

SkinEthic Laboratories, a Company Devoted to Develop and Produce *In Vitro* Alternative Methods to Animal Use

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Summary

SkinEthic Laboratories is a France-based biotechnology company recognised as the world leader in tissue engineering.

SkinEthic is devoted to develop and produce reliable and robust in vitro alternative methods to animal use in cosmetic, chemical and pharmaceutical industries. SkinEthic models provide relevant tools for efficacy and safety screening tests in order to support an integrated decision-making during research and development phases. Some screening tests are referenced and validated as alternatives to animal use (EpiSkin), others are in the process of validation under ECVAM and OECD guidelines.

SkinEthic laboratories provide a unique and joined experience of more than 20 years from EpiSkin SNC and SkinEthic SA. Their unique cell culture process allows in vitro reconstructed human tissues with well characterized histology, functionality and ultra-structure features to be mass produced.

Our product line includes skin models: a reconstructed human epidermis with a collagen layer; EpiSkin, reconstructed human epidermis without or with melanocytes (with a tanning degree from phototype II to VI) and a reconstructed human epithelium, i.e. cornea, and other mucosa, i.e. oral, gingival, oesophageal and vaginal.

Our philosophy is based on 3 main commitments: to support our customers by providing robust and reliable models, to ensure training and education in using validated protocols, allowing a large array of raw materials, active ingredients and finished products in solid, liquid, powder, cream or gel form to be screened, and, to provide a dedicated service to our partners.

Zusammenfassung: SkinEthic Laboratorien, eine Firma im Dienst der Entwicklung und Herstellung von Alternativen zu Tierexperimenten

SkinEthic ist ein französisches Biotechnologie-Unternehmen, das auf dem Gebiet der Gewebezucht weltweit führend ist. SkinEthic hat sich zur Aufgabe gestellt, zuverlässige und robuste in vitro Alternativen zu Tierversuchen in der kosmetischen, chemischen und pharmazeutischen Industrie herzustellen. SkinEthic Modelle stellen zuverlässige Werkzeuge dar, mit denen Wirksamkeits- und Sicherheitsprüfungen durchgeführt werden können, um während der Forschung und Entwicklung die richtigen Entscheidungen treffen zu können. Einige der Screening Tests sind anerkannt und validiert als Alternativen zum Tierversuch (EpiSkin), andere stehen im Validierungsprozess gemäss ECVAM- und OECD-Richtlinien.

Die SkinEthic Laboratorien weisen mit 20 Jahren EpiSkin SNC und EpiSkin SA eine einzigartige Erfahrung auf. Ihre Zellkulturen erlauben das Arbeiten mit rekonstruierten humanen Geweben mit gut charakterisierter Histologie, Funktionalität und Ultrastruktur-Eigenschaften, geeignet für die industrielle Produktion.

Die SkinEthic Produktlinien enthalten unter anderem eine rekonstruierte humane Epidermis mit einer Kollagenschicht, EpiSkin, rekonstruierte humane Epidermis mit und ohne Melanozyten (mit Bräunungseigenschaften vom Phototyp II bis VI) und ein rekonstruiertes humanes Epithel, wie Kornea und Mund-, Rachen-, Oesophagus- und Vaginalschleimhaut.

Die Philosophie des Unternehmens basiert auf drei Verpflichtungen: die Kunden mit robusten und zuverlässigen Modellen zu versorgen; Trainings- und Ausbildungsmassnahmen mit validierten Protokollen zu ermöglichen, um eine vielfältige Palette von Rohmaterialien, Wirkstoffen und Fertigprodukten in fester, flüssiger, pulverförmiger, creme- oder gelartiger Form prüfen zu können sowie einem Service für unsere Partner dem wir uns voll und ganz verschreiben.

Keywords: EpiSkin, reconstructed human epidermis, cornea, oral, gingival, oesophageal, vaginal

1 Who is SkinEthic Laboratories?

SkinEthic Laboratories is a biotechnology company based in France. SkinEthic is devoted to develop and produce reli-

able and robust *in vitro* alternative methods to animal use in cosmetic, chemical and pharmaceutical industries. SkinEthic Laboratories is an affiliated company of EpiSkin SNC, part of L'Oréal Research.

This cumulative experience of more than 20 years in tissue engineering allows us to provide a unique range of *in vitro* alternative methods based on 3D reconstructed skin and epithelial models to scientists from the industrial and academic communities.

2 What are the SkinEthic models?

The SkinEthic models are divided into 3 main categories (see Fig. 1):

- 1) models that are validated or in the validation process: Episkin, reconstructed human epidermis (RHE) and human corneal epithelium (HCE)
- 2) The skin models: Realskin a full thickness skin and pigmented epidermis RHE
- 3) The epithelial models: human oral, gingival, vaginal and oesophageal epithelium.

3 The models that are validated or in the validation process

In Europe the validation of these alternative methods is under the control of the ECVAM (European Centre for the Validation of Alternative Methods) with the participation of industrial companies and universities. The approval of the validation is the responsibility of an independent committee ESAC (ECVAM Scientific Advisory Committee).

3.1 Episkin:

is an *in vitro* reconstructed human epidermis from normal keratinocytes cultured on a composite collagen matrix at the air-liquid interface (see Fig. 2a). This model is histologically similar to the *in vivo* human epidermis (see Fig 2b).

Immunological, biochemical (keratins and lipid analysis) as well as genomic assays have shown the presence of the main epidermal differentiation markers: keratin 1, 10, 5 and 14, loricrin, filaggrin (see Fig. 3a and 3b), corneodesmosin, and capsase 14 as well as epidermal lipids including ceramide 1 implicated in barrier function.

Episkin is a validated model for skin corrosion (under OECD TG 431 guidelines) and skin irritation (validated by ECVAM). Episkin is a useful model for screening for acute and chronic skin irritation of topical formulations, for testing the phototoxicity of raw materials or finished products, for screening for genotoxicity potential of topically applied compounds or formulations, for ranking skin permeability and metabolism of products, for understanding the effects of

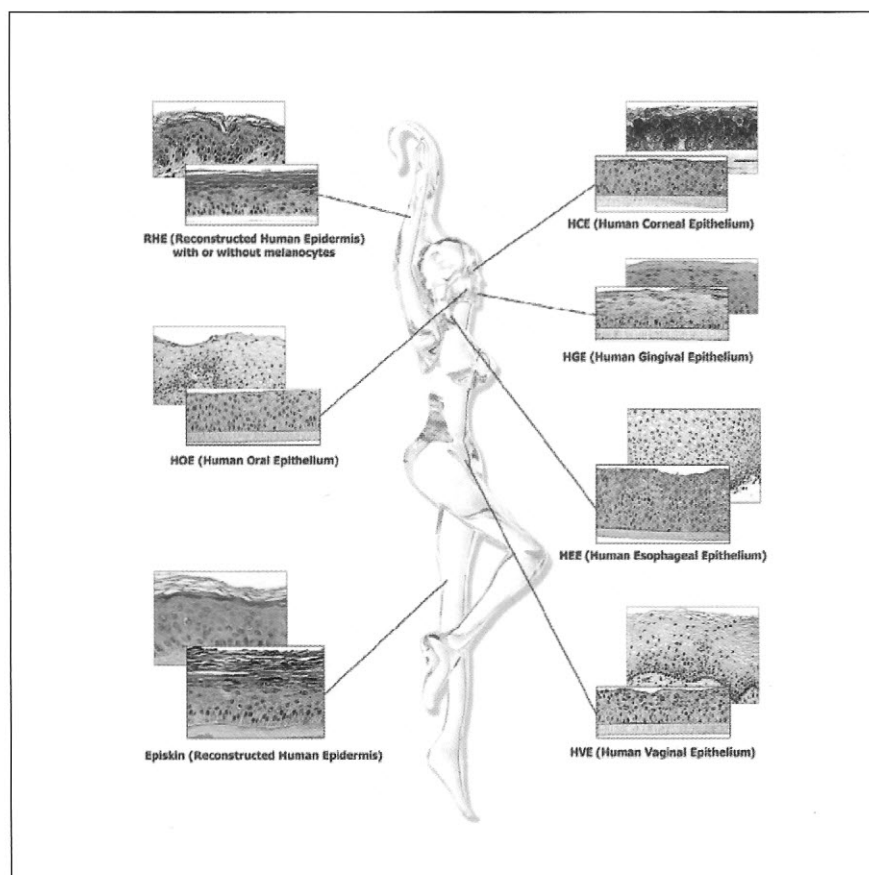


Fig. 1: The family of the SkinEthic models

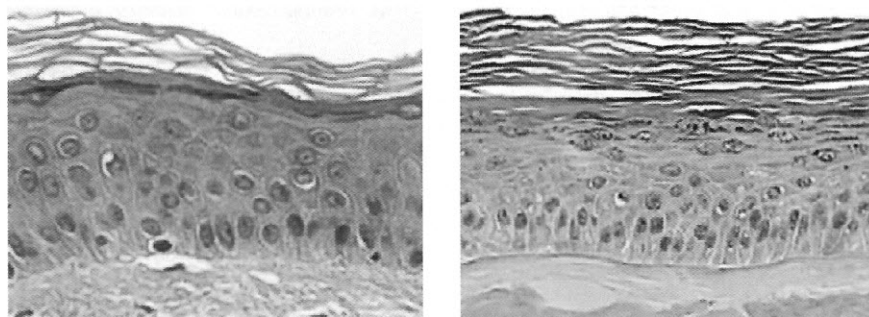


Fig. 2: Episkin. Reconstructed human epidermis (right) is very similar to human epidermis (left)

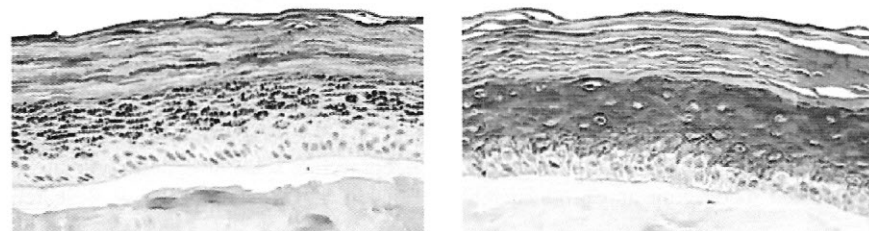


Fig. 3: Episkin. Detection of the epidermal markers filaggrin (left) and keratin (right)

UVA and UVB irradiation and UVB protection or for screening antibiotics. References concerning Episkin are: Flamand et al., 2006 ; Cotovio et al., 2005; Fentem et al., 1998 ; Tinois et al., 1994.

3.2 Reconstructed human epidermis (RHE)

The human epidermis is reconstructed from normal keratinocytes cultured on an inert polycarbonate filter at the air-liquid interface in a chemically defined medium (see Fig. 4a and 4b).

This epidermis expresses the major differentiation markers (filaggrin and involucrin in granular cell layers, transglutaminase 1 and keratin 10 in supra basal layers and loricrin in upper granular layers), and expresses the basal membrane markers (type IV collagen, integrin alpha 6, integrin beta 4, antigen BP, laminin 1 and laminin 5). Free fatty acids and ceramides are detected in the lipid profile. The ultrastructural features show secretion and normal arrangement of bilayered lipids in the intracellular spaces of the cornified cell layers (see Fig. 5a and 5b).

The RHE, a validated model for screening for skin corrosion (under OECD TG 431 guidelines), is under validation for skin irritation and is a useful tool for screening for acute and chronic skin irritation of topical formulations and for phototoxicity testing of raw materials or finished products. The RHE model can also be a useful model for understanding epidermal differentiation, the effects of UVA and UVB irradiation as well as UVB protection, for ranking epidermal permeability and metabolism of tested compounds or formulations, for performing the retinoid activity test and detecting genomic and transcriptomic signatures. References concerning RHE are: Kandarova et al., 2006a ; Kandarova et al., 2006b; Tornier et al., 2006 ; de Brugerolle et al., 1999.

3.3 Reconstructed human corneal epithelium (HCE)

When cultivated at the air-liquid interface, in a chemically defined medium, the transformed cell line HCE reconstructs a corneal epithelial tissue similar to the corneal mucosa of the human eye (see Fig. 6a and 6b).

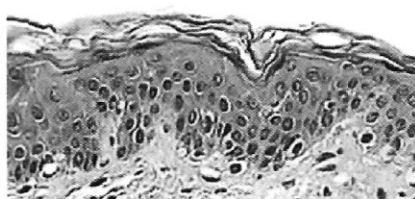


Fig. 4: Reconstructed human epidermis (RHE). The reconstructed epidermis (right) is very similar to human epidermis.

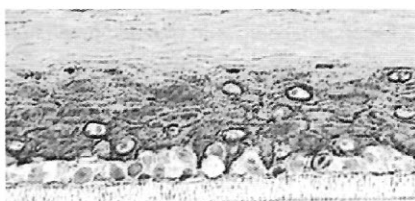
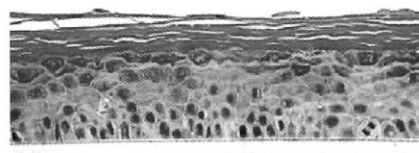


Fig. 5: Reconstructed human epidermis (RHE). Detection of the epidermal markers keratin 10 (left) and filaggrin (right).

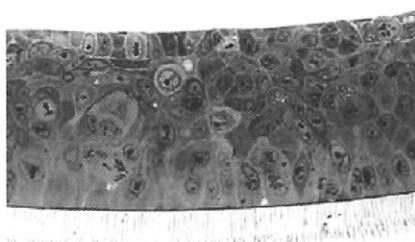
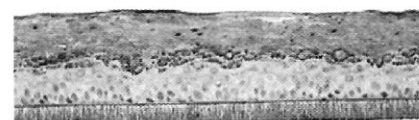


Fig. 6: Reconstructed human corneal epithelium (HCE). *In vitro* (semi-thin, left) and *in vitro* (light microscopy, right).

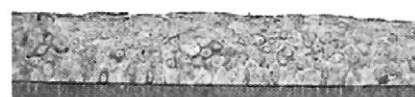
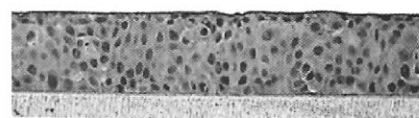


Fig. 7: Reconstructed human corneal epithelium (HCE). The HCE model expresses keratin (left) and CD 44 (right).



The morphological features show the typical presence of a columnar basal cell layer, 2-3 layers of transitional wing cells, and 2-3 layers of superficial squamous cells.

The HCE secretes the same mucins that are found in the human cornea *in vivo* and expresses CD44 and keratin (see Fig. 7a and 7b).

This reproducible, *in vitro* tissue model allows prediction of ocular irritation (in pre-validation phase by ECVAM) of raw materials and formulations, ranking of permeability and metabolism of tested compounds or formulations as well as

their impact on mucin production. References concerning HCE are: Van Goethem et al., 2006 ; Doucet et al., 2006; Nguyen et al., 2003.

4 Skin Models

4.1 Realskin a full thickness skin model

Realskin is an *in vitro* reconstructed human epidermis from normal human keratinocytes cultured on a lattice of collagen populated with normal human fibroblasts at the air-liquid interface.

Realskin is a new model that is undergoing a feasibility phase for validation for testing for the genotoxicity potential of topically applied raw materials or formulations.

4.2 Reconstructed human pigmented epidermal model

When cultivated at the air-liquid interface in a chemically defined medium, normal human keratinocytes cultured in the presence of melanocytes of phototypes II, IV and VI, form a 3D human pigmented tissue.

The different tanning degrees of these constructs correspond macroscopically to 3 different phototypes of human skin (see Fig. 9).

The reconstructed human pigmented epidermal model is characterized by a fully differentiated stratified epidermis where melanocytes are located in the basal cell layer. They synthesize and transfer melanin through their dendrites into the neighbouring keratinocytes (see Fig. 10a and 10b).

This model allows predicting and measuring the whitening effect of compounds or formulations as well as the pigmentation induced by UV irradiation or chemicals. References concerning the reconstructed human pigmented epidermal model are: Straube et al., 2006 ; Sahuc et al., 2004 ; Seiberg, 2003 ; Cappadoro et al., 2002.

4.3 Epithelial models

All epithelial models are reconstructed on a polycarbonate filter at the air-liquid interface in a chemically defined medium.

Except for the gingival epithelium, which is reconstructed from primary cells, all these epithelia are reconstructed from transformed cell lines, TR146 for the oral, A431 for the vaginal and Kyse 510 for the oesophageal epithelium.

These epithelial models are useful screening tools for corrosion, irritation, permeability and metabolism testing of raw materials and formulations. Other research interests are investigating the effects of anti-inflammatory or antibiotic compounds or formulations. References concerning epithelial models are: Bernhardt et al. 2004; Schaller et al. 2002, Schaller et al. 2005, Van de Vannet et al. 2005.

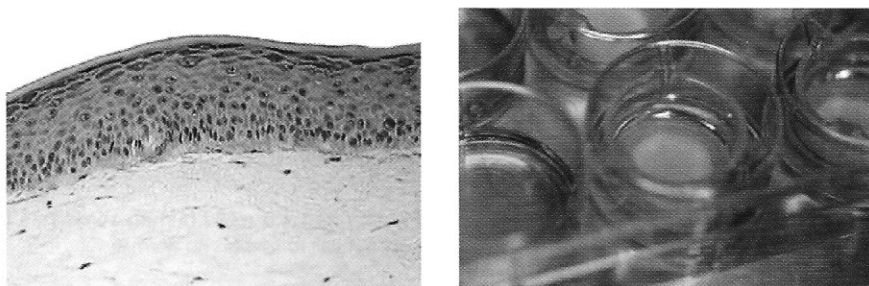


Fig. 8: Realskin. An *in vitro* reconstructed human epidermis (left) cultured on a lattice of collagen populated with normal human fibroblasts (right).

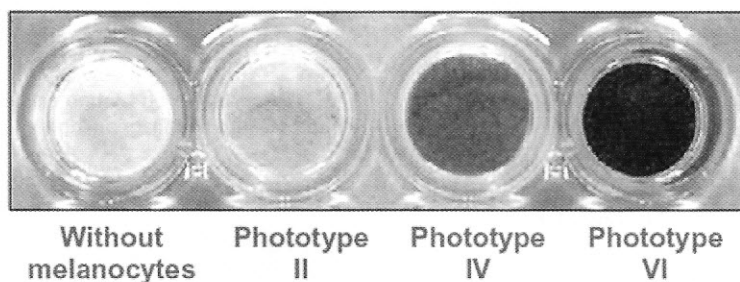


Fig. 9: Reconstructed human pigmented epidermal model. Normal human keratinocytes cultured in the presence of melanocytes of different phototypes of human skin.

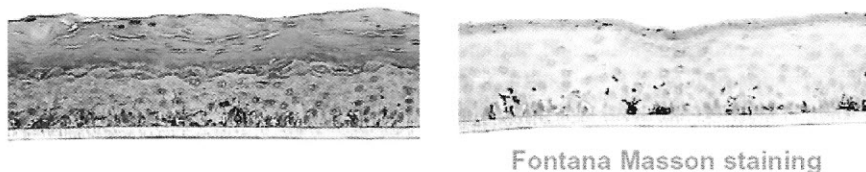


Fig. 10: Reconstructed human pigmented epidermal model. This model allows to predict and measure the whitening effect of compounds as well as the pigmentation induced by UV irradiation or chemical.

5 SkinEthic's philosophy

SkinEthic's philosophy is based on 3 main commitments:

- to develop and produce robust and valuable reproducible models,
- to ensure training and education on using validated protocols allowing the screening of large numbers of raw materials, active ingredients and finished products being either in a solid, liquid, powder, cream or gel form,
- to support our users and partners in providing a dedicated and customized service.

Please visit us at www.skinethic.com

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