Abstract

Human three-dimensional skin models have become an indispensable tool in current industrial and regulatory toxicity testing activities. Since the early 90’s, several models of the skin have been developed, characterized, validated and accepted as valid replacement method for animal experimentation. In addition current legislative dossiers such as REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), the Cosmetics Directive and the CLP (Classification, Labeling and Packaging) directive continue to stimulate the implementation of skin models in general. Part 1 of this publication will provide an update of the different commercially available skin models in terms of their basic characteristics and testing applications. Part 2, to be published later, will focus in detail on their use for dermal toxicity testing applications including corrosion, skin irritation, phototoxicity, dermal absorption, skin sensitization and genotoxicity testing.

INTRODUCTION

Skin model development in vitro goes back at least 30 years. One of the most important technological breakthroughs was probably the method developed by Pruniéras where human keratinocytes cultivated on a de-epidermised dermis were exposed to the air-liquid interface, which resulted in a fully-differentiated epidermis featuring characteristics of human epidermis in vivo (1, 2). Several other academic laboratories have reported successful reconstruction of epidermis using similar techniques (3, 4, 5, 6). Initially, in vitro epidermal reconstruction models were used purely for basic research interests aimed at obtaining a better overall understanding of skin physiology. Over time it became clear that chemicals could be tested for both efficacy and toxicity and replace current animal test methods.

Because of the attractive market opportunity based on the assumption that in vitro skin models would provide the ultimate research tool for industry to assess cutaneous toxicity and pharmacology of chemical substances, several commercial tissue engineering initiatives focused on “mass producing” 3D in vitro reconstructed human skin models, were started in the early 1990’s. The most important ones will be described below.

Today, in vitro reconstructed skin models are used globally in both industrial and academic research laboratories. In general terms, the implementation of these three-dimensional (3D) in vitro reconstructed human tissue models makes it possible for pharmaceutical, chemical and consumer product companies to:

- test the efficacy of newly developed formulations and products and thus to use them for claim support,
- reproducibly differentiate compounds that cause reversible and irreversible toxicity without resorting to in vivo animal testing,
- provide accurate and measurable mechanistic information that can be utilized to determine whether a molecule or compound can be altered to reduce irritation or toxicity, without loss of efficacy,
- objectively compare the toxicity and efficacy of newly-developed compounds with those already in use in the market place for the same purpose,
- reveal the precursor steps which lead to toxic reactions, thereby making reformulations possible, based on human response,
- evaluate the long term stability or shelf-life of finished products or raw materials,
- determine the potential health risks of employees exposed to chemicals (worker safety).

Furthermore, the use of 3D skin models enable groups to...
conduct safety studies at a fraction of the cost and time required for animal or human testing. Additionally, the methodology is user and implementation friendly, does not require a large staff, extensive facilities, or supplies. This unique species-correct model can reduce or replace animal tests while more accurately assessing the human safety of chemicals. In recent years national and European legislation has placed considerable constraints on industry to totally abandon animal-based test systems when assessing the toxicological potential of chemicals and finished products. Both the REACH directive (Registration, Evaluation, Authorization and Restriction of Chemicals), the Classification, Labeling and Packaging (CLP) directive and the EU Cosmetics directive demand for alternatives to animal testing. Hence they are creating a growing need for human reconstructed skin models, accelerating the adoption of these models in skin-related testing strategies required for regulatory purposes.

This paper, published as 2 parts, will provide an overview of the different skin models (part 1) and their dermal toxicity test applications (part 2) that have been developed over the last 20 years, which include skin corrosion, skin irritation, phototoxicity, dermal absorption, skin sensitization and genotoxicity tests. In addition, some important scientific considerations with regard to their applicability domain will be described.

COMMERCIALY AVAILABLE HUMAN SKIN MODELS IN THE EARLY 1990’S

One of first commercially available models was produced by Organogenesis, Canton, MA, USA and was called LSE (Living Skin Equivalent) and later Testskin I and II. This skin model was originally developed in 1991 (7, 8) and consisted of a differentiated epidermal tissue produced on a collagen lattice populated with human fibroblasts. Various test applications were developed using this skin construct including UV irradiation (9), skin irritation (10, 11), and dermal absorption (12). Unfortunately this model is no longer commercially available as the company decided to focus solely on clinical applications of its skin model called Apligraf®, which obtained FDA approval for the treatment of venous leg ulcers in 1998 and diabetic foot ulcers in 2000. At approximately the same time, another US based company called Advanced Tissue Sciences, La Jolla, CA, USA, launched a set of in vitro reconstructed human skin models under the brand name ‘Skin²RHE’. These full-thickness (dermis/epidermis) skin models called ‘Skin²RHE’ ZK1300 and ZK1350 were shipped worldwide and evaluated thoroughly, both morphologically and biochemically (13, 14), and used in a wide range of toxicological applications. These included skin corrosion for which regulatory approval was obtained by the US Department of Transportation (15), phototoxicity (16, 17, 18, 19), skin irritation (20, 21, 22, 23) and dermal absorption (24, 25, 26, 27). Unfortunately, despite the multiple successful validation efforts, the company’s shareholders decided to withdraw from the in vitro skin business in 1995 and focus entirely on the clinical skin equivalent called Dermagraft-TC, which was developed as a temporary wound cover for burn patients. In 2002, Advanced Tissue Sciences filed for Chapter 11 bankruptcy protection.

COMMERCIALY AVAILABLE SKIN MODELS TODAY

Today a variety of 3D in vitro reconstructed human skin models are commercially available on a routine basis (Figure 1).

Some were already developed in the early 1990’s including the EpiSkin™ model (L’Oréal, Lyon, France) and EpiDerm™ model (MatTek Corporation, MA, USA). Subsequently, additional human skin models were introduced to the commercial market, including the RHE or Reconstructed Human Epidermis (SkinEthic, Lyon, France), the EST1000® skin model (CellSystems, Troisdorf, Germany), the Phenion® Full-Thickness Skin Model and OS-Rep (Open Source Reconstructed Epidermis) model (Henkel, Düsseldorf, Germany), the Straticell model (Straticell, Les Isnes, Belgium), the Stratatest™ model (Stratatech, Madison, WI, USA) and the more recently introduced Labcyte model (Gamagori, Japan) and Vitrolife-Skin™ model (Kyoto, Japan). Some of the basic features as well as testing applications of each of these skin models are described in detail below in chronological order.

Figure 1. Examples of histological cross sections (Hematoxylin-Eosin staining) of 3D in vitro reconstructed skin models: EpiSkin (A), SkinEthic RHE (B), EpiDerm (C), EST1000 (D), Phenion®FT (E), OS-Rep (F), Straticell (G), Stratatest (H) and Sterlab (I) (Magnification not available)

All tissue models described in this paper reveal high similarity with native human skin both in histological architecture (Figure 2) and physiological properties.

The EpiSkin™ and SkinEthic RHE™ skin models
The EpiSkin™ model was originally developed by E. Tinois in 1991 and is composed of a collagen lattice containing human fibroblasts, surfaced with a film of type IV collagen, overlaid with a fully-differentiated human epidermis (28).

Figure 2. Histological cross section of human skin in vivo (Hematoxylin-Eosin staining). The multilayered epidermis (dark red) is cornified at its surface. The underlying dermis consists of fibroblasts embedded in a scaffold of extracellular matrix material. A few capillaries can be seen in cross section.
The models were initially produced by the company Imedex (Lyon, France), that became acquired by L’Oréal in 1997. The SkinEthic RHE™ model was developed by M. Rosdy in 1990. This epidermal construct generated by seeding human keratinocytes on inert synthetic filters, which are consequently exposed to the air-liquid interface and maintained using a chemically defined culture medium (29). The model became commercially available in 1993 by SkinEthic Laboratories, which also was acquired by L’Oreal (in 2006). Today, both the EpiSkin™ and SkinEthic RHE™ models are being produced by SkinEthic Laboratories. Lyon, France.

The EpiSkin™ and SkinEthic RHE™ models have been scientifically validated for skin corrosivity and skin irritation assessment by ECVAM, the European Centre for the Validation of Alternative Methods, and were adopted into Annex V of the Directive for Dangerous Substances Publication at the J.O. European Community Commission Directive 2000/33/EC and Directive 86/609/EC Guide line OECD 431 for corrosion testing (30, 31). In addition, based on the skin corrosion validation study, the EpiSkin™ method is able to distinguish Category 1A from Categories 1B/1C corrosives, mandatory for transportation of substances (US GHS and Transport of Dangerous Goods Regulations). In April 2007, ECVAM announced the validation of an ‘in vitro’ test (EpiSkin™) as a full replacement method for assessing the skin irritancy potential of chemicals, and in 2008, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC) endorsed the SkinEthic RHE™ assay ‘which measures or predicts the same biological or toxic effect as the fully validated and accepted reference method EpiSkin™. Both models are now integrated in OECD guidelines TG439 [32]. In addition, EpiSkin™ and SkinEthic RHE™ are also useful models for screening acute and chronic skin irritation of topical formulations (33), for testing the phototoxicity of raw materials or finished products (34, 35), for screening the genotoxicity potential of topically applied compounds or formulations (36), for ranking the skin permeability and metabolism of products (37), for understanding the effects of UVA and UVB irradiation and UVB protection (38), dermal penetration (39) and for detecting the genomic and transcriptomic signatures (40), for contact allergy profiles (41). Detailed information on the EpiSkin™ and SkinEthic RHE™ models can be found on http://www.cellsystems.de.

The EpiDerm™ skin models

The EpiDerm™ in vitro reconstructed skin model, produced by MatTek Corporation, Ashland, MA, USA was originally developed by J. Kubilus and M. Klausner. This model is also composed of human epidermal keratinocytes that develop a fully-differentiated epidermal tissue (fully-differentiated natural epidermis displaying a basement membrane, proliferating keratinocytes and a stratum corneum) after air-liquid interface cultivation on inert polycarbonate membranes. The EpiDerm™ model was introduced to the commercial market in 1993 and was first described by Cannon et al, (42). Later MatTek introduced a full-thickness model called EpiDerm-FT™ that featured both epidermal and dermal layers (43). Shortly after, a melanocyte containing epidermal model called MelanoDerm™ (44) was developed. For 15 years, the company has consistently mass-produced, in a highly reproducible way, the EpiDerm™ tissues (45).

The EpiDerm™ models are used by several industries in product development, claims substantiation, safety assessment and drug discovery and have been involved in several regulatory validation studies. These include skin corrosion (46, 47) and skin irritation (48, 49) for which the model obtained regulatory approval as full replacement method, and are referenced in the OECD TG 431 (skin corrosion) and OECD TG 439 (skin irritation). In addition, as demonstrated in two independent studies, in vitro skin irritation data obtained with the EpiDerm™ model correlate well with human dermal irritation effects caused by cosmetics and chemicals (50, 51). Also pre-validation studies on photoactivity (52, 53) and dermal penetration validation studies (54, 55) have been performed. A variety of additional testing applications using the EpiDerm™ models include cosmetic products testing (50), skin metabolism (56-57) genotoxicity (58), nanotoxicity (59, 60), and pharmacology (61). Detailed information on the EpiDerm™ models can be found on http://www.mattek.com.

The EST1000® skin model

The EST1000® skin model is an in vitro 3D reconstructed human epidermal model produced by Cell Systems, Troisdorf, Germany. This model has been commercially available since 2004 and is used in a wide range of regulatory toxicology tests (e. g., irritation, corrosion and phototoxicity studies). EST1000® was validated successfully and obtained regulatory approval for skin corrosion testing in accordance with the OECD test guideline 431 (62). Currently the EST1000® model is undergoing validation for skin irritation. In addition, the model was assessed for its utility in skin sensitization testing (63) and is currently taking part in a pre-validation study for skin sensitization [personal communication]. Other applications of both EST1000® model include phototoxicity and genotoxicity (64, 65, 66). Very recently the name of the EST1000® model was changed into epiCS®. More information can be found at http://www.cellsystems.de.

The Phenion® Full-Thickness Skin Model and the OS-REP model

The proprietary Phenion® Full-Thickness Skin Model (Phenion® FT model) was originally developed by scientists of the Henkel AG & Co. KGaA (Düsseldorf, Germany) in collaboration with the Universities of Frankfurt and Munich, and was introduced commercially in 2006. Human fibroblasts isolated from biopsies obtained from the...
Healthy donors are grown in a specially-produced stable matrix equivalent. After the development of this dermal equivalent, keratinocytes originated from the same donor are seeded on top. Within several days an epidermal tissue featuring all typical epidermal layers including a multilayered stratum corneum develops. The Phenion® Full-Thickness Skin Model exhibits structural and physiological properties comparable with native human skin (67). It is primarily used for efficacy studies, skin irritation (68), phototoxicity (69), dermal absorption (70), metabolism (71) and genotoxicity studies (72). Henkel also launched the so-called ‘Open Source Reconstructed Epidermis’, or OSRep model. The Open Source concept supports the idea of free public access to information and systems, currently best known in the computer software market. When applied to skin models “Open Source” means that, once a production method has been established, the know how becomes openly available in order to empower scientific groups throughout the world to produce the model ‘internally’, independent of any commercial supplier (73). The idea of a protocol for epidermal model production which can be applied by any experienced user was first introduced by Poumay et al. (74). Based on this protocol, which was optimized by Henkel regarding medium composition and cell quality, the OS-Rep model was established, featuring basic epidermal characteristics (75, 76). In addition, to expand the scientific knowledge base of this model, it is intended to be used for the toxicological assessment of substances, thus making it a viable alternative to animal testing. Clearly, especially for regulatory purposes, validated SOP’s and quality criteria for both OS model production as well as testing protocol have to be strictly respected. Recently the OS-Rep model underwent a successful catch-up validation for skin irritation assessment, which has been submitted to EURL-ECVAM for official regulatory acceptance (77). More information on the Phenion®FT and OS-Rep models can be found at http://www.phenion.com.

The StratiCELL® skin model
The company Straticeell, Les Isnes, Belgium, founded in 2005 as spin-out of the University of Namur, Belgium, has developed two proprietary in vitro skin models. The StratiCELL® reconstituted human epidermis with and without pigmentation, is composed of normal human keratinocytes and melanocytes cultured in a well-defined serum-free medium on a polycarbonate filter at the air-liquid interface. In addition, the company provides epidermal models from young/old & normal/diseased individuals (atopic dermatitis and psoriasis). Currently, the StratiCELL® skin model is preparing for catch-up skin irritation validation and the models were also evaluated for usefulness in nanotoxicology testing (78). Additional specific safety applications of the StratiCELL® skin model, for assessing genotoxicity and phototoxicity are currently being evaluated. For more information, please browse http://www.straticeell.com.

The StrataTest® skin model
The StrataTest® human skin model, developed and commercialized by the company StrataTech, Madison, WI, USA, is a fully-stratified, multi-layered human skin tissue composed of both epidermal and dermal components. The epidermal compartment is generated by the terminal differentiation of NIKS® keratinocytes, a spontaneously immortalized cell line, which serves as a unique, consistent and unlimited source of human keratinocyte progenitors (79). The dermal compartment of the model contains normal human dermal fibroblasts distributed throughout a fibrous collagen matrix. The NIKS® cells are non-tumorigenic, pathogen-free, and have been clinically evaluated as the epidermal component of StrataGraft® skin substitute tissue (80). Unlike skin models that must replenish keratinocyte sources periodically as stocks are depleted or utilize cells pooled from multiple donors, a uniform keratinocyte source is used to manufacture the StrataTest® human skin model. This provides a consistent, reproducible test platform. Several studies have described the biological response of NIKS® generated models to chemical compounds (81), antibiotic formulations (82), and surfactants (83), signaling proteins (84), and environmental factors including hypoxia (85, 86), ozone, cigarette smoke, and ultraviolet light (87). An additional unique feature of the NIKS® keratinocytes resides in their ability to be genetically-modified by non-viral means to generate stably-transfected clones (88, 89).

In vitro skin irritation data generated with the StrataTest® model are comparable to human in vivo scores (90). Currently the model is preparing for catch-up skin irritation validation.

More information can be found at http://www.stratatech.com.

The LabCyte™ skin model
The LabCyte™ skin model (EPI-model) is produced by Japan Tissue Engineering Co., Ltd., Gamagori, Japan, by culturing human epidermal cells on inserts. After human epidermal cells have proliferated, exposure to the air-liquid interface causes it to keratinize, creating a cultured epidermis model similar to the human epidermis (91). The EPI-model has undergone validation for skin corrosion in accordance with the OECD test guideline 431 (92). More recently the LabCyte™ model has undergone a formal catch-up validation study for skin irritation (93) and has been included into a new draft version of the OECD TG 439 (94).

More information can be found at http://www.jpte.co.jp/.
The Vitrolife-SKIN™ skin model
The Vitrolife-SKIN™ skin model, produced by Gunze Corporation Ltd, Kyoto, Japan, is a commercially available 3D reconstructed skin model, which is supplied as a kit containing 24 collagen sponges without cells and culture medium. The models need to be prepared in the laboratory (cultivation of cells in sponges) resulting in a 3D skin model composed of a dermis and an epidermis with cornified layers as described in the literature (95, 96). The model was recently validated for corrosivity (97, 98) and obtained the JACVAM (Japanese Centre for the Validation of Alternative Methods) validity statement that the Vitrolife-SKIN™ corrosivity assay complies with the OECD guideline TG 431 (99). Additional information on the model can be found at http://www.gunze.co.jp/e/medical/.

OTHER SKIN MODELS
In addition to the skin models described above which can be considered the most widely used models in industry today, several other in vitro models of human skin have been developed. For instance, the Company Bioalternatives, Gençay, France, has developed an in-house reconstructed epidermis model, called EPI-8a, however this skin model is mostly used for pre-clinical efficacy studies (100, 101). More information at http://www.bioalternatives.com/

The Swiss-based company CellNtech Advanced Cell Systems AG, Bern, commercializes a 3D epidermal ‘kit’ which contains all necessary materials and reagents, including epithelial cells, to reconstitute a 3D epidermal model in the laboratory (102). More recently, Sterlab, a tissue engineering laboratory based in Sophia Antipolis, Nice, France, provides 3D tissue models ‘on demand’, including skin models using its proprietary chemically defined medium. Both epidermal, dermal-epidermal models with or without the presence of melanocytes, or Langerhans cells are proposed for research and preclinical testing applications, including toxicity and efficacy evaluations. Additional information on the Sterlab skin models can be found at http://www.sterlab.com/ingenierie_tissulaire/uk/home.html. Finally, the UK-based biotech company Evocutis has developed a full-thickness skin equivalent called LabSkin™ consisting of a fully differentiated epidermis on top of a dermal compartment composed of fibroblasts embedded in a bioartificial matrix. Originally developed as a platform to study the microbial system of human skin in vitro and eventually to develop anti-microbial products, the applications of LabSkin™ have extended to wound healing studies, basic and applied skin research, structural, metabolic and physiological studies and the testing of ingredients and product efficacy. More information can be found on www.evocutis.com.

CONCLUSION
Commercially available 3D skin models have become reliable tools in today’s toxicity and efficacy testing for both preclinical and regulatory purposes. In this publication (Part 1), we have provided an overview of the mostly used skin models describing their most important features. Part 2 of this publication, to be published soon, will focus in detail on the different testing applications including skin corrosion, skin irritation, phototoxicity, dermal absorption, skin sensitization and genotoxicity tests. In addition, some important scientific considerations with regard to their applicability domain will be described.

ACKNOWLEDGEMENTS
We would like to thank the following friends/colleagues for their assistance to the realization of this paper: Dr. Michel Salmon of Stratatcell, Dr. Cathy Rasmussen of Stratatech, Dr. Helena Kandarova of the MatTek Corporation, Dr. Oliver Engelking of Cell Systems GmbH, Dr. Kerstin Reisinger and Dipl. Biol. Lars Vierkotten of Henkel AG & Co. KGaA.

REFERENCES AND NOTES
2. E. Bell, S. Sher et al., J Invest Dermatol. 81(1 Suppl):2s-10s, 1983.

The complete list of references is available on our website: www.hpc-today.com
The building blocks defined in the late 20th century such as SPF and UVA standard tests, new sunscreen actives, greater product efficiency, improved product textures and presentations and more, have created technology platforms that should help us launch the next generation of sun protection products.

The general trend towards health and wellbeing is fundamental to what we are trying to achieve in sun protection. The controversy around vitamin D deficiency and sun exposure has raised serious questions concerning possible over-avoidance of sun and the role of sun protection products, whilst on the other hand skin cancer is increasing even though there is a greater awareness of sun damage to the skin. On top of this we have questions of safety of sunscreen actives, in particular the oestrogenic characteristics and the implied effects on water ecology.

It is clear that the sun care industry must continue to improve products and test methods and that we need to work together with other disciplines to create holistic approaches to sun exposure and protection. There are questions on the quality and quantity of sun protection required; the role of regulations as a driver of change, and the development and application of technology to create even better and new types of products.

The Sun Protection Conference will update attendees on the state of the art in sun protection concepts, technology, regulations and product testing and we will discuss and explore the vision of sun protection for the 21st century.

Day 1 topics and speakers:

**Session 1: Vitamin D, skin cancer and sun protection**
Chairman: Dr. Jack Ferguson, SkinInnovation Ltd, UK
KEYNOTE - Influencing sun protection behaviour – Lessons learnt, future directions
Craig Sinclair, Cancer Council Victoria, Australia

Vitamin D – does diet matter?
Professor Helen Macdonald, University of Aberdeen, UK

Sun, sunscreens and vitamin D
Professor Antony Young, King’s College London, UK

Vitamin D, photoprotection and Fitzpatrick skin type
Dr. Steven G. Wang, Memorial Sloan-Kettering Cancer Center, USA

**Session 2: Assessing effectiveness of sun products**
Chairman: TBC

Benefits of daily photoprotection
Professor Paul Matts, Procter & Gamble, UK

The history and future of SPF testing
Bernd Herzog, BASF, Germany

Predicting the efficacy of sunscreens in vivo veritas
Marc Pissavini, Coty - Lancaster, Monaco

Assessment of the Sun Protection Factor (SPF) via Diffuse Reflectance Spectroscopy (DRS)
Eduardo Ribeiro, Johnson & Johnson, USA

Discussion
Four topical sun protection issues will be discussed by an expert panel.

Day 2 topics and speakers:

KEYNOTE - The evolution of human skin pigmentation and its implication for health in the modern world
Professor Nina Jablonski, The Pennsylvania State University, USA

**Session 3: Regulation and safety of sunscreens and standardisation of sun product testing**
Chairman: TBC

Sunscreen regulations worldwide including emerging markets
Debra Redbourm, Dr Cosmetics Regulations, UK

Sunscreen safety in the EU
Professor Vera Rogiers, Vrije Universiteit Brussel, Belgium

Update on ISO test methods for sun protection and review of their adoption worldwide
Dr. Dominique Moyal, L’Oreal Research & Innovation, France

Interlaboratory comparisons of functional tests on sunscreen products
Bruno Berken, BIPEA, France

**Session 4: Sun protection products: A future review**
Chairman: Dr. Jack Ferguson, SkinInnovation Ltd, UK

Beyond SPF – Getting that tan back
Jahn Stanion, Dermatest Pty Ltd, Australia

What are the future sun protection products expected by the social media users?
Anne Caret-Charpentier, Attraction, France

Formulating sun protection products for the 21st century – Efficacy, elegance and globality
Julian P. Hewitt, JPH SunCare Technologies Ltd, UK

Who should attend?
This will be an important meeting for all professionals interested in sun protection, including R&D managers and directors, dermatologists, marketing and product managers, retailers of sun care products, regulatory affairs personnel, formulation chemists, product valuation scientists, research scientists, raw materials suppliers and suppliers of sun product testing apparatus.

Speakers are of international reputation and include scientists, regulators, sun protection specialists, dermatologists, national regulatory authorities and product developers from the sun care industry.

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