Human induced pluripotent stem cell differentiation for in vitro skin modelling

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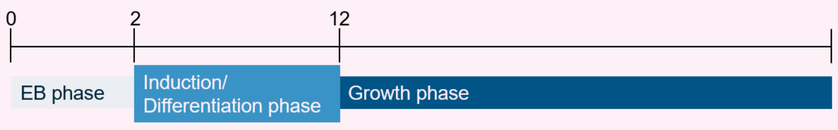
Motivation:

As one of the most important organs of the human body, the skin protects internal tissue from external influences. In case of disorders, personalized medicine can support efficient treatment and patient-specific skin equivalents can be used for drug testing and the investigation of disease progression. The use of primary keratinocytes for such models, yields in a low number of equivalents from one donation, as the maintenance and amplification. By implementing skin cells derived from human induced pluripotent stem cells (hiPSC) to generate scaffold-based skin model, higher yield and therefore high-throughput drug testing can be achieved.

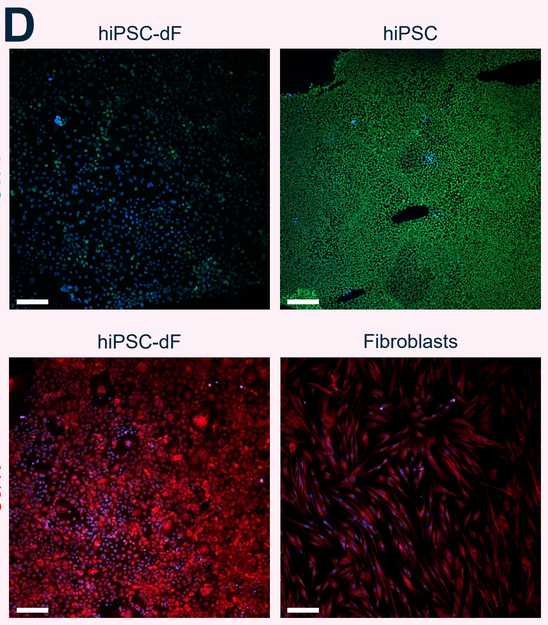
Methods:

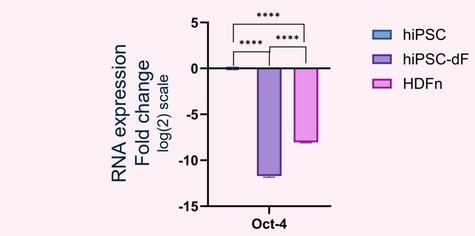
Protocols for the differentiation of hiPSCs towards dermal fibroblasts[1] (hiPSC-dF) and epidermal keratinocytes[2] (hiPSC-dK) were established. The derived skin cells were characterised by RT-qPCR and immunofluorescence (IF) staining. Skin equivalents were generated to evaluate cellular functionality to build a dermal equivalent and to stratify, respectively. In the dermal model hiPSC-dF were seeded on microporous scaffold (Alvetex®) for 14 days. The stratification was performed by seeding hiPSC-dK on dermal models from human dermal fibroblasts, followed by 3 days of submerged culture and 21 days at the air-liquid interface.

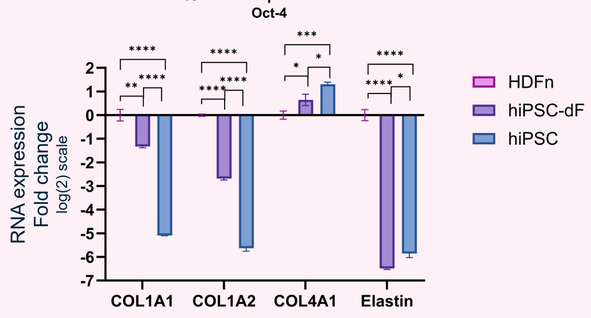
hiPSC-derived fibroblasts



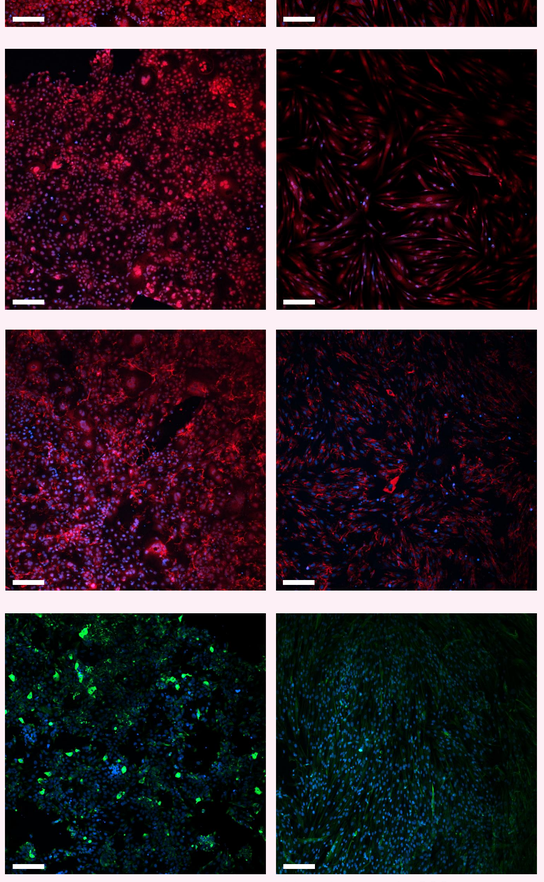
2D

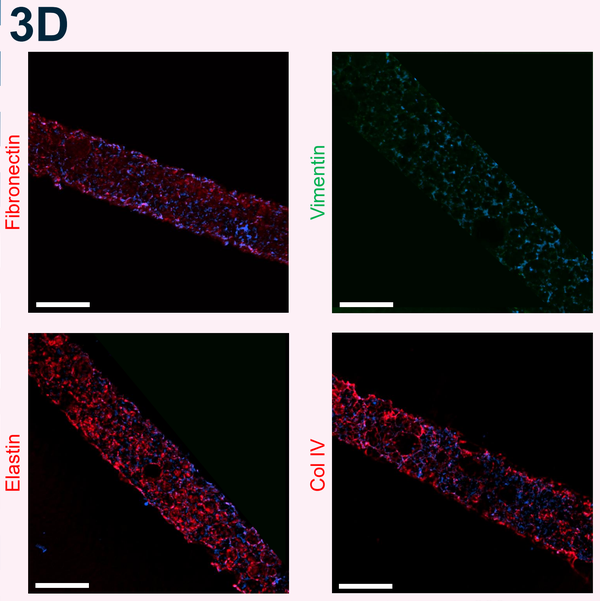




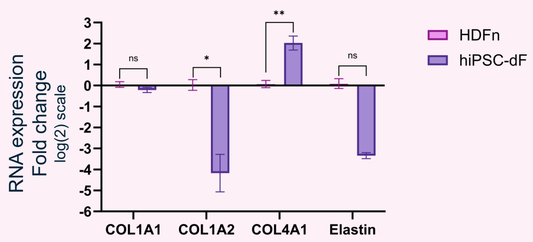


3D

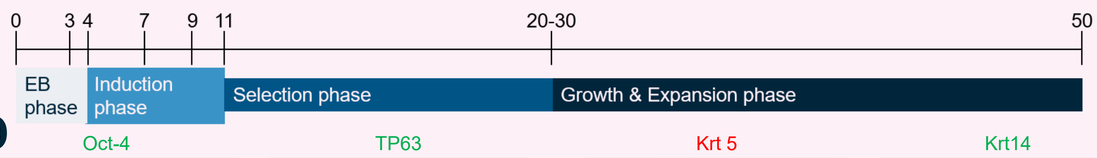




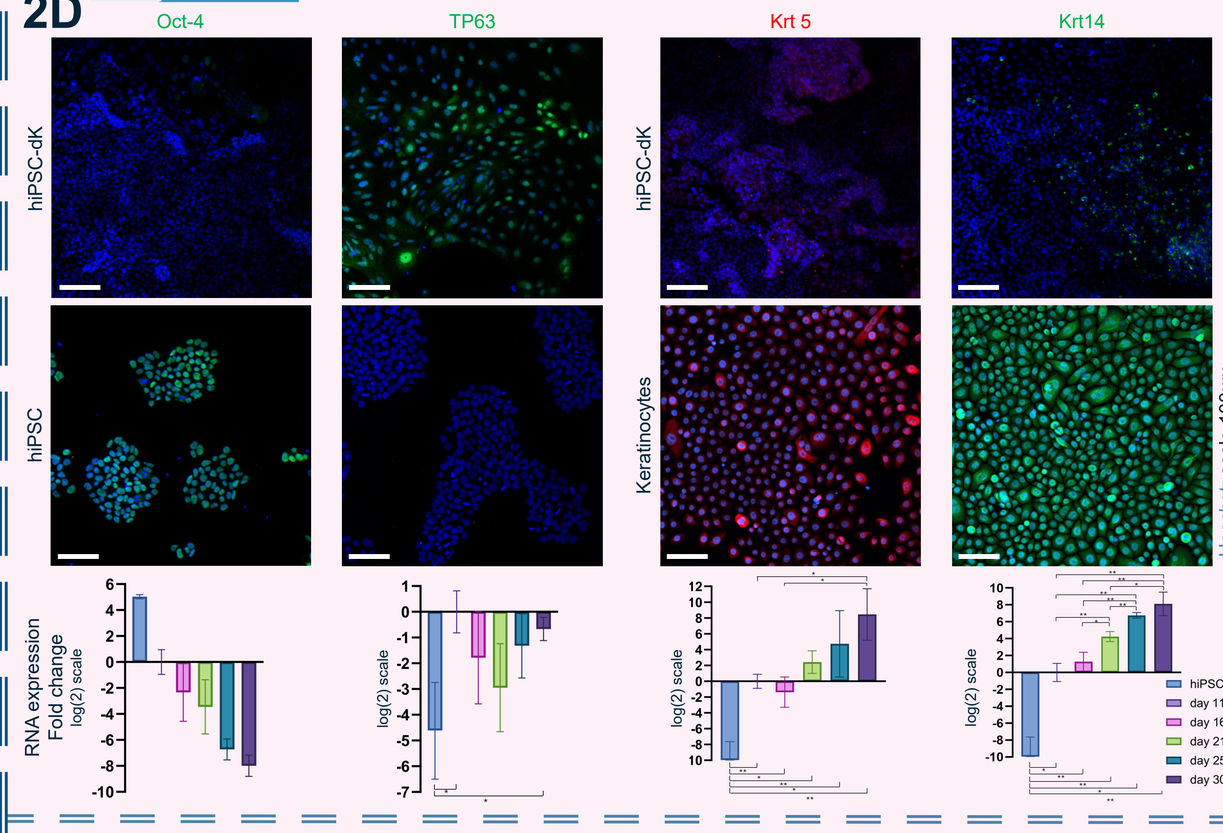
Hoechst; scale 200µm



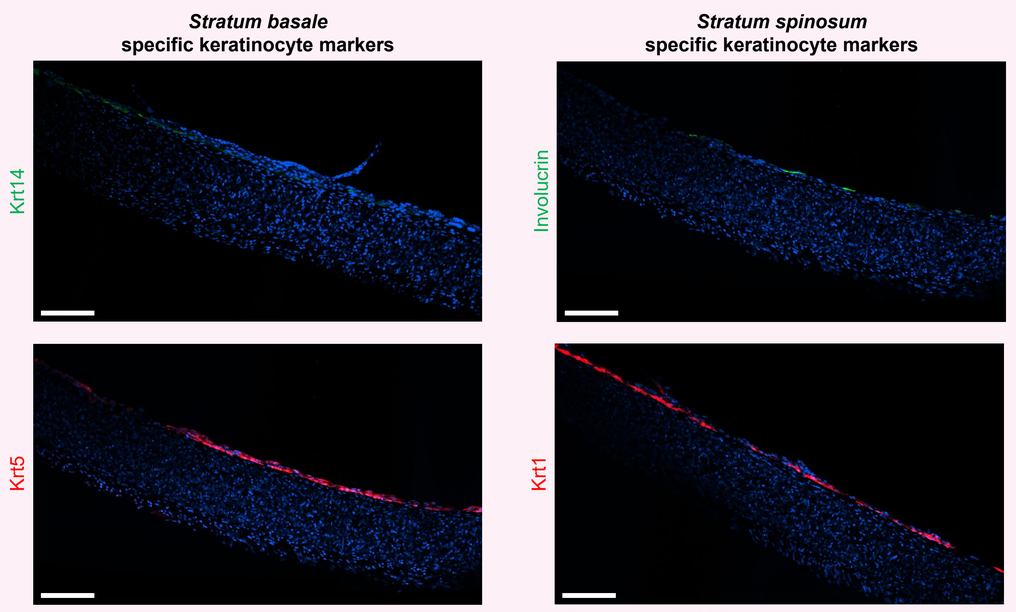
hiPSC-derived keratinocytes



2D



3D



Hoechst; scale 200µm

Conclusion:

Functional skin cells were differentiated from hiPSCs, enabling the generation of dermal models from hiPSC-derived fibroblasts, evaluated by the expression of ECM proteins in 2D culture and in the dermal model.
hiPSC-derived keratinocytes (hiPSC-dK) were characterised by the downregulation of pluripotency markers and the upregulation of keratins 5 & 14. Introducing hiPSC-dK onto a dermal model succeeded in a partly stratified skin model after 38 days.

Outlook:

An *in vitro* skin model fully created from hiPSCs can be developed, a huge progress in patient-specific skin models. This model further provides a foundation to be expanded, i.e. by inclusion of early-stage hiPSC-dK, which are hypothetically epidermal stem cells, leading to an even more physiological model. These personalised skin equivalent might also be adapted for transplantiation.

[1] Itoh, M., Umegaki-Arao, N., Guo, Z., Liu, L., Higgins, C. A., & Christiano, A. M. (2013). Generation of 3D skin equivalents fully reconstituted from human induced pluripotent stem cells (iPSCs). PloS one, 8(10), e77673. https://doi.org/10.1371/journal.pone.0077673
[2] Koch, P. J., Webb, S., Gugger, J. A., Salois, M. N., & Koster, M. I. (2022). Differentiation of Human Induced Pluripotent Stem Cells into Keratinocytes. Current protocols, 2(4), e408. https://doi.org/10.1002/cpz1.408

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN

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