Immunocompetent and vascularized skin-on-chip

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Introduction

On a daily basis, our skin is exposed to several substances such as chemicals, cosmetics, drugs, but also **medical devices**. This contact can lead to adverse effects, such as allergic contact dermatitis, also called **skin sensitization**.

Skin sensitization is characterized by four main **key events** (OECD Test No. 406: Skin Sensitisation), the covalent binding of an hapten to skin proteins (KE1), the activation of keratinocytes (KE2) and dendritic cells (KE3) and the activation and proliferation of T-cells (KE4).

It is important to evaluate the sensitization potential of chemicals and medical devices that may come into contact with the skin. Standards governing such tests have authorized ***in vitro* methods** for chemicals (OECD test no. 442 E), either built on 2D models[1,2], or on 3D models based on reconstructed epidermis[3,4] targeting one key event at a time. For medical devices, although ISO guidelines only recognize *in vivo* tests (GPMT, Buehler and LLNA), *in vitro* methods are not excluded, but their use must be supported by validation data confirming their equivalence or superiority to *in vivo* methods (ISO 10993).

The objective of this project was to develop a 3D **immunocompetent** and **vascularized** skin-on-chip based on **cell lines** to perform sensitization tests for medical devices, targeting key events 1 to 4.

Methods

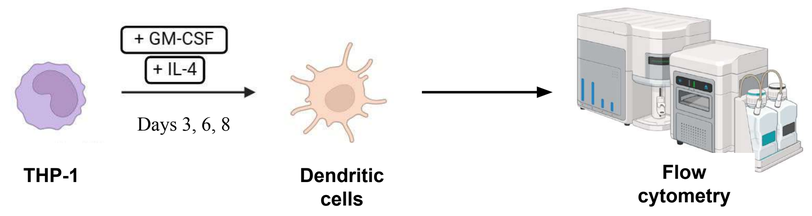

Figure 1. Differentiation of THP-1 into dendritic cells.

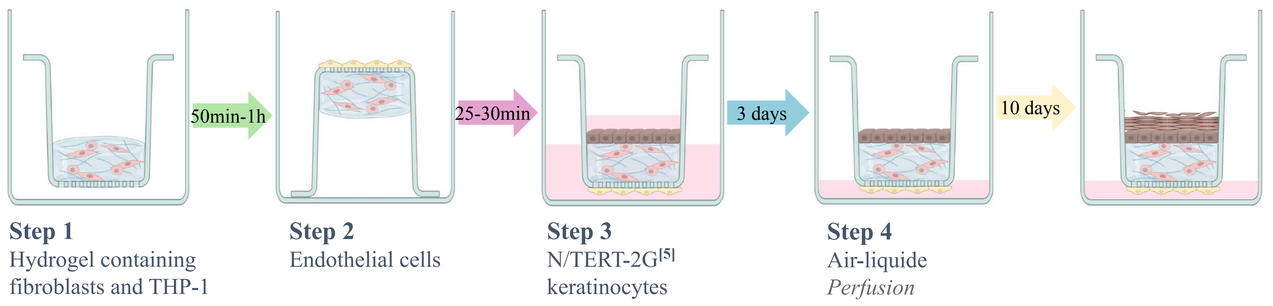

Figure 2. Building of the 3D skin model. Hydrogel is made of collagen, fibrinogen and thrombin.

Results

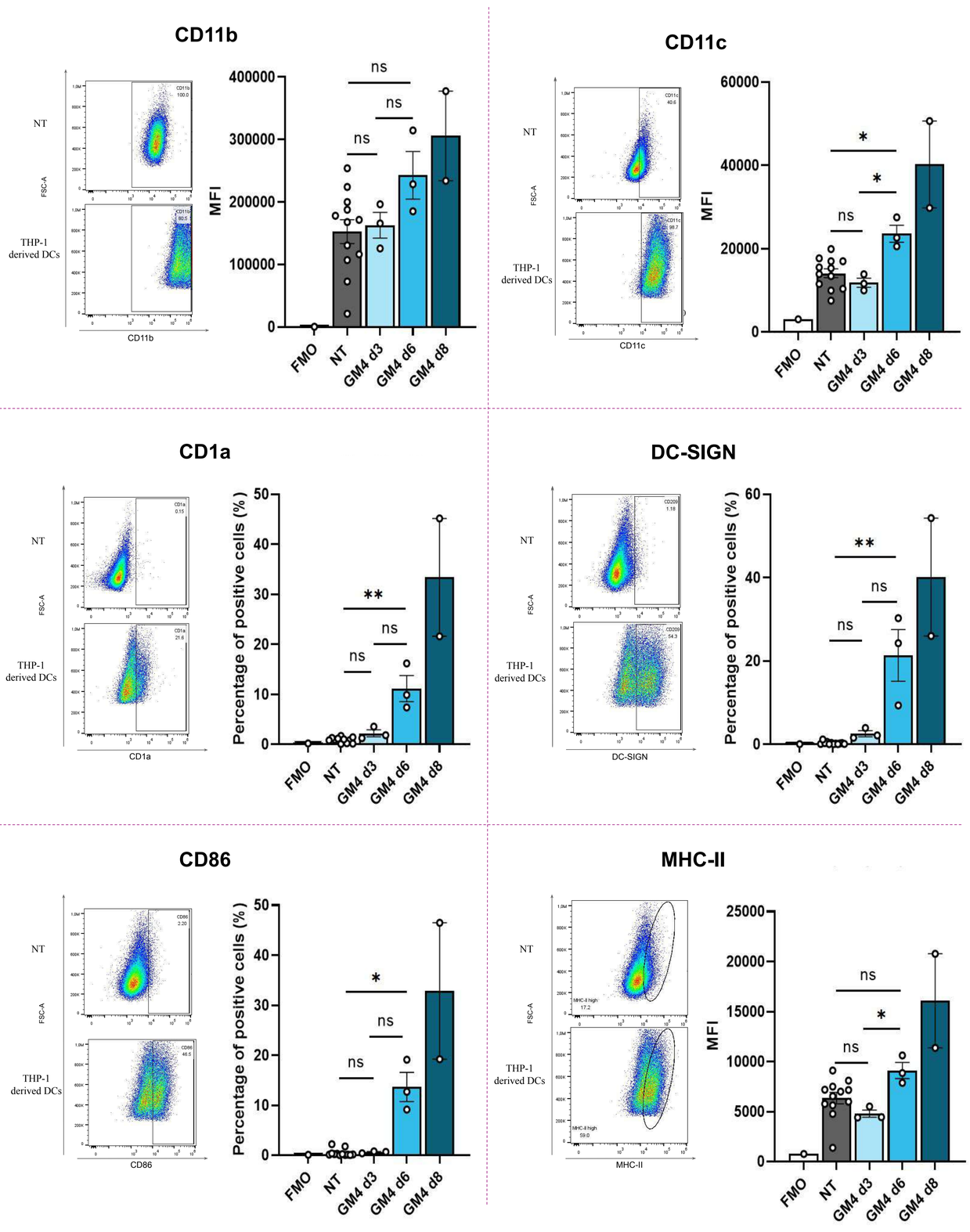

Figure 3. Flow cytometry analysis of THP-1 and THP-1 derived dendritic cells (DCs) based on myeloid markers (CD11b and CD11c), phenotypic markers (CD1a and DC-SIGN) and maturation markers (CD86 and MHC-II). *p ≤ 0.05; **p ≤ 0.01; n.s., non-significant, p > 0.05; error bars are standard errors; n ≥ 3.

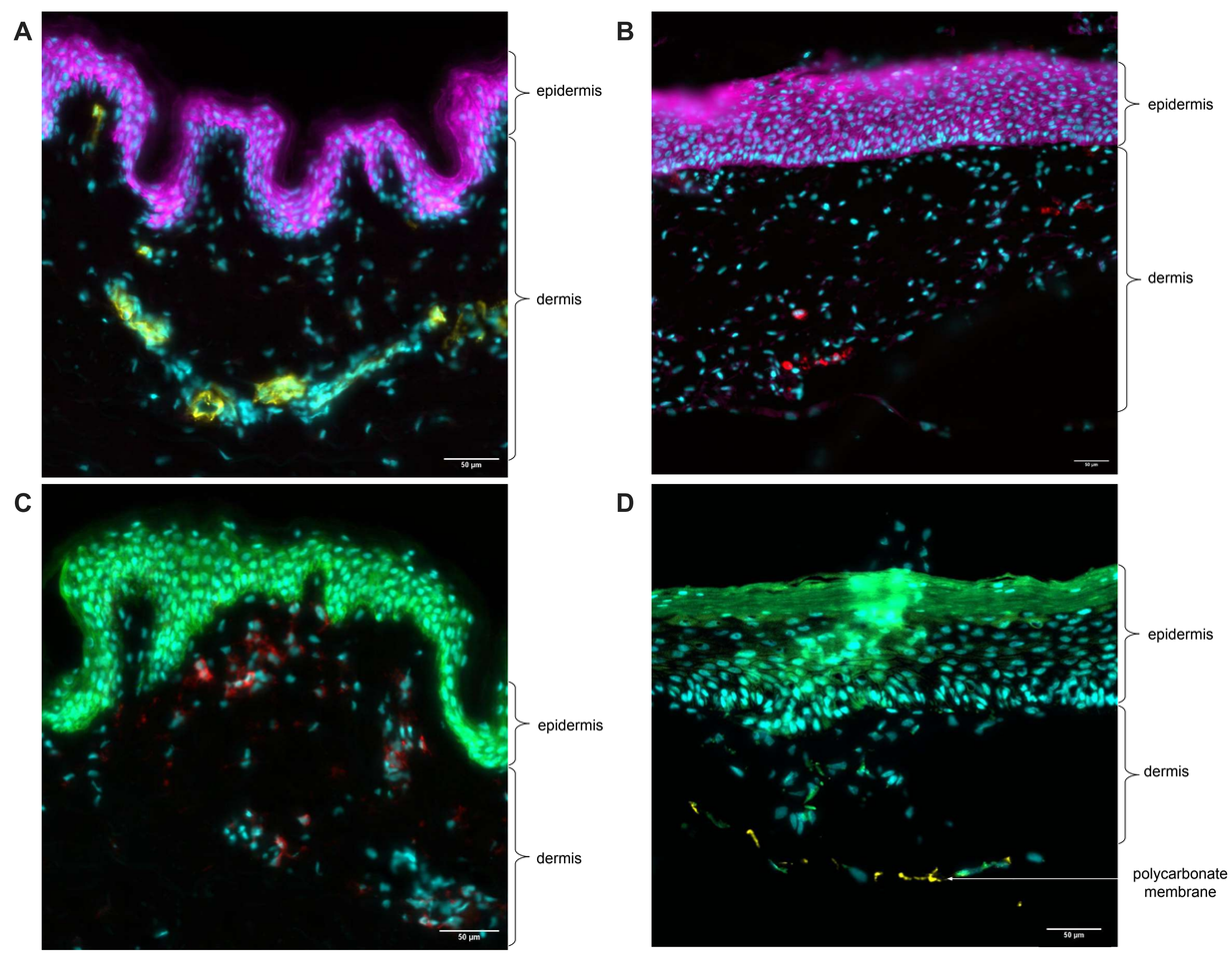

Figure 4. Immunofluorescence staining of **(A,C)** human skin punch and **(B, D)** 3D reconstructed skin. Epidermis layers, *Stratum basale* and *Stratum granulosum/spinosum* were stained with respectively, K14 (green) and K10 (magenta). THP-1 was stained with CD45 (red), HUVECS with CD31 (yellow) and nuclei with DAPI (cyan).

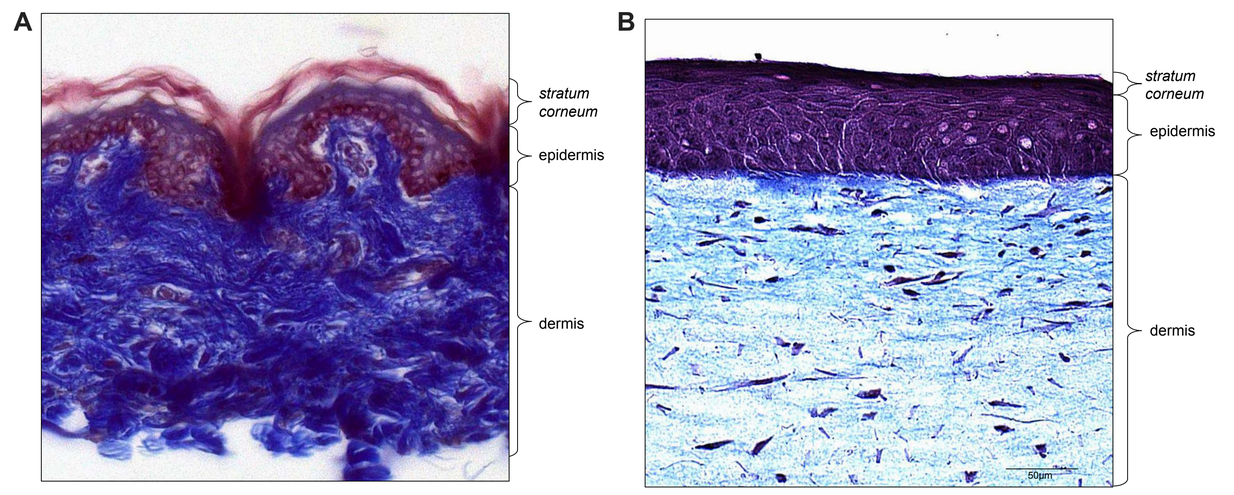

Figure 5. Histology sections of **(A)** dermatomized human skin and **(B)** 3D reconstructed skin generated using primary fibroblasts and HUVECs, THP-1 and N/TERT-2G cells lines. Masson's Trichrome coloration stains keratin present in the epidermis in red, extracellular matrix of the dermis in blue and nuclei in black.

Discussion

- ✓ THP-1 differentiated using GM-CSF and IL-4 express characteristic markers of **dendritic cells**. Non-differentiated cells remain in the 3D model after 13 days of culture.
- ✓ N/TERT-2G cell line is able to build a **3D stratified epidermis**, comprising a *stratum corneum.*
- ❑ Differentiation of THP-1 into **macrophages** and **Langerhans cells**;
- ❑ Use a microfluidic device for the **perfusion** of the model;
- ❑ Test a panel of known molecules (sensitizers and non-sensitizers).

References

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