A New alternative method for skin sensitization and safety assessment based on an in vitro 3D fully differentiated skin model

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Abstract

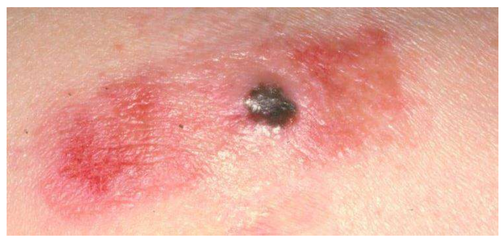


Image of an allergic contact dermatitis induce by a bandage

Prevalence of allergic contact dermatitis is increasing, justifying the need to assess skin sensitization potential for any chemical products. Current assessments of potential sensitization events of new compounds are performed according to three guidelines (OECD 442 C, D and E) and a battery of *in vitro* tests. Despite the advances of skin model equivalents (SME) validated by the governmental organizations, none of them consider the interplay between the epidermis/dermis cells and the resident immune cells. The aim of our study was to develop a SME that includes an endothelial barrier and some relevant resident immune cells involved in the skin sensitization cascade.

The model integrated adult Normal Human Epidermal Keratinocytes, Normal Human Dermal Fibroblasts, Human Umbilical Vein Endothelial cells, THP-1 (a human leukemia monocytic cell line), and THP-1 derived macrophages. Structure, cells and tissue-specific markers were revealed by immunocytochemistry techniques.

Our approach resulted in a multilayered, differentiated epidermis proliferating on top of the dermis as revealed by immunolabelling analyses. Presence of specific markers of human epidermis/dermis as well as presence/activation of immune resident cell phenotype was verified using immune labelling techniques.

Methods and Materials

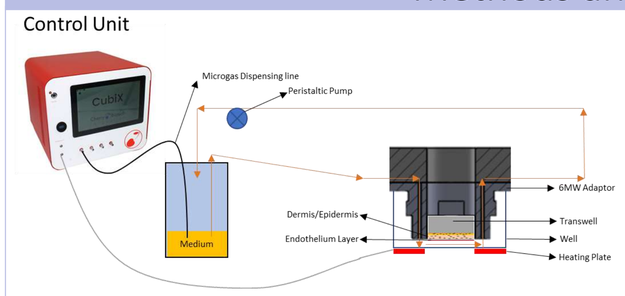


Figure 1: Schematic representation of the Cubix configuration used to perfuse the model and condition the cell medium

All individual biological features have been made in **standard 6 multi-well plates (MW)** by means of our microenviromentally controlled **microphysiological system (MPS) (CubiX).** The skin model was constructed (Figure 1) using an 8um porous Brandt insert 2in1 for 12 MW (BrandTech Scientific, Essex)

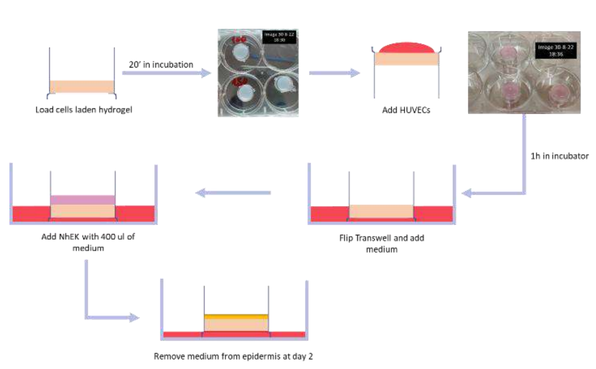
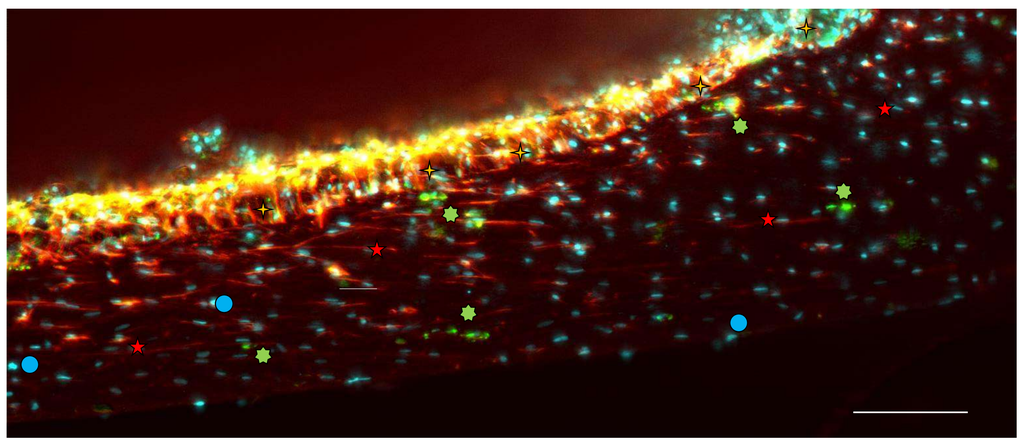


Figure 2: Graphical representation of the protocol steps used to obtain the reconstructed skin model equivalent.

The model integrated Normal Human Epidermal Keratinocytes (NHEK, 750K.mL^{-1}), Normal Human Dermal Fibroblasts (NHDF, 400K.mL^{-1}), Human Umbilical Vein Endothelial cells (HUVECS, 49,1K.cm^{-2}), and THP-1 (a human leukemia monocytic cell line, 30K.mL^{-1}) from PromoCell, Lonza and ATCC respectively. The model is constructed layer by layer (Figure 2). The CubiX system allows the control of gas concentration in the medium; we used 20% O2, 4% CO2 and 76% N2 and a flow of 150ul/min.

Results

1) Reconstructed human epidermis/dermis interface



After air-exposure, the keratinocytes start to differentiate to form a stratum corneum. Images of whole mount sections revealed the distribution and localization of cells included in the models as well as the presence of involucrine, a standard marker of keratinocytes differentiation as visible on Figure 3 & 4

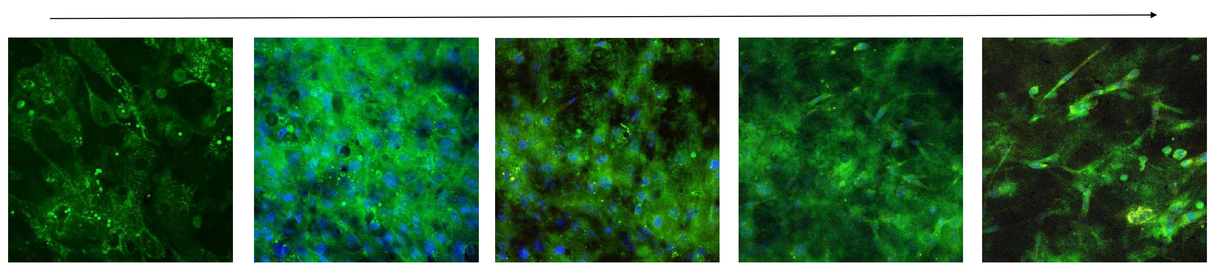
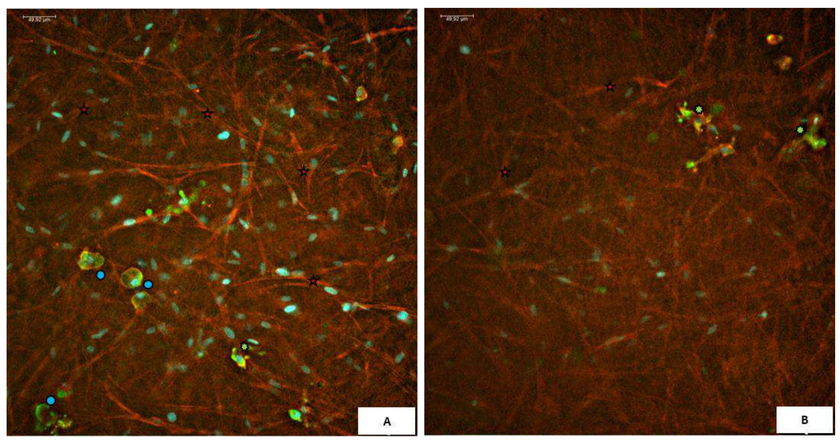


Figure 3 (top left) : Cross section IF micrograph of a of a whole mount skin model. Red channel = ActinRed; cyan channel = NucBlue; green channel = CD206; yellow channel = involucrine; cyan circles = putative THP-1; green stars = putative macrophages; red stars = fibroblasts; yellow star = keratinocytes. Scale bar = 100 um. Figure 4 (bottom right). Raman microscopy image of the epidermis/dermis interface. Green: cytoplasm, blue nuclei.

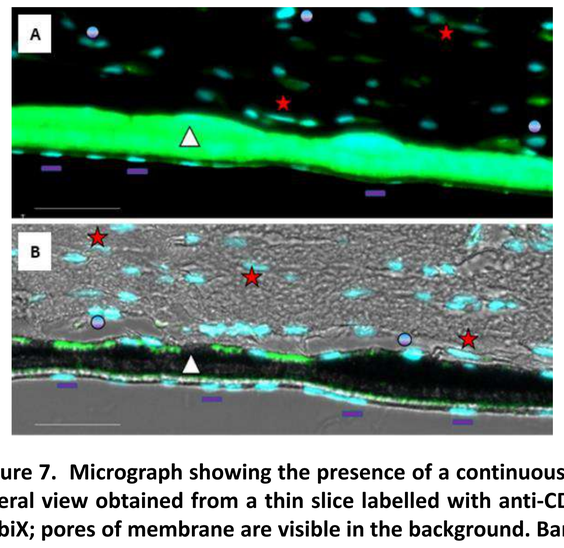
2) Reconstructed immunocompetent dermis



Fluorescent imaging highlighted a physiologically mimicking network of dermal fibroblast in the reconstructed dermis equivalent as visible on Figure 6. Additionally, our data suggest a not homogeneous distribution of THP-1 and macrophages as visible in Figure 5. The presence of THP-1 was found more localized between the endothelial barrier and the first half of the dermis whereas the macrophages seemed more abundant in the dermis layer closer to the keratinocytes.

Figure 5 (Left). Bottom up IF optical sections obtained in the middle of the dermis (A) and close to the epidermis (B) of a whole mount skin model. Red channel = ActinRed; cyan channel = NucBlue; green channel = CD206; cyan circles = putative THP-1; green stars = putative macrophages; red stars = fibroblasts. Figure 6. Raman microscopy of the dermis equivalent in second harmonic emphasizing the extra cellular matrix (collagen) density.

3) Dermis vascular interface



The presence of a continuous and cohesive monolayer of endothelial cells can be observed. Images obtained on the whole model using ActinRed as well as CD-31 immunolabelling on slices showed the presence of a well tight and aligned layer of cells on the bottom side of the transwell as shown on Figure 7. A specific CD31 signal in some putative fibroblasts inside the dermis layer as well as the presence of the peculiar large monocyte nuclei were also observed.

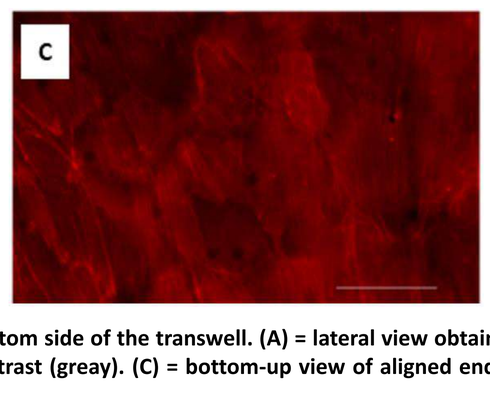


Figure 7. Micrograph showing the presence of a continuous and aligned monolayer of endothelial cells on the bottom side of the transwell. (A) = lateral view obtained from a thin slice labelled with anti-CD31 (green) and NucBlue (Nuclei, cyan). (B) = lateral view obtained from a thin slice labelled with anti-CD31 (green) and NucBlue (Nuclei, cyan) and phase contrast (greay). (C) = bottom-up view of aligned endothelial cells cytoskeleton revealed by ActinRed ICC after 5 days of perfusions using CubiX; pores of membrane are visible in the background. Bar = 50 microns.

Conclusion

To date most of the chemical risk assessment (independently of their field of application) rely on the use of animal models that have the drawback of raising more and more ethical concerns as well as potential translatability of the results. To overcome those drawback new alternative methods are/have been developed.

Our data demonstrate the capability to obtain a partially immunocompetent models that recapitulate the main structure and functional elements of human skin. The presence of a micro/milli fluidic circuit allowed us to obtain a continuous and aligned endothelial cells interface. The model is highly customizable in terms of cells population as well as vasculature to tissue ratio: this is expected to bring benefits when traying to recreate in vivo not only healthy and young skin models but also models with a pre-inflammatory condition or skin fragility. The next steps will be to assess the capability of our skin model to recapitulate a sensitization episode.

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