

Development and characterization of an immunocompetent and vascularized skin model

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Introduction

On a daily basis, the skin is exposed to many substances such as **chemicals, cosmetics, drugs, or medical devices**. Such exposure can lead to adverse effects, including allergic contact dermatitis, which arises from **skin sensitization**. As defined by the Organisation for Economic Co-operation and Development (OECD test procedure #406), skin sensitization can be subdivided into four main Key Events: 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).

The sensitization potential of chemicals and medical devices that may come into contact with the skin must be evaluated. Standards governing such tests have authorized **in vitro methods** for chemicals (OECD test no. 442), 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). Our current goal is to develop a 3D **immunocompetent and vascularized skin model based on cell lines**, which will be adapted into a skin-on-chip sensitization test covering KE1-3.

Methods

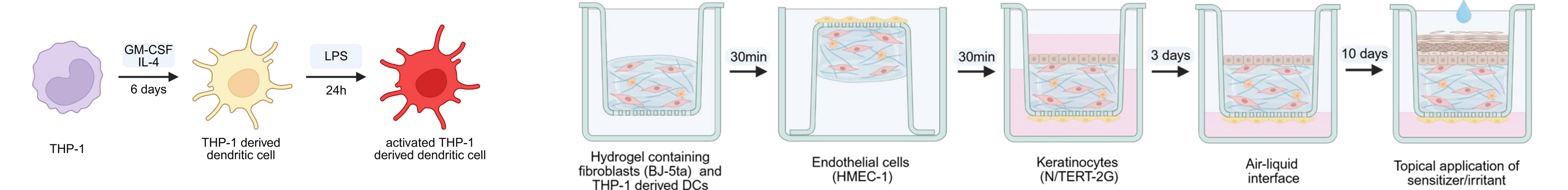


Figure 1. Differentiation of THP-1 into dendritic cells and activation.

Figure 2. Building of the 3D reconstructed skin model. Hydrogels were made of collagen, fibrinogen and thrombin. Sensitizers and irritants were deposited on top of the epidermis and washed away 1h later.

Results

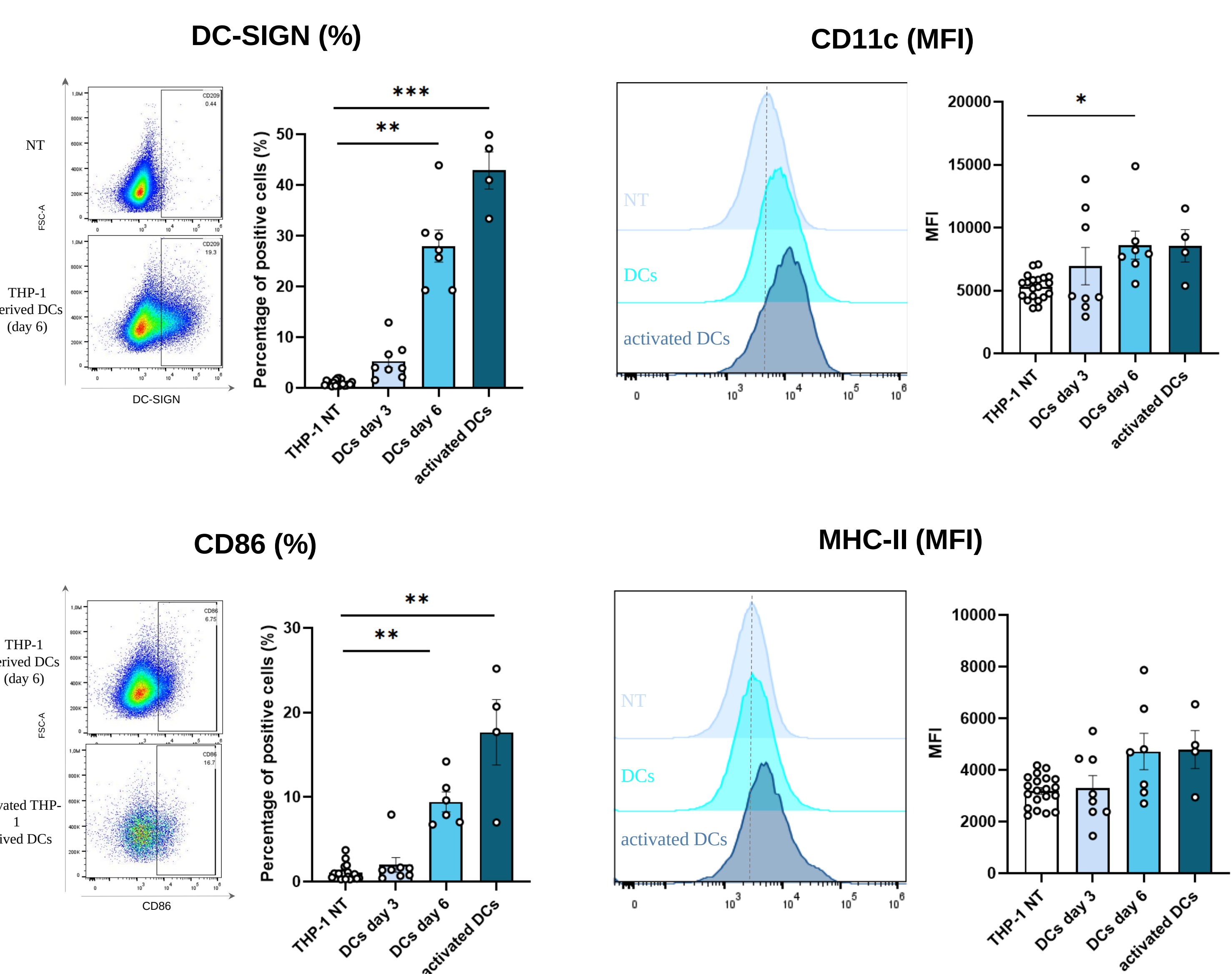


Figure 3. Analysis of THP-1 and THP-1-derived dendritic cells (DCs). Flow cytometry stainings of phenotypic markers (CD11c, DC-SIGN) and maturation markers (CD86, MHC-II). Quantification was based upon the percentage of positive cells (%) or the mean fluorescence intensity (MFI). Statistical comparisons by Kruskal-Wallis tests: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. Error bars represent standard error of the mean; n ≥ 4.

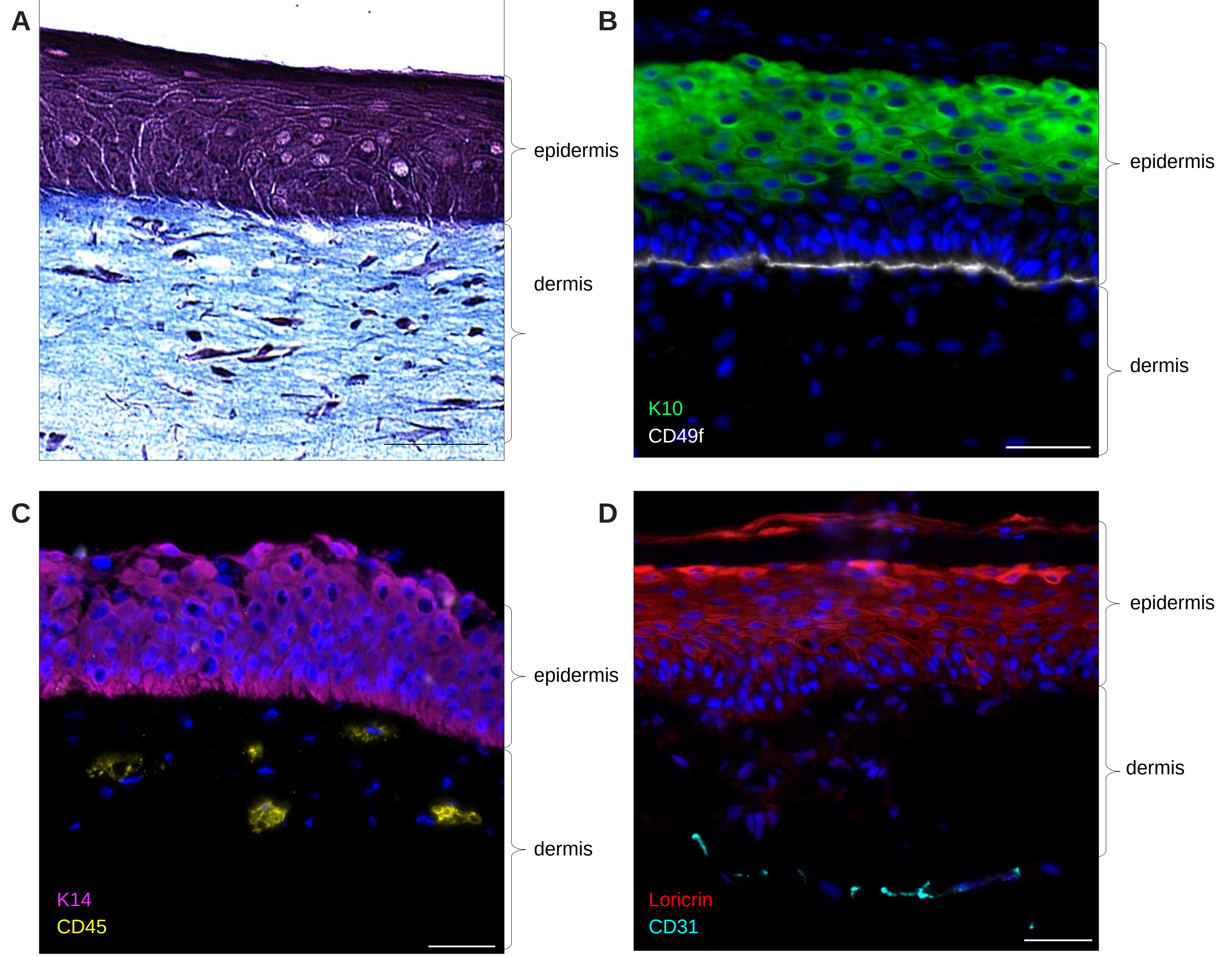


Figure 4. Structural characterization of the 3D skin model. (A) Masson Trichrome coloration and (B,C,D) immunofluorescence staining of skin models. Epidermis was stained with Keratin 14 (basal keratinocytes, magenta), Keratin 10 (differentiated keratinocytes, green), CD49f/a6 integrin (basal keratinocytes, white) and Loricrin (terminally differentiated keratinocytes, red). Immune cells, including THP-1, were stained with CD45 (yellow), endothelial cells with CD31 (cyan) and nuclei with DAPI (blue). Scale bar = 50µm.

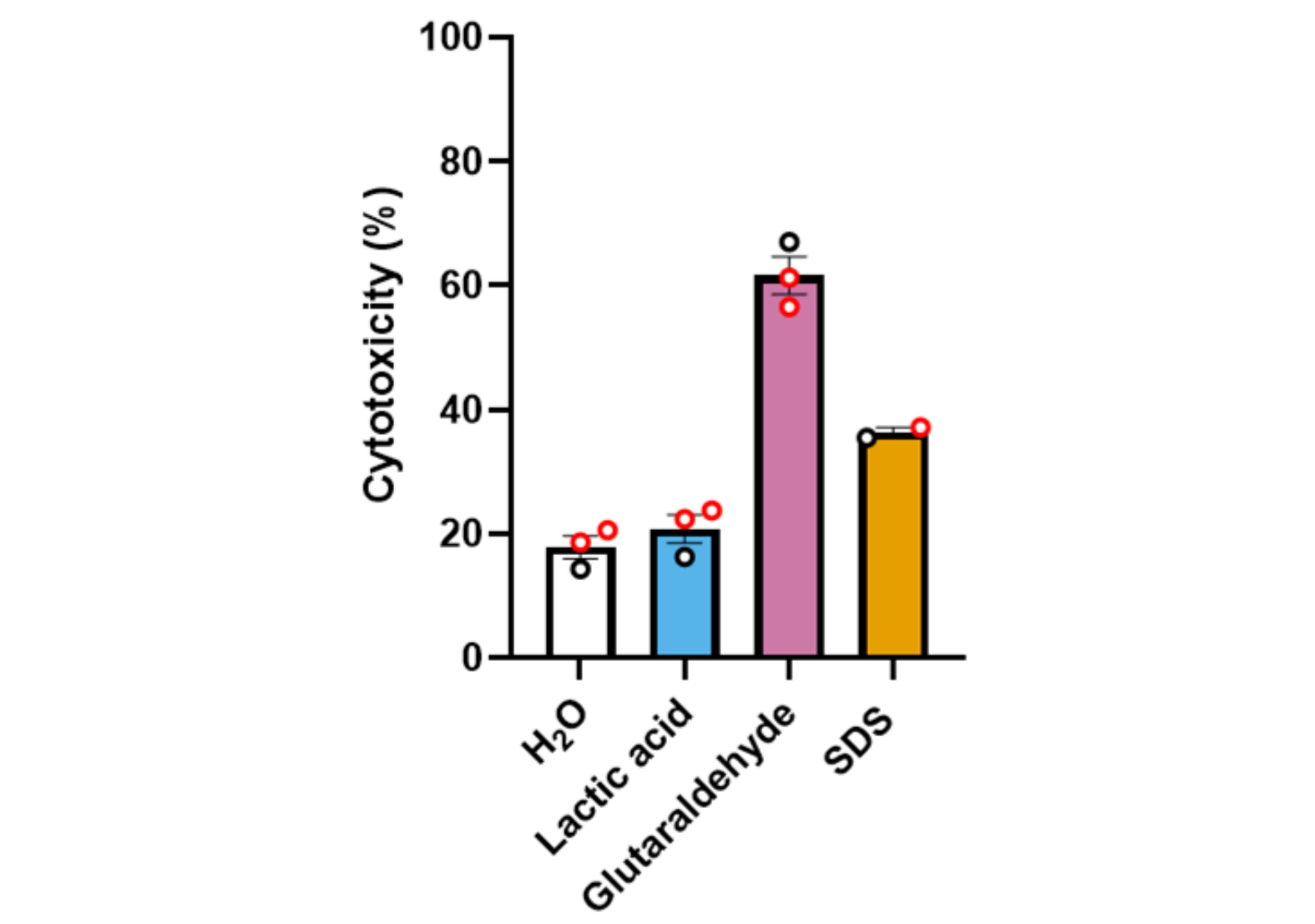


Figure 5. Toxicity upon topical exposition to chemicals. LDH assay on supernatants harvested 24h after application of H₂O (vehicle), lactic acid (non-sensitizer), glutaraldehyde (strong sensitizer) and SDS (irritant). One dot = one model. Black dots = models without endothelial cells.

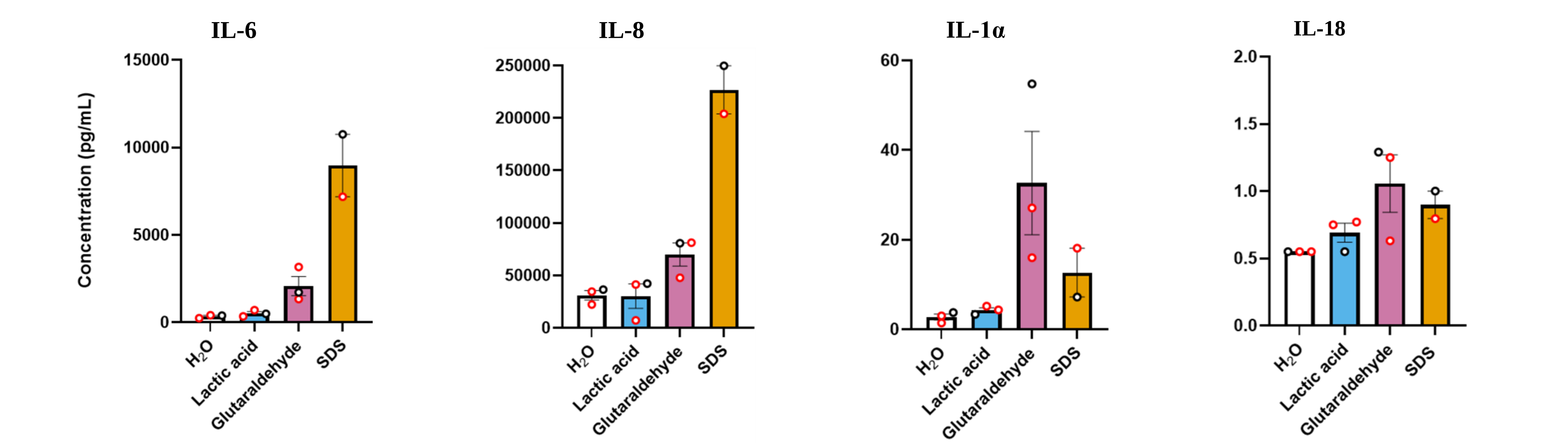


Figure 6. Inflammatory cytokines release upon topical exposition to chemicals. Cytokines were quantified in cell culture supernatants using LegendPlex assays 24h after topical application of H₂O, lactic acid, glutaraldehyde and SDS. One dot = one model. Black dots = models without endothelial cells.

Conclusions and perspectives

- ✓ THP-1 differentiated using GM-CSF and IL-4 express characteristic markers of **dendritic cells** and have the potential of being activated.
- ✓ N/TERT-2G cell line is suitable for building a **3D stratified epidermis**, comprising a **stratum corneum**.
- ✓ Inflammatory cytokines released after topical application of sensitizers and irritants show the potential of the model to respond to activation by initiating an innate immune response.
- Other sensitizers and irritants will be tested to validate the model.
- Models will be **perfused** using a microfluidic setup.

References

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