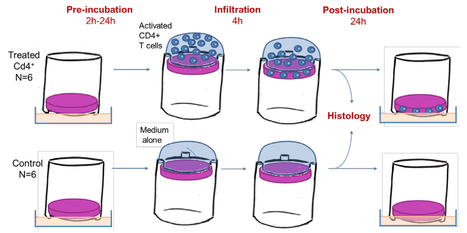
The cell migratory insert presents two levels of porosity. The same than the classical model and a second one with a lower density (between 250 and 1000 pores /inserts) and a wider diameter (>3µm) to allow immune cells circulation.

Electronic microscopy view of the double porosity of the polycarbonate membrane

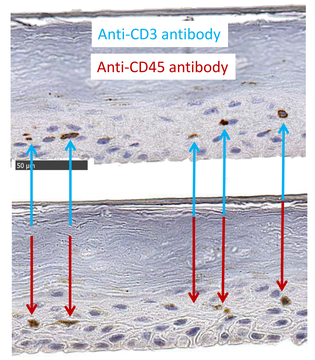


The proof of concept of colonization of the epidermal model by immune cells have been done in collaboration with GSK laboratories (Les ullis, France).

SkinEthic™ RHE-CMM inserts are turned upside down and 200µl of medium (RPMI + 10% FCS) with $1.4x10^6$ of activated CD4+ T cells are deposited on the polycarbonate membrane. After 4h of incubation the inserts are returned and put in 1ml of fresh maintenance medium for a 24 hours post-incubation step in the incubator.



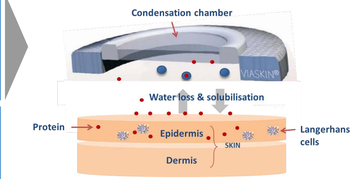
Infiltrated CD4+ lymphocytes are visualized by double labelling to ensure specific labelling onto serial 7 µm slices, stained with H&P (heamatoxylin-phloxin).



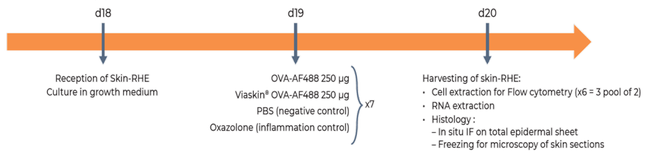
SkinEthic™ RHE-LC & Epicutaneous immunotherapy – Viaskin Technology

Viaskin provides allergenic information to immune system without entering the blood stream

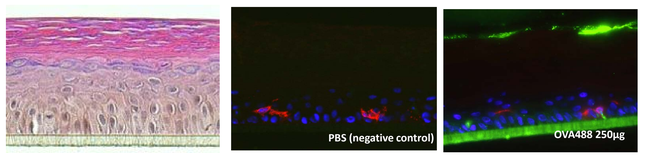
Use of SkinEthic™ RHE-LC model for mechanistic investigation on on antigen delivery in human

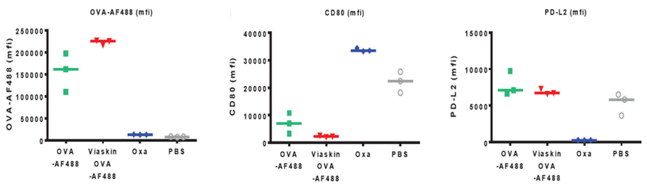


Experimental design for the in vitro evaluation of antigen uptake after Viaskin® application



Capture of allergen by Langerhans visualized by immunohistochemistry and flow cytometry

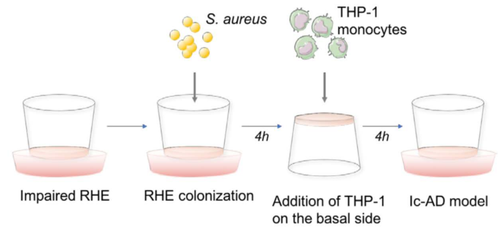




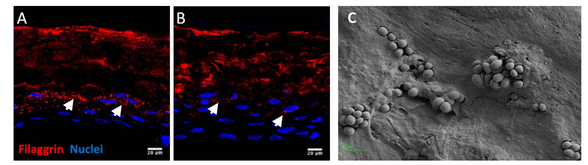
SkinEthic™ RHE-CMM & Atopic dermatitis model

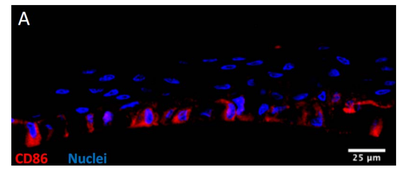
Atopic dermatitis (AD) is a common skin disease linked to a dysregulation of the immune system and an impaired epidermal barrier. Barrier impairment and immune modulation are thought to create a vicious circle leading to AD.

Colonization of a reconstructed human epidermis, SkinEthic™ RHE-CMM, by *Staphyloccocus Aureus* (1.10^6 CFU/RHE) for 4 hours and the basal infiltration of immune cells (THP-1 monocyte) induces a phenotype of atopic dermatitis by recapitulating biological cross-talk in a 3D environment.



Immunostaining of filaggrin (red signal) on cryosections of (A) normal RHE and of (B) atopic model at 16h showing alteration of epidermal barrier function in the AD model colonized by *S.aureus* (C) and THP-1.





Infiltration of immune cells in the SkinEthic™ RHE-CMM model as indicated by the immunostaining against CD86