Evaluation of Vaccine Potency with High-Dimensional Immune Profiling Using an Innovative Bio-stabilized Human Skin Model

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Scientific context

In the rapidly evolving field of vaccine research, the quest for innovative methodologies that more closely mimic human immune responses prior to entering clinical trials is paramount. To overcome this challenge, our research introduces a novel approach utilizing bio-stabilized, injectable *ex vivo* human skin models (HypoSkin®) to closely study the human immune response to vaccines within its natural tissue context. This approach offers a dual-scale investigative lens—spanning organ-wide to single-cell resolutions—to meticulously chart the immune landscape's dynamics in response to vaccination.

Using advanced techniques like spatial biology and transcriptomics analysis, we demonstrate that Hyposkin® maintains its viability and functionality for up to ten days post-injection. Specifically, the administration of the mRNA-1273 COVID-19 vaccine triggered specific cytokine and chemokine responses essential for recruiting T cells and monocytes. Longitudinal single-cell analysis demonstrated significant, time-dependent shifts in the activation states of immune cells, indicating a robust and coordinated immune response at the injection site.

This innovative framework, VaxSkin, enhances our understanding of immune responses to vaccines in their native tissue environment and has significant translational potential. It offers a new standard for preclinical vaccine evaluation, aiming to speed up the development of new vaccines by providing a more accurate evaluation of human immune responses.

1 HypoSkin®: A bio-stabilized natural human skin platform for studying immune response to drugs

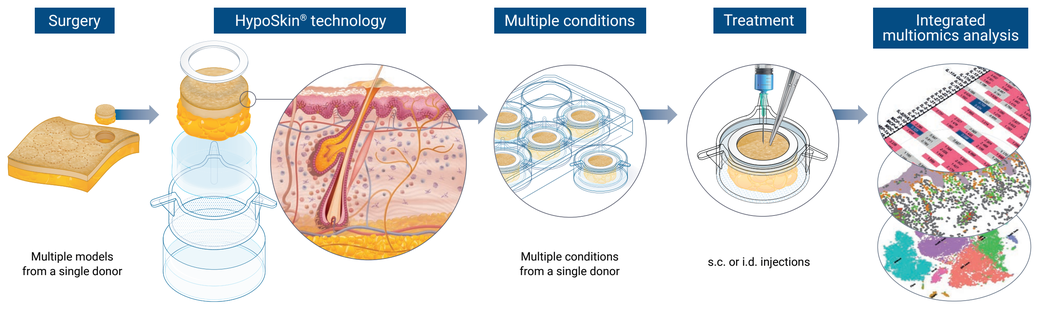

Figure 1: Experimental workflow for the HypoSkin® validation as a suitable platform to study the early steps of immune response to injectable drugs.

2 HypoSkin® is stable over a period of 10 days & is suitable for subcutaneous injection into the hypodermis

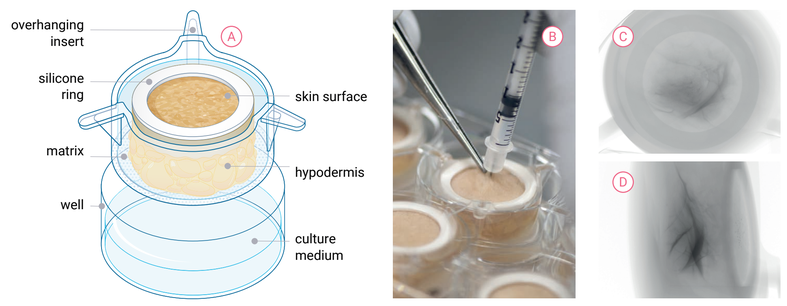

Figure 2: HypoSkin® is an injectable human skin model.
(A) Schematic representation of HypoSkin® model. (B) Photo of a subcutaneous injection with an insulin syringe. (C) Top view of X-ray tomography of the bolus formed in the hypodermis following subcutaneous injection of a potassium iodide solution (in black). (D) Side view.

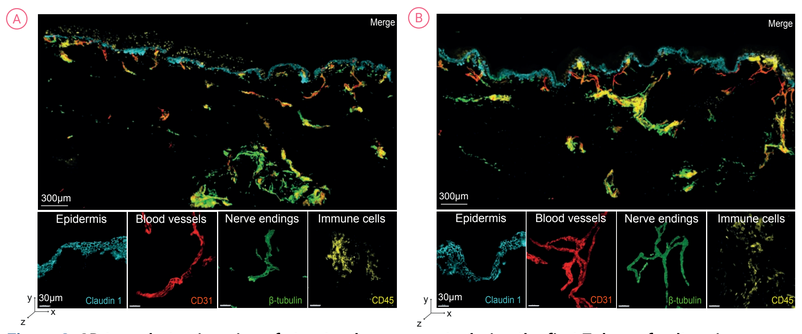

Figure 3: 3D two-photon imaging of structural components during the first 7 days of culture in HypoSkin® models. Co-staining of immune cells (CD45, yellow), vessels (CD31, red), nerve endings (Beta-tubulin, green), and epidermis (Claudin, blue), on 100 µm skin sections and observed under a two-photon microscope. (A) Day 0. (B) Day 7.

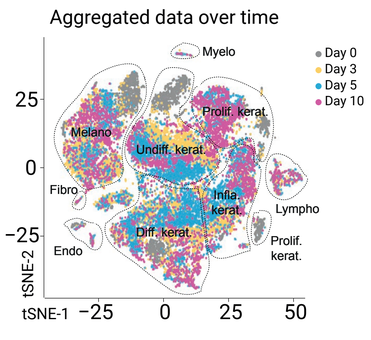

Figure 4: Characterization of immune & structural cell populations by single cell RNA sequencing in the HypoSkin® model.
t-SNE plot showing the distribution of cells in the different populations, colored according to the day of analysis of culture samples.

- **HypoSkin® is a human skin model which allows subcutaneous injections to be performed similarly to what is performed in clinic.**
- **HypoSkin® allows for maintainance of skin structure and integrity for up to 10 days.**
- **scRNAseq analysis was used to confirm that the HypoSkin® model contains most of the immune cells that are naturally present in human skin.**
- **All identified cell types were present over the course of the 10 day *ex vivo* culture.**

3 Skin secretome analysis following vaccine injection

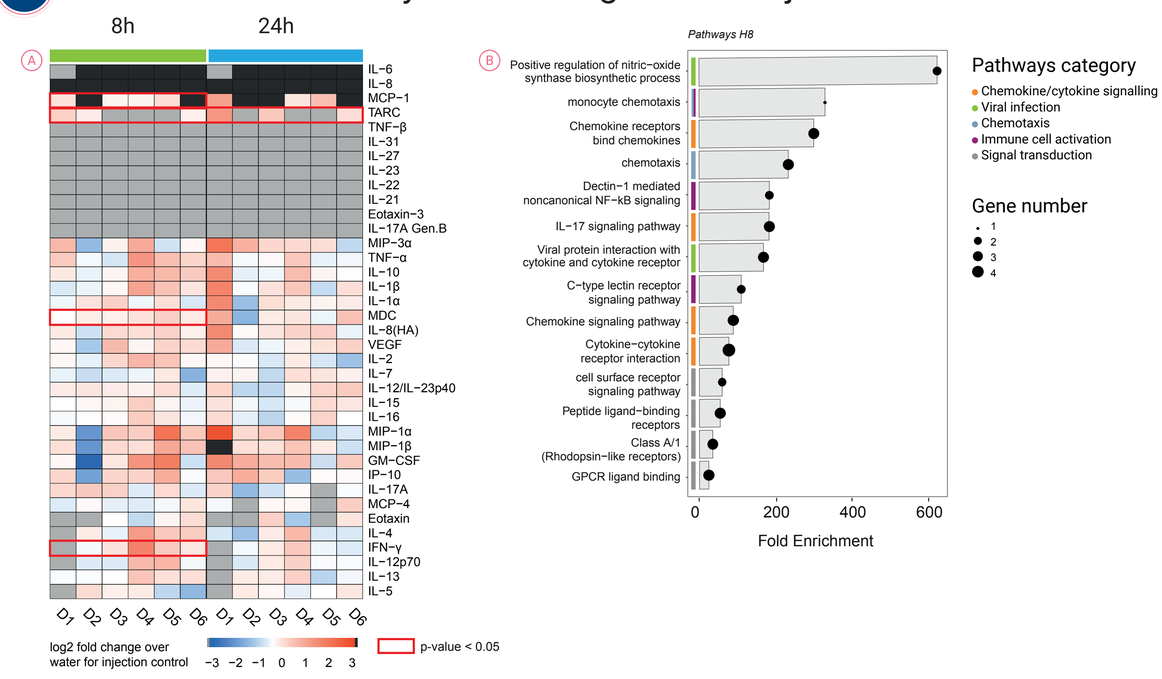

Figure 5: Analysis of cytokine release from HypoSkin® model injected with water or Moderna COVID-19 vaccine at different times.
(A) Heatmap showing the ratio between the cytokine concentration for the vaccine-injected condition and the cytokine concentration for the water injected condition per donor. Cytokines were measured in the surrounding gel matrix post-injection at indicated time points. Red rectangular outlines indicate statistically different cytokines between the two conditions. The ratio is expressed as a log2 scale. (B) Biological pathways activated by the injection of the Moderna vaccine in HypoSkin® models. Pathways were generated based on the cytokines found to be statistically different between the two conditions.

Cytokines released by HypoSkin® at 8 hours following Moderna vaccine injection:

MCP-1:
- Recruitment of monocytes and macrophages to the site of skin inflammation
- Modulation of T cell, mast cells and dendritic cells activity

MDC:
- T cell recruitment and activity

TARC:
- T cell recruitment

IFN−γ:
- Regulation of the immune response
- Bridge between innate and adaptive immunity

4 Single-cell analysis of skin-resident immune cells over time after vaccine injection

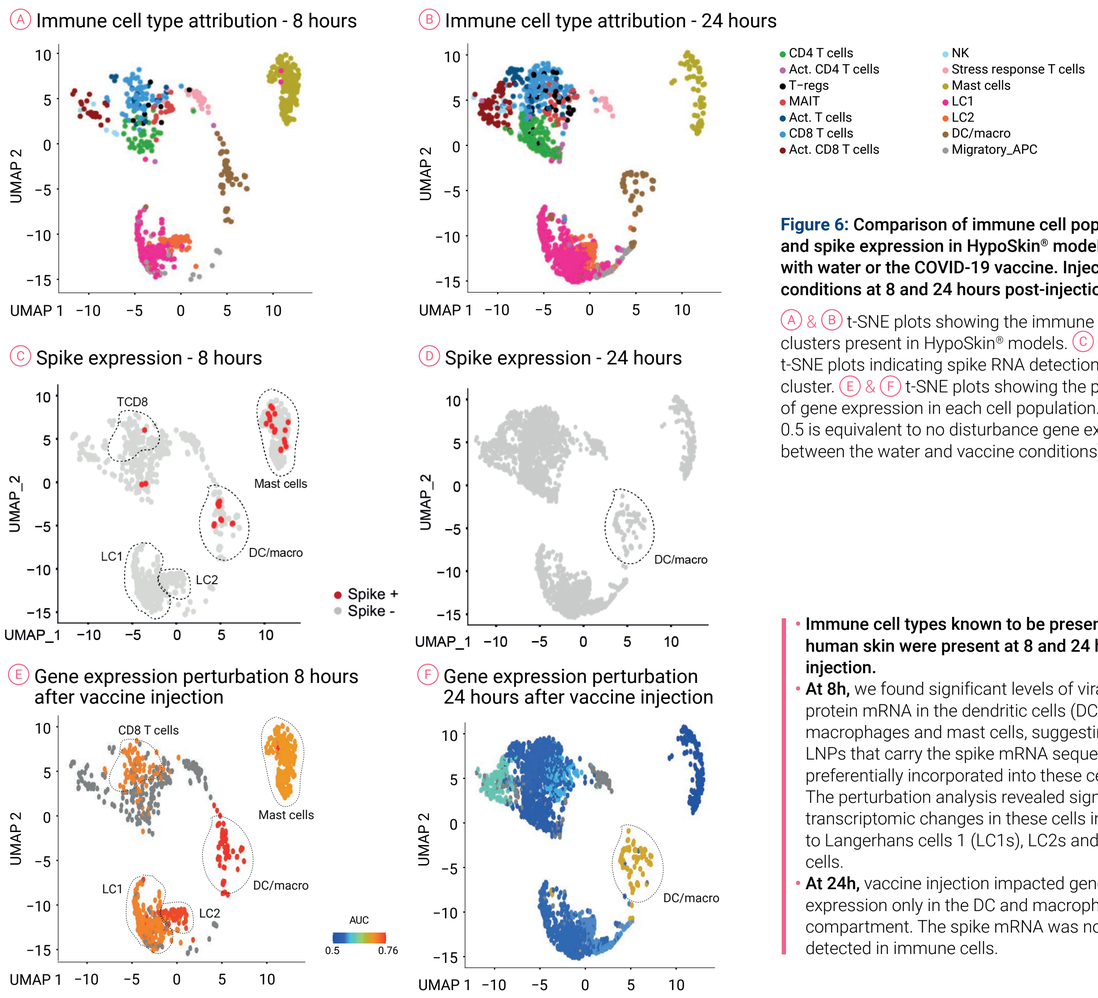

Figure 6: Comparison of immune cell populations and spike expression in HypoSkin® models injected with water or the COVID-19 vaccine. Injected conditions at 8 and 24 hours post-injection.
(A) & (B) t-SNE plots showing the immune cell type clusters present in HypoSkin® models. (C) & (D) t-SNE plots indicating spike RNA detection in each cluster. (E) & (F) t-SNE plots showing the perturbation of gene expression in each cell population. (AUC = 0.5 is equivalent to no disturbance gene expression between the water and vaccine conditions).

- **Immune cell types known to be present in human skin were present at 8 and 24 hours post-injection.**
- **At 8h,** we found significant levels of viral spike protein mRNA in the dendritic cells (DCs), macrophages and mast cells, suggesting that the LNPs that carry the spike mRNA sequences were preferentially incorporated into these cell types. The perturbation analysis revealed significant transcriptomic changes in these cells in addition to Langerhans cells 1 (LC1s), LC2s and CD8 T cells.
- **At 24h,** vaccine injection impacted gene expression only in the DC and macrophage compartment. The spike mRNA was no longer detected in immune cells.

5 Antigen presenting cell (APC) activation analysis with multiplex spatial imaging (MANTIS®) 24 hours following vaccine injection

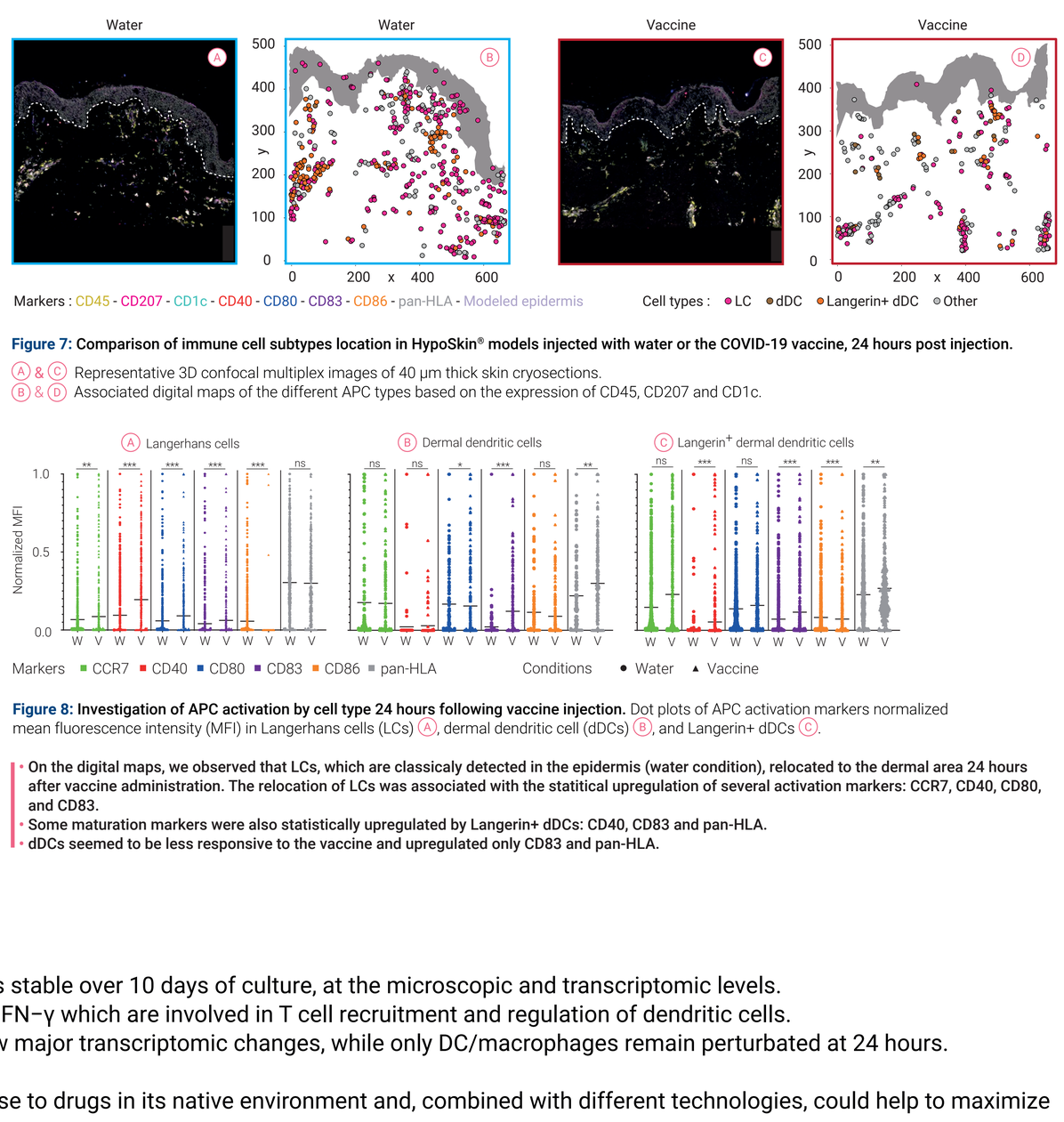

Figure 7: Comparison of immune cell subtypes location in HypoSkin® models injected with water or the COVID-19 vaccine, 24 hours post injection.
(A) & (C) Representative 3D confocal multiplex images of 40 µm thick skin cryosections.
(B) & (D) Associated digital maps of the different APC types based on the expression of CD45, CD207 and CD1c.

Figure 8: Investigation of APC activation by cell type 24 hours following vaccine injection. Dot plots of APC activation markers normalized mean fluorescence intensity (MFI) in Langerhans cells (LCs) (A), dermal dendritic cell (dDCs) (B), and Langerin+ dDCs (C).

- **On the digital maps, we observed that LCs, which are classicaly detected in the epidermis (water condition), relocated to the dermal area 24 hours after vaccine administration. The relocation of LCs was associated with the statitical upregulation of several activation markers: CCR7, CD40, CD80, and CD83.**
- **Some maturation markers were also statistically upregulated by Langerin+ dDCs: CD40, CD83 and pan-HLA.**
- **dDCs seemed to be less responsive to the vaccine and upregulated only CD83 and pan-HLA.**

Conclusions

HypoSkin® is an injectable human skin model composed of a natural multicellular ecosystem that remains stable over 10 days of culture, at the microscopic and transcriptomic levels.

- The Moderna COVID-19 vaccine induced a significant increase in the release of MDC, MCP-1, TARC and IFN−γ which are involved in T cell recruitment and regulation of dendritic cells.
- 8 hours after vaccination, many immune cell populations such as APCs, mast cells, and CD8 T cells show major transcriptomic changes, while only DC/macrophages remain perturbated at 24 hours. This process could be indicative of antigen presentation following exposure to vaccine mRNA.
- The HypoSkin® technology provides a unique platform for the study of the early steps of immune response to drugs in its native environment and, combined with different technologies, could help to maximize specific immune targeting or evaluate adjuvant potency ahead of clinical trials.