The use of SkinEthic reconstructed human Epidermis (RHE) to study immunocompetent cells trafficking and recruitment between skin compartments

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Introduction:

Several skin diseases such as psoriasis or atopic dermatitis are associated with a chronic skin inflammatory state in which recruitment of specialized immunocompetent cells plays a central role. The ability to have an *in vitro* model to reproduce the different steps of recruitment, activation, migration and interaction of these inflammatory cells with keratinocytes of a living epidermis could be very useful to better understand these pathologies and to develop new treatments to target specific engagement steps.

This poster present an EPISKIN model, SkinEthic™ RHE, with a modified insert which allows cell trafficking between the living epidermis and the underlying compartment.

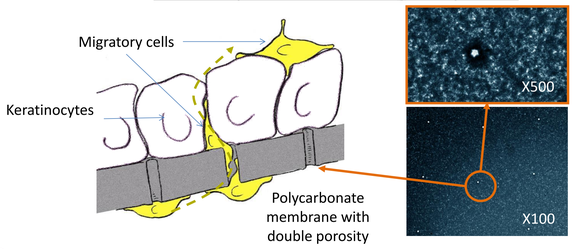
Using this model, a protocol have been developed in collaboration with GSK R&D laboratories to mimic epidermal infiltration of human lymphocytes T.

The modified membrane of the SkinEthic™ RHE is incubated four hours with activated CD4+ T cells. Twenty four hours later we observed by CD3/CD45 immunolabelling the infiltration of the CD4+ T cells in the suprabasal layers of the epidermis.

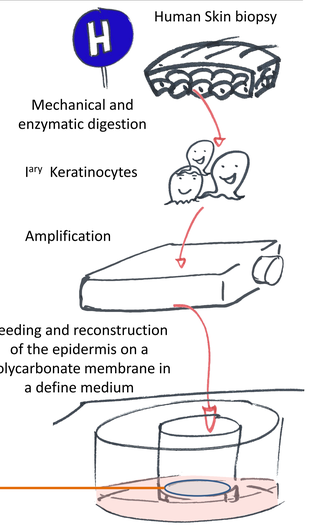
Migratory insert:

The density and the diameter of the pores of the membrane of the SkinEthic™ RHE model allow medium diffusion for cell nutrition but are too small for cells circulation. The migratrory insert presents two level 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 cells circulation.

Microscopic view of the migratory insert

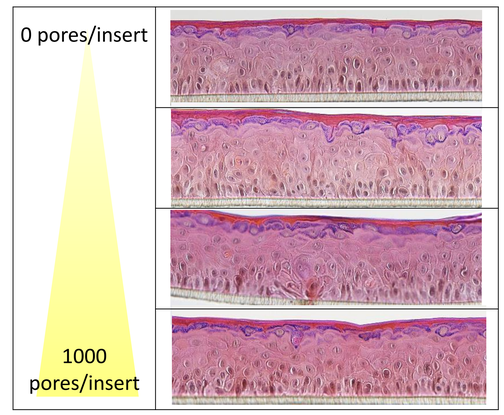


Reconstruction of SkinEthic™ RHE



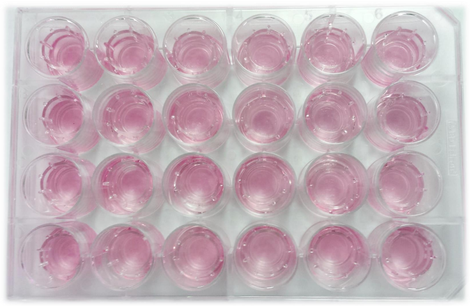
HES coloration of SkinEthic™ RHE

Different densities of pores have been tested with few impact onto the morphology of the reconstructed epidermis. We can see when the section crossed a pore, a local perturbation of the keratinocytes arrangement.

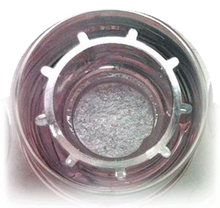


SkinEthic™ RHE

SkinEthic™ RHE is a model of epidermis reconstructed from human primary keratinocytes seeded on a polycarbonate membrane in a define medium of culture. It's produced for more than 20 years in an industrial scale with a high level of reproducibility. It can be used to test substances, chemicals, cosmetics, drugs, medical devices independently of their forms (liquid, powder, gel, solid) and their solubility (hydrophilic, lipophilic).



SkinEthic™ RHE small model (0.5 cm²) in a 24 wells plates



SkinEthic™ RHE is validated by ECVAM and OECD to assess skin corrosion/irritation potential of chemicals. This model is also used in dermatological research for its capacity to mimic human epidermal morphology and physiology. The presence of a functional stratum corneum[1] allows natural conditions of exposure, penetration, distribution and metabolism. Characterization studies of this model showed the same xenobiotic[2] metabolism than in human skin.

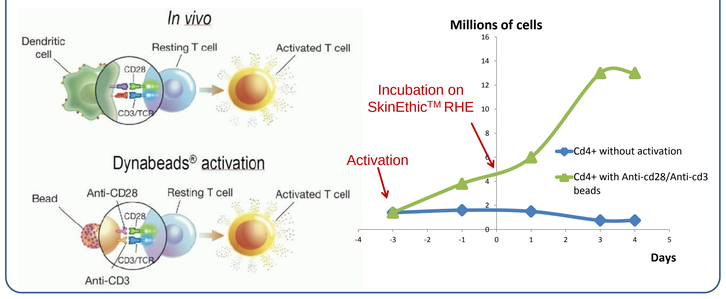
Epidermis infiltration protocol:

The SkinEthic™ RHE models are used after a 2 hours or overnight pre-incubation step in an incubator (37°C, 95% humidity, 5%CO_2). Three days before the infiltration step, normal peripheral blood CD4+ human lymphocytes obtained from AllCell (Alameda, CA) are activated by Dynabeads® Human T-Activator CD3/CD28 system (ratio 3/1 beads/cells). For the infiltration step, the 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. Control inserts are incubated with the medium alone. After 4h of incubation half of the inserts are kept for histology studies and the others are returned and put in 1ml of fresh maintenance medium for a 24 hours post-incubation step in the incubator. Then the epidermis are separated from the insert with a scalpel and immerged in a formalin solution for histology studies. The infiltrated CD4+ are visualized by double immunolabelling on serial 7 µm slices, stained with H&P (heamatoxylin-phloxin) to visualize histopathological changes in tissues. The first slice is labelled with and anti-CD3 antibodies and the following with anti-CD45 antibody to insure specific labelling.

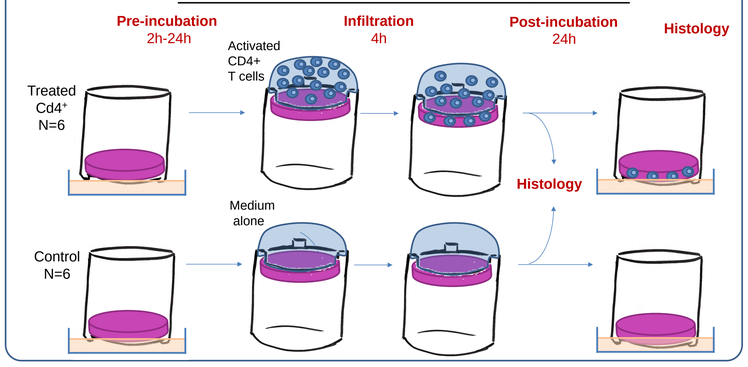
3 days before :

Activation and expansion of T-cells with cd28/cd3 beads

Ration 3:1 (beads/cells)



Incubation of activated Cd4+ with SkinEthic™ RHE



Conclusions

We showed that using this new migratory insert patented by EPISKIN it is possible to reconstruct the SkinEthic™ RHE with few impact on the organization of the reconstructed epidermis.

The preliminary results of the migratory protocol show that 24h after the incubation step the lymphocytes CD4+ have migrated in the living epidermis leading to resident immunocompetent cells in the suprabasal layers.

Complementary studies are underway to confirm these preliminary results and to quantify the number of migrated cells. These study will be completed by transcriptomics analysis to characterize keratinocytes answers at the level of cytokines, AMP and different markers of differentiation .

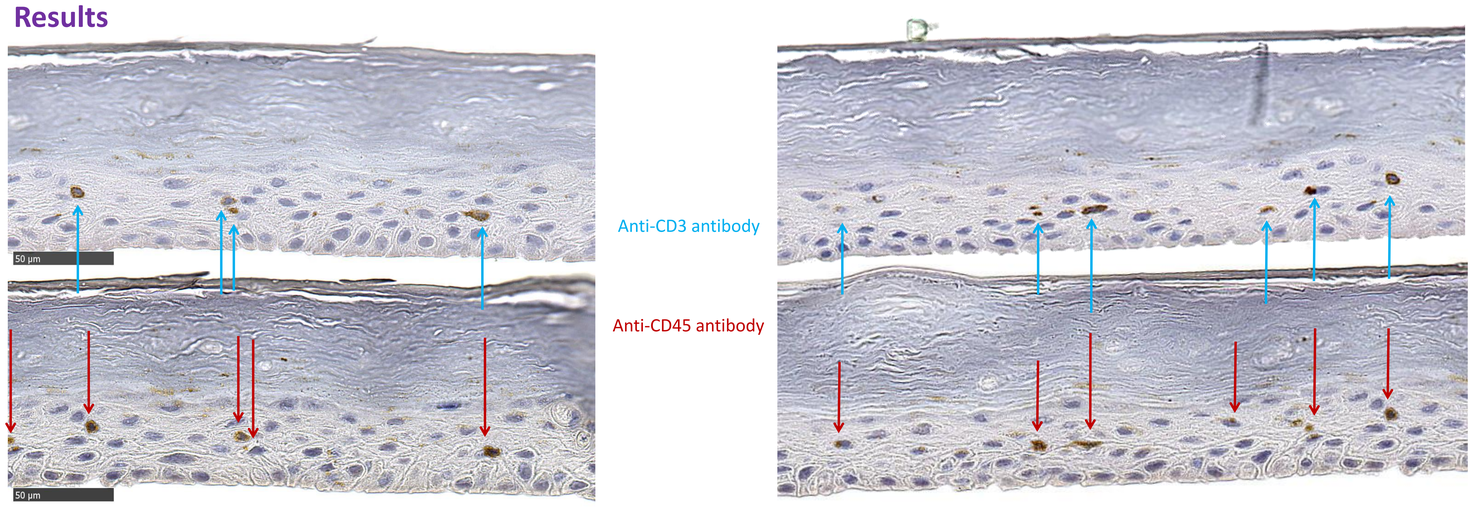
The SkinEthic™ RHE migratory protocol opens the door to innovative methods to study complex interactions between living epidermis and inflammatory cells.

References:

1- The human epidermis models EpiSkin, SkinEthic and EpiDerm: An evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. F.Netzlaff et al. 2005 , European Journal of Pharmaceutics and Biopharmaceutics .

2-Expression profiles of phases 1 and 2 metabolizing enzymes in human skin and the reconstructed skin models Episkin™ and full thickness model from Episkin. V.Luu-Theet al. 2009, The Journal of Steroid Biochemistry and Molecular Biology.

Results



Infiltrated CD4+ lymphocytes in the migratory SkinEthic™ RHE model are visualized by double labelling onto serial slices. No specific immunolabelling is detected in the control inserts. No absolute quantification of the number of infiltrate lymphocyte T have been done but when incubated with CD4+ we observe infiltration at T Cells at 4h and at 24 hours.