INTRODUCTION

In the quest for alternatives to the use of laboratory animals for the testing of toxic actions on skin (sensitization, irritation and corrosion tests) we were interested to establish techniques in our laboratory for the preparation of 3D-Reconstructed Human epidermis (RHE) models from primary human keratinocytes. We report here on the preparation and characterisation of 3D-RHe from Normal Human Epidermal Keratinocytes (NHEK). We have confirmed the skin irritation potential responses of the NHEK-3D-RHE to a recognized proficiency panel of chemicals (OECD TG 439 RHE). We also present here data on the induction of cytochrome (CYP) P450 isoytes in an in vitro human skin model (EPISKIN) by standard inducing agents.

RESULTS

Figure 1: Culture of 2D and 3D-Epidermis derived from primary keratinocytes.

NHEK cells are seeded and cultured on petri dishes in KBM-Gold medium for amplification until 70% confluence. In the immersion phase, primary keratinocytes are seeded in the medium on thin inserts at 37°C for 72h (A). On day 3, the medium is changed to CnT-02 and cells are incubated for 24 hours (B). On day 4 the CnT-02 is discarded (from the insert only) and then the air-exposed phase for induction of vertical differentiation begins. This phase takes 2 weeks and leads to the 3D epidermids.

Figure 2: Skin architecture of Reconstructed Human Epidermis (RHE) derived from primary human keratinocytes.

Hematoxylin and eosin staining was performed. On the control Episkin epidermis, staining shows a structure with its basal, spinous, granular and cornified layers (A). On the NHEK-3D epidermis, staining shows that all layers of the structure are present (B).

Figure 3: K10/K14 immunofluorescence of 3D epidermis derived from primary human keratinocytes.

Immunostaining for K10/K14 on control Episkin epidermis shows a structure with a basal layer expressing K10, spinous and granular layers expressing K10 (A), and on NHEK-3D epidermis immunofluorescence demonstrates that the basal layer is present and the keratinocyte differentiation has occurred (B).

Methodology 1

We have established techniques for the preparation of 3D-Reconstructed Human Epidermis from NHEK primary keratinocytes, and characterized this model. Key stages in the cell culture of 2D and 3D epidermids were identified, and a range of quality controls were performed on the RHE we prepared.

• The 3D-RHE showed the correct histological structure containing all layers of human epithelium.
• Immunofluorescence staining of keratin 10 and keratin 14 maturation markers confirmed that horizontal and vertical differentiation was successfully achieved.
• In tests performed with a proficiency panel of chemicals (OECD TG439 RHE), the 3D RHE correctly identified the skin irritation potential of the test chemicals.
• We demonstrated the sensitivity of the 3D-RHE model to the induction of CYP-450 isoytes CYP 1A and CYP 1B.

CONCLUSION

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REFERENCES


Table 1: TGN-439-Panel of chemicals for proficiency testing.

<table>
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<tr>
<th>Chemical Name</th>
<th>Class</th>
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The in vitro skin metabolism assay consists of skin treatment with compounds at specific concentrations for 48h. Following exposure, mRNA transcript levels of relevant CYP genes are quantified by RT-qPCR. The CYP induction is based on expression relative to vehicle control (0% DMSO). This value should show a more than 2-fold mRNA level of 0.1% DMSO. After 48h treatment with Dexamethasone, CYP1A1 and CYP1B1 mRNA expression levels are induced. There is no induction of CYP2C9, CYP2C19, CYP2A5 or CYP2E1 mRNA expression levels with their respective established inducers (100 µM Rifampicin, 50 µM Dexamethasone) (n = 3).