RECONSTRUCTED HUMAN EPIDERMIS: AN EFFICIENT PREDICTION TOOL

INTRODUCTION

Pharmaceutical and Dermocosmetic industries are increasingly using the Reconstructed Human Epidermis (RHE) as a model for performing tests, such as skin-penetration rates of various agents, inflammatory reactions, effects of UV irradiation, hormone metabolism. Continuous improvements of this model show it to be an efficient prediction tool for research and development.

Reconstructed Human Epidermis (RHE) is human skin tissue obtained from an *in vitro* process in which human keratinocytes are cultured on an inert polycarbonate medium.

RHE has the ultimate advantage to grow donor epidermal cells in a serum-free culture environment: after rapidly proliferating preparative keratinocyte cultures have been obtained, the yielded epidermal cells are seeded on inert filter substrates that are lifted to the air-liquid interface in a humidified-air incubator. A fully-defined nutrient medium feeds the basal cells through the filter substratum. After 14 days, a stratified epidermis has formed that closely resembles human epidermis *in vivo*. Morphologically, these cultures exhibit a well-stratified epithelium and cornified epidermis with significantly improved barrier function and metabolic activity. Differentiation markers such as suprabasal keratins, integrin β4, integrin α6, fibronectin, involucrin, filaggrin, trichohyalin, type I, III, IV, V and VII collagen, laminin, heparan sulfate and membrane-bound transglutaminase have been found to be expressed similar to those of the epidermis.

IRRITATION & INFLAMMATION

The totally defined and serum-free culture environment allows the detection of very small quantities of inflammatory mediators, cytokines or growth factors secreted by the epidermis in response to topical application of test substances, and in a very reproducible way.

In the field of irritation/inflammation, the combination of dose-dependent cell viability measurements, with IL-1α and IL-8 quantification, can provide enough information to allow, in a single assay, *in vitro* detection, discrimination and classification of irritant and sensitizing agents. In parallel, we have developed protocols in order to evaluate and/or to discriminate the potency of formulations to inhibit epidermal inflammation *in vitro*, by using IL-1α and PGE_2 as endpoints.

A FLEXIBLE MODEL

An advantage of RHE is the possibility of incorporating various additional cell types in combination with keratinocytes or the creation of pathological models.

Melanocytes can be successfully isolated and cultured from the human epidermis and so can be diluted into a suspension of cultured keratinocytes in order to obtain a 3 D culture. As in the *in vivo* state, melanocytes appear as dendritic cells and are polarized to the basal layers maintaining close contacts with both the basal lamina and neighbouring keratinocytes. These cultures provide an attractive *in vitro* system to study the regulation of melanogenesis and melanocyte-keratinocyte interactions but also to test the phototoxic or photoprotective potential of various compounds. More recent advances in culture techniques have made it possible to develop reconstructed epidermis containing not only keratinocytes but melanocytes and Langherhans cells. Cord blood-derived CD34+ hematopoietic progenitor cells induced to differentiate by GM-CSF and TNFα are seeded onto a reconstructed epidermis composed of keratinocytes and melanocytes.

This culture system gives rise to a reconstructed *in vitro* model displaying a pigmented epidermis with melanocytes in the basal layer and resident epidermal

Langherhans cells located suprabasally and expressing major histocompatibility complex class II, CD1 antigen, and Birbeck granules.

PSORIASIS MODEL

In vitro 3D models of psoriasis can also be developed in a serum-free environment. This model is composed of psoriatic keratinocytes cultured on the surface of a gel comprising fibroblasts of the same origin or on a polycarbonate membrane alone. The characterization of this model by immunohistochemistry shows that classical markers of keratinocyte differentiation exhibited similar patterns of distribution in the psoriatic models to those derived from normal cells. However, some crucial differences are observed. Notably, the chemokine receptor CXCR2 is overexpressed in the psoriatic models, and is localized to the granular layer of keratinocytes as seen in psoriasis *in vivo*. Pro-inflammatory genes TNF-a, IFN-y, and IL-8 are expressed at high levels in the psoriatic models, but were only minimally expressed in the normal models.

RHE was also used to predict the phototoxicity potential of raw materials using a library of 13 non-phototoxic and phototoxic compounds, applied topically on the epidermal tissues. Because the epidermal tissues are highly resistant to UVA irradiation, it was possible to increase irradiation by (at least) 3-fold without decrease in tissue viability. In such conditions, the phototoxicity assay is more sensitive, so that the model can be used for the detection of strong (e.g. chlorpromazine or 8-MOP), but also of weak phototoxic compounds such as 6-methylcoumarin or bithionol. Additionally, the epidermal model allows the evaluation of the phototoxic potential of finished products (skin care formulations), as published by Medina et al.

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CONTACT INFORMATION

EUROPE
+ 33 1 53 78 18 79
clinicalresearch@sgs.com

NORTH AMERICA
+ 1 877 677 2667
clinicalresearch@sgs.com

WWW.SGS.COM/CRO