

Application of artificial skin models to investigate innovative cosmetics

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Introduction

Innovative products developed by the cosmetic industry are tested for irritation, phototoxicity, genotoxicity of various ingredients by *in vitro* analysis as well as *in vivo* tests. The *in vitro* methods of their assessment have been essential for development of new high quality products. However, the *in vitro* analyses are very expensive and time – consuming and the benefits for the producer are not obtained instantly.

Human skin is the best possible model for *in vitro* tests [1]. Cadaver skin, biopsy material or plastic surgery can be sources of human tissue, however, its use is subject to many legal and ethical concerns. The European Union prohibits financial gain through the application of human tissue, making a widespread use no matter for which purpose very complicated. Until recently, animal skin (e.g. rabbit, mouse, rat skin) was an alternative. The Draize albino rabbit test has been the most frequently used, reliable and accurate to distinguish the potential irritant from nonirritant ingredients in cosmetic formulations [2]. Since the mid-1980s, efforts to achieve a reduction in animal testing have slowly been gaining momentum in Europe. These efforts were initiated under the pressure of animal welfare groups, public opinion and ethical debates. In 1986, the EU directive on the protection of animals used for experimental purposes (Directive 86/609/EEC) banned the application of animal experiments when a scientifically approved alternative existed. The 7th Amendment to the Cosmetics Directive (Directive 76/768/EEC) predicted a complete ban on animal testing for cosmetic ingredients from 2009 for all human health-related effects [2]. The cosmetic industry became pioneer in limiting the number of animals used for safety assessment of chemical substances and stimulated development of new *in vitro* techniques to be applied by both scientific and industrial communities [3].

In recent years several artificial human skin models have been proposed, and some of them are commercially available. They must mimic the relevant properties of human skin as closely as possible [1]. If the model is to be used for regulatory purposes, it needs to be validated, that is its relevance and reliability should be established following the internationally recognized procedures. If the model is to be applied for research on irritant contact dermatitis, it has to be physiologically relevant [2].

Human skin consists of three layers: epidermis, dermis and the subcutaneous tissues (Fig. 1). The organization of the skin into epidermal and dermal layers that differ in thickness, strength, and flexibility allows to determine the specific functions of these skin components [4]. Two functions of human skin are the most important: prevention of desiccation and protection against environmental hazards such as bacteria, chemicals and UV radiation. Artificial skin models, which are now extensively developed, should ensure a competent barrier function and show the same reaction to environmental hazards as human skin.

Until now the EpiSkin[®], EpiDerm[®] and SkinEthic[®] models have been validated by EVCAM (European Centre for the Validation of Alternative Methods). Epidermis created in *in vitro* conditions retains its barrier functions and its lipid profile is almost identical to that manifested by *in vivo*.

The main purpose of this article is to provide a review on the morphology, lipid composition and application of EpiSkin[®], EpiDerm[®], SkinEthic[®] and EpiDermFT[®] models, which currently are the most important existing artificial skin models.

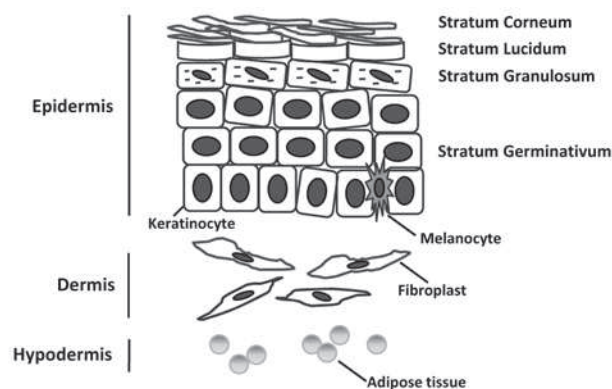


Fig. 1. Skin structure [4]

Artificial Skin Models

The epidermis, dermis and full thickness skin equivalent models are meant to offer the opportunities to enrich the knowledge concerning the processes occurring in the skin. Therefore they should fulfil the following criteria:

- the stratum corneum should contain lipids, with viable cell layer below
- cell viability should be high enough to enable distinction between controlled substances inducing positive and negative effects
- results should be repeatable for a wide range of different chemical substances under defined conditions.

The human epidermis model

• EpiSkin[®]

Attempts at obtaining the human epidermis model were first undertaken by E. Tinois in the 1980s. Nowadays EpiSkin[®] is produced in the Tissue Engineering Laboratories in Lyon, which is a wholly owned subsidiary of L'Oreal group, well-known for studies on reconstructing of epidermis and mucous membrane and responsible for product commercialization [5]. EpiSkin[®] model is commercially available not only for scientific purposes but also for the needs of pharmaceutical, cosmetic and chemical industries.

EpiSkin[®] is a substitute of native epidermis and consists of stratum basale, stratum spinosum, stratum granulosum and stratum corneum. However, the model seems to be simplified because the stratum corneum is thicker and more permeable in comparison to its native equivalent [6].

The preparation of epidermis model EpiSkin[®] starts with covering the bottom of the special vessel with a mixture of lyophilized type I and III collagen, representing the dermis. Subsequently the film of type IV human collagen mimicking the basilar membrane is surfaced and upon it keratinocytes are applied. The culture is grown as long as the special vessel is filled with continuous cell layer. Further cell division and cellular differentiation can be continued, if the culture is placed at the interface between the culture medium (containing higher concentration of calcium ions) and the air. The experiments proved

that after 7÷10 days a highly organized structure (containing type IV and VII collagen, integrin, laminin and hemidesmosoms) mimicking the native epidermis, is obtained [7, 8].

The studies have confirmed that all major epidermal classes were found in the EpiSkin® model such as phospholipids, ceramids, glucosphingolipids and cholesterol. However, in the EpiSkin model the amount of di-/triglyceride and cermides was higher than in normal human epidermis [1]. Additionally, these components are not well organized that affects the penetration and absorption of different chemical compounds.

- **EpiDerm®**

The EpiDerm® model was first developed and described by Cannon et al. in 1994 [9]. The model is produced by MatTek Corporation, Ashland, MA, USA. Nowadays the EpiDerm® is used in laboratories by such companies as Clariol, Johnson&Johnson, Procter&Gamble, Revlon, Unilever and Dr Eris.

According to MatTek Corporation, the EpiDerm® model is composed of normal human derived epidermal keratinocytes, which have been cultured to form a multilayered, highly differentiated human epidermis model. The EpiDerm® model has 3-dimensional structure comparable to EpiSkin®. It consists of 8÷12 cell layers mimicking stratum basale, stratum spinosum, stratum granulosum and stratum corneum. The procedure of obtaining EpiDerm® model is similar to that of the above mentioned EpiSkin®. However, the cells are not surfaced on the collagen film but on the nylon net covered with collagen. The EpiDerm® model includes all major lipid classes in the amounts similar to those in native equivalent. The studies proved a slightly elevated amount of glucosylceramides and lower contents of free fatty acids with reference to native human epidermis [1]. Additionally, it was established that after 14 days the stratum corneum of EpiDerm® model was thicker than its natural equivalent. The proposed explanation of these phenomena was the disability of corneocyte to peel in the EpiDerm® model [10, 11].

- **SkinEthic®**

The SkinEthic® Laboratories was founded in Nice, France by Martin Rosdy in 1992 to design and develop the artificial equivalent of human skin [1]. SkinEthic® model is known as epidermis reconstructed by normal human keratinocytes, which has three dimensional structures, highly similar to human epidermis. All the major components of native epidermis can be found in the SkinEthic® model, such as stratum corenum, stratum granulosum, and stratum spinosum. Additionally, the well-developed hemidesmosomes were identified in the structure by electron microscopy. Despite high similarity of SkinEthic® model to native epidermis, some differences were recognized. Lipid droplets have been found in all layers of the equivalent model which were not encountered in natural epidermis. The highest frequency of lipid droplets was identified in stratum basale. The stratum corneum was thicker in comparison to that in native epidermis [1].

Full thickness skin equivalent

Commercially available full thickness skin substitute has a complex structure consisting of dermis equivalent containing fibroblasts, basilar membrane and epidermis equivalent with keratinocytes.

The most common skin substitute produced by MatTek Corporation is known as EpiDermFT®. Its structure consists of epidermis with all layers. The well-developed basilar membrane and dermis contain fibroblasts. Furthermore, the lipid amount in the stratum corneum is similar to that in the native skin [6]. Additionally, epidermis and dermis interact, which strongly influences the processes taking place in the skin [6].

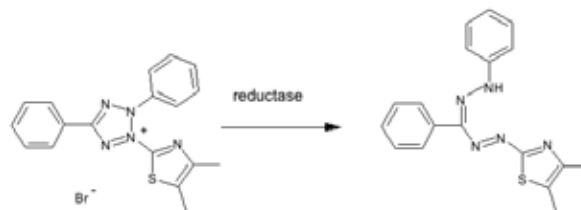
The EpiDermFT® model can be stored under laboratory conditions for about 28 days, which enables the repeatable application of a cosmetic formulation examined at regular time intervals like during the real application of the cosmetic product on the skin, which seems to be the main advantage of this model. However, similarly as for the

models of epidermis, in EpiDermFT® the stratum corneum is not peeled off and its thickness increases, which has a definite influence on permeability of chemical compounds and does not fully correspond to the *in vivo* conditions.

Application of Artificial Skin Models

Irritancy testing

In irritancy tests three-dimensional models of reconstructed epidermis such as EpiSkin®, EpiDerm®, SkinEthic® can be used. The testing for irritating properties of chemical substances in cosmetic products is based on the assessment of cytotoxic action on the epidermal cells known as the MTT (mitochondrial tetrazolium salt) assay, in which the tetrazolium salt is reduced by metabolically active cells. In the first step, a small amount of the test product is deposited onto the surface of the epidermis model (minimal amount 25 mg/cm²). Then the cultures are incubated at 37°C for a specific time depending on the type of epidermis model (from 10 minutes up to 6 days), after which they are analyzed for cell viability by the MTT assay giving repeatable and accurate results. The pre-incubated epidermis model is placed in the MTT solution (0.3÷1 mg/ml) at 20÷28°C. The tissue is reacted with the compound known as MTT which is converted by mitochondrial reductases to dark purple formazan salt.



After 3 hours the purple product, formazan, is extracted with an appropriate solvent such as isopropanol and its concentration is determined by optical density at 545÷595 nm. The lack of dark purple colour in the sample is the indicator of decreased viability and the component tested is classified as an irritant if the tissue viability determined by MTT assay in percent is close to 50% relative to the negative control [12].

Phototoxicity testing

The aim of this test is to determine the relative phototoxic potential of a cosmetic formulation after topical application on different epidermis models. The product examined is placed onto the surface of the six reconstructed epidermal tissues (size 0.5 cm²). All cultures are incubated at 37°C, 5% CO₂ for 24 hours. Then three tissue samples are irradiated with UVA (6 J/cm²) and the other three non-irradiated tissue samples are stored in the dark at ambient temperature. After the UVA treatment the test product is removed and all cultures are incubated for another 24 hours at 37°C, 5% CO₂. Then the irradiated and non-irradiated tissues are assessed for their viability and histology. The epidermis models should be capable of discriminating between phototoxic and nonphototoxic compounds [13, 14].

Penetration assay

The aim of this test is to determine the ease and depth of penetration of the cosmetic product after topical application on the epidermis model. The product examined is applied on two reconstructed human epidermal tissue samples. Untreated vehicle control should be run simultaneously. The medium underneath the tissues is collected and replaced at the time intervals of 1, 2, 3, 6 and 24 hours and it is used for further analysis. After 24 hours of the experiment one tissue is subjected to histology test and the other one to viability assessment. The amount of the compound which has been transported through the model is measured as a function of time [15].

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English translation by the Author

Anna OLEJNIK M.Sc. is a PhD student in the Applied Chemistry Group, at the Faculty of Chemistry, the Adam Mickiewicz University in Poznan. She obtained the Master's degree in 2008, while in 2009 she graduated from additional studies - Chemistry of Cosmetics. Her research is focused on determination of low molecular peptides in cosmetic formulation and their penetration abilities through synthetic membranes. She is a co-author of 2 publications from the ISI master Journal List, 6 articles and 6 presentations at national and international conferences.

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Events IYC'2011

February 2011

The Big Picture - Feb 09, 2011 - Michael Fowler Centre, Wellington, New Zealand

The Chemical Heritage Foundation's Year-long IYC 2011 Celebration - Feb 10, 2011 - Chemical Heritage Foundation
315 Chestnut Street Philadelphia, PA 19106

The Chemical Heritage Foundation's Year-long IYC 2011 Celebration - Feb 14, 2011 - Philadelphia

Chemistry Principles in Action - Feb 14 - Mar 04, 2011 - Tohatchi

The Chemical Heritage Foundation's Year-long IYC 2011 Celebration - Feb 15, 2011 - Chemical Heritage Foundation, Philadelphia

Twelve Months of Chemistry (2011 = Chemistry x 12) - Feb 16, 2011 - Department of Chemistry, University of Aveiro

Sustainable Chemistry, Innovative and Competitive Companies. 2nd edition - Feb 17 - Feb 18, 2011 - Barcelona, Spain

Can Chemistry Be Green? Nottingham Chemistry Public Lecture Series - Feb 17, 2011 - Lecture Theatre XI, School of Chemistry, University of Nottingham, University Park, Nottingham, UK.

Chemistry for All! 'Atoms of Delight' - Feb 17, 2011 - Filmhouse Cinema, Edinburgh

Celebrate the International Year of Chemistry at the AAAS 2011 Annual Meeting Chemistry--Our Life, Our Future - Feb 17 - Feb 21, 2011 - Walter E. Washington Convention Center, Washington

Creating Awareness About the Need to Celebrate Chemistry - Feb 19, 2011 - National Institute Of Technology Karnataka, Mangalore, India