Pharmaceutical Research, Vol. 20, No. 8, August 2003 (© 2003)

Review

Xenobiotic Bioconversion in Human Epidermis Models

Audra L. Stinchcomb[1,2]

Received February 4, 2003; accepted April 17, 2003

There is a great need for alternative experimental methods for measuring percutaneous xenobiotic biotransformation. Animal testing and excised human skin studies have been the historical standards for confirmation of therapeutic and toxic effects that occur in the skin as a result of drug and other chemical metabolism. Human skin epidermal bioequivalents have become progressively more used for these types of pharmacological/toxicological studies in recent years. These epidermal models have been used in the form of cell culture, tissue sheets, and highly differentiated epidermal and epidermal/dermal systems. This review highlights the existing published data on the utility of these skin bioequivalent models for various types of metabolism and toxicology studies that should be of interest to the dermatopharmaceutical scientist.

KEY WORDS: human-skin culture; skin equivalent; percutaneous absorption; cutaneous pharmacology; cutaneous metabolism.

INTRODUCTION

Human skin equivalents are desirable tools for measuring percutaneous absorption and other biochemical processes, as shown in Fig. 1. Over the years, a standard keratinocyte culture experimental system has evolved into highly differentiated skin equivalents containing epidermal and even dermal layers. Although all of these tissue equivalent types are useful for a variety of experimental subsets that are described in more detail throughout this minireview, there still remains a certain amount of dissatisfaction in the drug transport community regarding the "leakiness" of many of the available intact tissue models. The barrier properties of many of the skin bioequivalent systems are in fact not equivalent to the human skin, and the reasons for this have been investigated by several researchers inside and outside of the tissue engineering field (1–3). It appears that the essential intercorneocyte lipids are not of the same quantity and quality in many of these *in vitro* systems; however, steady progress toward a human skin match has been made over the last several years (4–12). Skepticism about the utility of these tissue models for permeability measurements should not overshadow their ideal utility for xenobiotic bioconversion studies.

Activation and deactivation of drugs and other chemicals in the skin via metabolic processes are critical factors relevant to therapeutic and toxic outcomes. It is very important that the pharmaceutical scientist have other metabolism experiment alternatives to animal and human studies. Human skin bioequivalent tissue can provide an alternative that can be considered superior to animal experiments because it is derived from human tissue, and also superior to excised human tissue experiments because it is 100% viable. The purpose of this review is to highlight some of the xenobiotic bioconversion studies that have been performed recently in human skin tissue and cell culture models. These experimental systems are very valuable to dermatopharmaceutical science researchers, especially as a supplement to the limited supply of elective surgery human tissue specimens available, and as a way to reduce animal experimentation as well.

HUMAN EPIDERMIS MODEL SYSTEMS

Keratinocyte Culture

Keratinocytes are the primary cells of the epidermis and the most often used cell culture type for xenobiotic metabolism studies. Rheinwald and Green established the first human keratinocyte cultures for experimental use in 1975 (13). The keratinocyte sources for these cultures consist of skin from biopsies and elective surgery (breast and abdominal), as well as foreskin. Culture media has developed over the years into simpler systems with serum-free formulas and time-saving commercial kits (e.g., Cambrex Corporation's Clonetics™ systems; Ref. 14). Comprehensive reviews on keratinocyte culture methods and applications can be found in the literature (15,16). In addition, the HaCaT spontaneously immortalized human keratinocyte cell line, first described in 1988 by Boukamp *et al.* (17), has also been used for dermatopharmaceutical studies (18–21). The HaCaT cell line was the first human skin-derived epithelial cell line that exhibited a full epidermal differentiation capacity when transplanted onto nude mice.

Reconstructed Skin Models

The initial reconstructed skin models were developed during the 1980s. Reconstituted epidermis from hair follicle outer root sheath cells provided the source for the first skin

[1] Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky Lexington, Kentucky 40536-0082.
[2] To whom correspondence should be addressed. (e-mail: astin2@email.uky.edu)

0724-8741/03/0800-1113/0 © 2003 Plenum Publishing Corporation

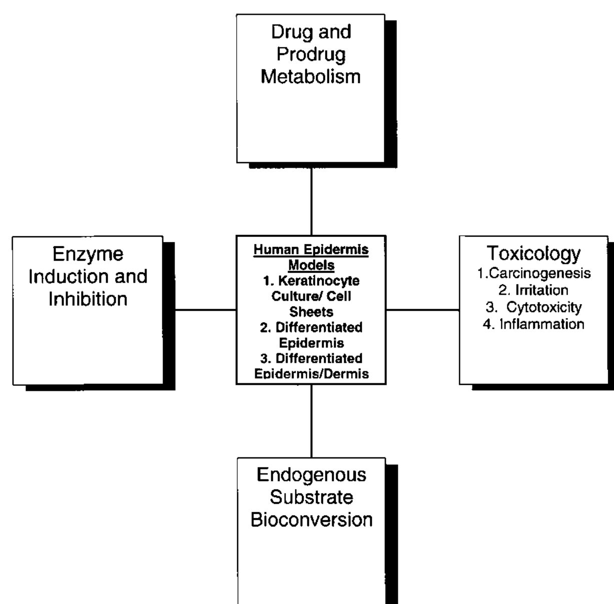

Fig. 1. Schematic of the abundant uses for human epidermis models.

equivalent model for a drug metabolism study in 1990 (22). Some of the more common commercially available reconstructed skin models available today are listed in Table I. Detailed biochemical descriptions of these models can be found at the suppliers' web sites, and comprehensive reviews of the tissue technology have been published (23). Epidermis overlaying a tissue layer containing dermal fibroblasts has become useful for researchers interested in skin irritation, sensitization, and other types of testing where immune response is a driving force of the experimental outcome (12,24). This dermal-epidermal interaction has been shown to be important in retinoid metabolism as well (25). Most drug metabolism studies should not be significantly affected by use of an epidermis-only model unless there is evidence that the compound of interest may be influenced by dermal fibroblast and epidermal keratinocyte cross talk, as may be the case with retinol. Pigmented skin equivalents are also available from the MatTek and SkinEthic companies. These pigmented equivalents can be useful if skin exposure to the sun may influence the experimental outcome, e.g., sunscreen studies, other UV irradiation studies where melanin synthesis is integral (26), and drug metabolism/toxicity studies in areas of sun-exposed skin (27).

SKIN ENZYMES INVOLVED IN DETOXIFICATION AND DRUG BIOAVAILABILITY

Xenobiotic removal from the body via metabolism is mainly accomplished by the liver, but many other tissues in the body also have metabolic capability. The skin has many of the same types of enzyme systems as the liver, but it also has some enzymes that are unique. Common enzymes include the cytochrome P-450–dependent monooxygenases and epoxide hydrolases. Specific activities of these cutaneous enzymes are usually less than 10% of the specific activity of the liver enzymes; however, there are some exceptions. Typical conjugation reactions also take place with glucuronide, sulfate, and glutathione. Some toxicities can also result from enzymatic reactions in the skin, e.g., when compounds are activated into allergens or carcinogens via oxidation (28). Transport proteins (organic anion transport protein [OATP B,D,E], multidrug resistance protein 1 [MDR1 formed after dexamethasone induction], and multidrug resistance-associated proteins [MRP1,3-6]) also exist in the viable keratinocytes of the skin (29). However, no downregulation of these transport proteins occurs in the presence of cytokines as compared with the cytokine-mediated downregulation that occurs in hepatocytes and tumor cells.

CURRENT DATA AVAILABLE FROM HUMAN EPIDERMIS MODELS

Drug Metabolism

One of the earliest reports of comprehensive drug metabolism studies in a human epidermis model was published in 1990 by Pham *et al.* (22). This research group used a reconstituted epidermis model from the outer root sheath cells of human hair follicles. Phase I and Phase II drug metabolism reactions were observed in this model system, including oxidation, reduction, glucuronidation, glutathione conjugation, sulfatase hydrolysis, and epoxide hydrolysis.

The earliest use of a commercially available skin equivalent model for drug metabolism investigation was reported by Ademola *et al.* in 1993 (30). This study included a comparison of an Organogenesis skin equivalent and its homogenate, a basal keratinocyte monolayer cell culture, and split-thickness human skin and its homogenate. The metabolites produced by the intact skin, cell culture, and skin equivalent were similar in quality and quantity. However, a de-ethylation of 7-ethoxycoumarin in human skin and skin equivalent homogenates revealed a 3- to 8-fold increase in enzymatic activity for the skin equivalent over the human skin homogenate. This de-ethylation reaction has been studied as a method for xenobiotic detoxification in the skin (31). Some researchers have argued that observed metabolic activity differences between human skin equivalent models and excised human tissue are the result of the skin equivalents' deficiencies. In cases where the metabolic activity of the skin equivalent is higher than the human tissue, as with this example of de-ethylation,

Table I. Commercially Available Reconstructed Skin Models

Product	Supplier	Web site
EpiDerm™	MatTek Corporation, Ashland, MA, USA	www.mattek.com
EPISKIN™	EPISKIN SNC (L'Oréal), Lyon, France	www.loreal.com
SkinEthic™ HRE	SkinEthic Laboratories, Nice, France	www.skinethic.com
Testskin™II	Organogenesis, Inc., Canton, MA, USA	www.organogenesis.com
$Skin^2$ ZK1300™	Advanced Tissue Sciences, LaJolla, CA, USA[a]	www.advancedtissue.com

[a] Currently under Chapter 11 reorganization.

another argument may be true. It seems logical that living skin equivalents are a more viable system than excised human tissue, with the capacity to have greater metabolic activity. Experiments with skin equivalents are usually performed with the tissue culture system still intact (a 100% viable system) or immediately after excision from the tissue culture plates. Excised human skin experiments are rarely performed at this level of viability, as some significant type of lag time is usually experienced between harvesting the tissue and beginning the experiment. When fresh human surgical waste tissue is used, there is a lag time of at least 1 to 24 h before experiments are initiated. Obviously, the level of viability can be significantly reduced when tissue is harvested from cadavers, a common experimental practice. Tissue sampling times postmortem should be carefully recorded and studied for the effect on metabolic capacities of the enzyme system of interest.

Prodrug bioconversion in a commercially available skin equivalent model was first described in 1994 by Lamb *et al.* (32). A comparison of the prodrug hydrolysis between a human surgical waste skin homogenate and an intact LDE Testskin system showed that the metabolic activity in the Testskin system was one-fifth that of the homogenate. This is not surprising, as intact skin is known to provide decreased drug metabolism as compared to skin homogenates (18,33). Homogenization of tissue exposes enzymes that may not be available to the drug during transit through intact membranes. Maximum metabolism rates have been quickly reached in keratinocyte homogenates, whereas intact keratinocyte cell sheets provided metabolism rates below saturation (18). Substrate diffusion in intact tissue can definitely provide a rate-limiting step to metabolism. Furthermore, it is also possible that reconstructed skin models, with substrate diffusion rates that often exceed diffusion rates in human surgical waste skin, may also exhibit metabolism rates closer to saturation levels than what is seen with the surgical tissue. In this case metabolite levels observed in the reconstructed models may overpredict metabolism in humans. These overestimates of substrate flux and metabolism can be accounted for and incorporated into the interpretation of epidermal model data.

Sandoz Pharma conducted drug metabolism studies in two of Advanced Tissue Sciences' Skin²™ models to compare the potential efficacy of topical treatments for psoriasis (34). These *in vitro* models worked well as a method to compare the rate and extent of cutaneous biotransformation of two cyclosporins.

A peptidomimetic compound, L-Ala-4-methoxy-2-naphthylamide, has been used as an example drug for creating a diffusion and concurrent metabolism model in cutaneous tissue (19). Cultured HaCaT (human transformed keratinocytes) cell sheets were used as part of the model validation. These cell sheets were much more permeable to L-Ala-4-methoxy-2-naphthylamide than stripped human epidermis.

Keratinocyte cultures have been a good source for drug biotransformation studies. Even keratinocytes from postmortem skin have been deftly cultured and used for metabolism studies (27). 7-ethoxyresorufin-*O*-deethylase, phenacetin de-ethylase, procainamide *N*-acetyltransferase, paracetamol sulphotransferase, glucuronidation, and glutathione *S*-transferase (GST) activity were quantitated in these postmortem keratinocytes. Enzymatic activities comparable to those observed in keratinocytes from fresh tissue were found in these post-mortem (<50 h) keratinocytes. Drug metabolizing enzyme activities (except for paracetamol sulfotransferase) in these cultures were significantly higher in skin cells that had been harvested from sun-exposed areas of skin vs. sun-protected sites. The authors of this study believe this activity increase could be a protective response to chemical exposure, as higher amounts of chemicals have been reported to be able to penetrate and accumulate in the epidermis of photoaged skin. Unfortunately, the authors also pointed out, some of the enzymatic activity increases could lead to toxic and mutagenic effects through binding of intermediates to tissue proteins and DNA. Another biotransformation study in keratinocyte cultures has included retinol conversion to 3,4-didehydroretinol (35).

Another significant milestone study in human keratinocyte cultures demonstrated the existence of multidrug resistance-associated transport proteins, as well as the expression of multiple cytochrome P450 enzymes (29). Expression of CYP1A1, CYP1B1, CYP2B6, CYP2E1, CYP3A5, multidrug resistance-associated transport proteins 1 and 3-6, and lung resistance protein were found in keratinocytes using the reverse transcription-polymerase chain reaction analysis. Additionally, dexamethasone induction in this experimental system exhibited expression of cytochrome 3A4 and multidrug resistance 1. Many of these reverse transcription-polymerase chain reaction results were confirmed by immunoblots and immunohistology.

The drug efflux transporter p-glycoprotein (MDR-1) has a unique purpose in the skin, as compared to observed MDR-1 function in other tissues. Recent reports have described the significance of MDR-1 in the immune function of the skin (36). In this study, surface MDR-1 on dendritic and T cells was identified in cultures of split-thickness cadaver skin. MDR-1 antagonists prevented the epidermal dendritic cells and associated T cells from migrating to the lymph nodes to commence an immune response. It seems to be possible to exploit this MDR-1 migration mechanism for enhancing the duration and expression level of therapeutic gene delivery to the skin (37). This immune function behavior has not been observed with dendritic cell multidrug resistance-associated protein (38).

Gene therapy transduction can be studied in human epithelial cell lines (HaCaT, A549; Ref. 20). Improvements in gene transfer can be important for overexpression of intracellular or surface protein within the keratinocytes to modify cellular features to protect against apoptosis and T-cell–mediated cytotoxicity.

Induction and Inhibition

Cultured keratinocytes have been used to examine induction of the phase I enzymes, CYP1A1 (cytochrome P4501A1) and NADPH reductase, and the phase II enzymes UDP-glucuronyltransferase and GST (39). These enzymes are of special interest because they are active in carcinogenic chemical metabolism. The chemicals used for induction in these studies included 3-methylcholanthrene, dimethylbenz-[*a*]anthracene, phenobarbital, clofibrate, and all-*trans* retinoic acid. The CYP1A1 expression in the keratinocytes correlated with the CYP1A1 mRNA levels.

Another cultured keratinocyte enzyme induction study is

of particular interest because the data suggest that enzyme activities were either slightly or insignificantly reduced after freezing the keratinocyte cultures (40). Storing frozen keratinocyte cultures that will still be viable upon thawing adds some convenience and flexibility to experimental design. Several enzymes were investigated, including ethoxyresorufin-*O*-de-ethylase, GST, phenacetin de-ethylase, procainamide *N*-acetyl transferase, and paracetamol sulfotransferase. Induction of vitamin D_3 25-hydroxylase (CYP27) mRNA by vitamin D_3 and UVB radiation has also been demonstrated in keratinocyte culture (41).

Human skin equivalents have been used to study the activation of vitamin D_3 to calcitriol (42). In this enzymatic reaction, vitamin D_3 is hydroxylated to 1α,25-dihydroxyvitamin D_3, calcitriol. The hydroxylation in this study was inhibited by the P-450 oxidase inhibitor ketoconazole. Another investigation by these researchers showed that provitamin D_3 (7-dehydrocholesterol) could be photo-activated by ultraviolet B irradiation to form calcitriol as well (43). Ketoconazole was also capable of inhibiting this reaction in the tissue.

SkinEthic RHE tissue has been shown to express the steroid 5α-reductase isoenzyme 1 (44). This reductase enzyme is responsible for activation of testosterone to 5-dihydrotestosterone, the more potent form of testosterone. Finasteride, a drug used to treat male-pattern hair loss (androgenetic alopecia), inhibited the activity of the 5α-reductase isoenzyme 1 in the skin equivalent. This same inhibitory effect of finasteride had been previously observed in the HaCaT keratinocyte cell line as well (21).

Carcinogenesis

Not only is it important to understand cutaneous processes for the purpose of topical drug bioavailability, it is even more important to understand how cutaneous processes may affect the safety profiles of topically applied drugs, and other xenobiotics that come into contact with human skin as well. Safety profiles for topical skin exposure can include testing for irritation, inflammation, and cytotoxicity; but accurate genotoxicity assessment can probably be considered the most critical testing procedure for topical compound safety. Animal testing has been the mainstay for *in vivo* genotoxicity assessment for many years. Non-animal testing alternatives are constantly sought after for this research milieu.

One valuable study that examined the use of a commercial human skin equivalent for carcinogenesis evaluation reported a successful correlation with previously published human and murine skin results (45). In this study, known carcinogens were tested for their genotoxic effects in the MatTek EpiDerm model. Benzo[*a*]pyrene, ultraviolet B radiation, ultraviolet A radiation, and psoralen-ultraviolet A radiation effects were evaluated by identifying markers such as DNA adducts, c-*fos*, and *p53* proteins. Ultraviolet irradiation studies have been very successful in the human skin equivalents (26,46–48).

Endogenous Substrate Bioconversion

Although a comprehensive examination of cutaneous endogenous compound biochemical reactions is beyond the scope of this review, it is important to discuss some of the studies that have been done in human skin equivalents. Much of the topical drug delivery and toxicity knowledge base available to the pharmaceutical scientist originated from studies of dermal endogenous peptides, hormones, lipids, proteins, nucleic acids, cofactors, and carbohydrates.

Human keratinocyte cultures have been used to study fatty acid metabolism (49). An active metabolite of the psoriasis treatment drug, etretinate, was found to change fatty acid profiles in keratinocytes and interfere with arachidonic acid esterification into nonphosphorous lipids. Phospholipid oxidation has also been studied in human keratinocyte cultures (50). Preferential oxidation of phosphatidylserine was observed in the Shvedova *et al.* study after treatment with cumene hydroperoxide. Additionally, the role of a lipid metabolizing enzyme transcription factor, peroxisome proliferator-activated receptor-α, has been studied in a reconstructed epidermis model (51). Reconstructed human epidermis has also been used as a model system to demonstrate the requirement of vitamin C in the stratum corneum lipid formation (52).

Skin equivalents are useful for examining immune response in contact dermatitis and skin infections like cutaneous candidosis (53,54). Aspartyl proteinase, a hydrolytic enzyme and virulence factor secreted by *Candida albicans*, has been identified in a SkinEthic tissue model of cutaneous candidosis (53). The ability to use these *in vitro* models, which exhibit tissue behavior under the influence of a disease state, should be helpful in the evaluation of drug treatments and possibly help to save some clinical trial expenses. Development methods for effective topical drug treatments for cutaneous infections and irritation should account for enzymes and other immune factors present in the diseased tissue. Treatment of diseased dermal tissue can present a very different microenvironment to drug therapies than those that are designed to be used on healthy tissue (e.g., transdermal patches for systemic drug delivery).

Irritation markers have been identified in commercial skin equivalents (55). Levels of mRNA for interleukin-1α (an early marker for irritation) were compared in EpiDerm and excised human skin after irritation induction with sodium dodecyl sulfate (SLS) and the water vehicle (56). Water did not induce irritation in the EpiDerm cultures, as the 100% humidity incubation conditions for this system are essentially equivalent to surface water exposure. A 3-fold increase in IL-1α mRNA was observed in SLS treated tissue vs. water vehicle treated tissue for excised human skin and EpiDerm. SLS concentrations were adjusted in these experiments for the permeability differences that exist between human skin and EpiDerm. Overall, EpiDerm was much less resistant to the damage caused by SLS than excised human skin. After the SLS-treatment adjustment for the 10- to 20-fold difference in EpiDerm permeability, similar levels of irritation markers were observed in both tissue types.

Cytotoxicity from wound dressing materials can be investigated in human skin equivalents by quantifying DNA synthesis (57). Similar results have been obtained when human skin and human skin equivalents have been treated with hyaluronidase (58). Endogenous hyaluronic acid and its receptor, CD44, can be studied in the human skin equivalents. Hyaluronic acid is important in tissue growth, wound repair, and cosmetic applications as well.

CONCLUSIONS

Epidermal skin equivalents are becoming more popular for investigating substrate bioconversion that results in therapeutic and toxic effects. As long as carefully controlled experiments are completed with these tissue substitutes, very valuable information about drug delivery and exposure can be obtained. The complete replacement of excised human tissue and animal experimentation has not occurred, but great advances in the development and utilization of the models has taken place over the last twenty-five years. Corrections for permeability differences can be made and incorporated into the data analysis and conclusions drawn from these tissue culture experiments. The dermatopharmaceutical scientist is encouraged to take advantage of these model systems for the investigation of topically applied drugs and other xenobiotics.

ACKNOWLEDGMENTS

Literature searching was facilitated by the efforts of Dana C. Hammell, D. Caroline Earles, and Amanda J. Dean. Financial support provided to ALS by The American Cancer Society (RPG-00-027-01-CDD) and NIH (AA13853 and DA13425).

REFERENCES

1. R. Pouliot, L. Germain, F. A. Auger, N. Tremblay, and J. Juhasz. Physical characterization of the stratum corneum of an in vitro human skin equivalent produced by tissue engineering and its comparison with normal human skin by ATR-FTIR spectroscopy and thermal analysis (DSC). *Biochim. Biophys. Acta* **1439**:341–352 (1999).
2. F. P. Schmook, J. G. Meingassner, and A. Billich. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. *Int. J. Pharm.* **215**:51–56 (2001).
3. M. Ponec, S. Gibbs, G. Pilgram, E. Boelsma, H. Koerten, J. Bouwstra, and M. Mommaas. Barrier function in reconstructed epidermis and its resemblance to native human skin. *Skin Pharmacol. Appl. Skin Physiol.* **14**(Suppl. 1):63–71 (2001).
4. M. Rosdy and L. C. Clauss. Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air-liquid interface. *J. Invest. Dermatol.* **95**:409–414 (1990).
5. L. M. Wilkins, S. R. Watson, S. J. Prosky, S. F. Meunier, and N. L. Parenteau. Development of a bilayered living skin construct for clinical applications. *Biotechnol. Bioeng.* **43**:747–756 (1994).
6. D. Asselineau, B. Bernhard, C. Bailly, and M. Darmon. Epidermal morphogenesis and induction of the 67 kD keratin polypeptide by culture of human keratinocytes at the liquid-air interface. *Exp. Cell Res.* **159**:536–539 (1985).
7. A. Casasco, M. Casasco, N. Zerbinati, A. I. Cornaglia, and A. Calligaro. Cell proliferation and differentiation in a model of human skin equivalent. *Anat. Rec.* **264**:261–272 (2001).
8. E. Boelsma, S. Gibbs, C. Faller, and M. Ponec. Characterization and comparison of reconstructed skin models: morphological and immunohistochemical evaluation. *Acta Derm. Venereol.* **80**:82–88 (2000).
9. A. H. Kennedy, G. M. Golden, C. L. Gay, R. H. Guy, M. L. Francoeur, and V. H. W. Mak. Stratum corneum lipids of human epidermal keratinocyte air-liquid cultures: implications for barrier function. *Pharm. Res.* **13**:1162–1167 (1996).
10. M. Michel, L. Germain, P. M. Belanger, and F. A. Auger. Functional evaluation of anchored skin equivalent cultured in vitro: percutaneous absorption studies and lipid analysis. *Pharm. Res.* **12**:455–458 (1995).
11. A. El-Kattan, K. Creek, P. Wertz, and B. Michniak. Evaluation of a human bio-engineered skin equivalent for drug permeation studies. *Pharm. Res.* **17**:1092–1097 (2000).
12. A. Black, O. Damour, and K. Schlotmann. Investigating human skin barrier lipids with in vitro skin models. In T. Förster (ed.), *Cosmetic Science and Technology Series, Cosmetic Lipids and the Skin Barrier,* Marcel Dekker, New York, 2002, pp. 121–147.
13. J. G. Rheinwald and H. Green. Serial cultivation of strains of human keratinocytes: the formation of keratinizing colonies from single cells. *Cell* **6**:331–344 (1975).
14. S. T. Boyce. Methods for the serum-free culture of keratinocytes and transplantation of collagen-GAG-based skin substitutes. *Methods Mol. Med.* **18**:365–389 (1999).
15. I. A. Bernstein and F. L. Vaughan. Cultured keratinocytes in in vitro dermatotoxicological investigation: a review. *J. Toxicol. Environ. Health. Part B. Cri. Rev.* **2**:1–30 (1999).
16. Y. Barlow and R. J. Pye. Keratinocyte culture. In *Methods in Molecular Biology.* **75**:117–129.
17. P. Boukamp, R. T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, and N. E. Fusenig. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol.* **106**:761–771 (1988).
18. I. Steinsträsser, K. Koopmann, and H. P. Merkle. Epidermal aminopeptidase activity and metabolism as observed in an organized HaCaT cell sheet model. *J. Pharm. Sci.* **86**:378–383 (1997).
19. P. Boderke, K. Schittkowski, M. Wolf, and H. P. Merkle. Modeling of diffusion and concurrent metabolism in cutaneous tissue. *J. Theor. Biol.* **204**:393–407 (2000).
20. C. Doebis, T. Ritter, C. Brandt, B. Schönberger, H. D. Volk, and M. Seifert. Efficient in vitro transduction of epithelial cells and keratinocytes with improved adenoviral gene transfer for the application in skin tissue engineering. *Transpl. Immunol.* **9**:323–329 (2002).
21. R. Altenburger and T. Kissel. The human keratinocyte cell line HaCaT: an in vitro cell culture model for keratinocyte testosterone metabolism. *Pharm. Res.* **16**:766–771 (1999).
22. M. A. Pham, J. Magdalou, G. Siest, M. C. Lenoir, B. A. Bernard, J. C. Jamoulle, and B. Shroot. Reconstituted epidermis: a novel model for the study of drug metabolism in human epidermis. *J. Invest. Dermatol.* **94**:749–752 (1990).
23. R. Roguet and H. Schaefer. Overview of in vitro cell culture technologies and pharmaco-toxicological applications. *Toxicol. In Vitro* **11**:591–599 (1997).
24. L. P. Bernhofer, M. Seiberg, and K. M. Martin. The influence of the response of skin equivalent systems to topically applied consumer products by epithelial-mesenchymal interactions. *Toxicol. In Vitro* **13**:219–229 (1999).
25. D. Hoeller, B. Huppertz, T. C. Roos, P. P. Gutierrez, H. F. Merk, J. Frank, and F. K. Jugert. An improved and rapid method to construct skin equivalents from human hair follicles and fibroblasts. *Exp. Dermatol.* **10**:264–271 (2001).
26. M. Archambault, M. Yaar, and B. A. Gilchrest. Keratinocytes and fibroblasts in a human skin equivalent model enhance melanocyte survival and melanin synthesis after ultraviolet irradiation. *J. Invest. Dermatol.* **104**:859–867 (1995).
27. B. Hirel, E. Watier, C. Chesne, M. Patoux-Pibouin, and A. Guillouzo. Culture and drug biotransformation capacity of adult human keratinocytes from post-mortem skin. *Br. J. Dermatol.* **134**: 831–836 (1996).
28. H. Mukhtar, R. Agarwal, and D. R. Bickers. Cutaneous metabolism of xenobiotics and steroid hormones. In H. Mukhtar (ed.), *Pharmacology of the Skin*, CRC Press, Boca Raton, Florida, 1991, pp. 89–109.
29. J. M. Baron, D. Höller, R. Schiffer, S. Frankenberg, M. Neis, H. F. Merk, and F. K. Jugert. Expression of multiple cytochrome P450 enzymes and multidrug resistance-associated transport proteins in human skin keratinocytes. *J. Invest. Dermatol.* **116**:541–548 (2001).
30. J. I. Ademola, E. Bloom, A. E. Maczulak, and H. I. Maibach. Skin penetration and metabolism: comparative evaluation of skin equivalent, cell culture, and human skin, *J. Toxicol-Cut. Ocular Toxicol.* **12**:129–138 (1993).
31. S. J. Moloney, J. M. Fromson, and J. W. Bridges. The metabolism of 7-ethoxycoumarin and 7-hydroxycoumarin by rat and hairless mouse skin strips. *Biochem. Pharmacol.* **31**:4005–4009 (1982).
32. K. A. Lamb, S. P. Denyer, F. D. Sanderson, and P. N. Shaw. The metabolism of a series of ester pro-drugs by NCTC 2544 cells, skin homogenate and LDE testskin. *J. Pharm. Pharmacol.* **46**: 965–973 (1994).

33. N. Higo, R. S. Hinz, D. T. W. Lau, L. Z. Benet, and R. H. Guy. Cutaneous metabolism of nitroglycerin *in vitro* I. Homogenized versus intact skin. *Pharm. Res.* **9**:187–190 (1992).
34. A. E. M. Vickers, W. A. Biggi, R. Dannecker, and V. Fischer. Uptake and metabolism of cyclosporin A and SDZ IMM 125 in the human *in vitro* Skin2™ dermal and barrier function models. *Life Sci.* **57**:215–224 (1995).
35. O. Rollman, E. J. Wood, M. J. Olsson, and W. J. Cunliffe. Biosynthesis of 3,4-didehydroretinol from retinol by human skin keratinocytes in culture. *Biochem. J.* **293**:675–682 (1993).
36. G. J. Randolph, S. Beaulieu, M. Pope, I. Sugawara, L. Hoffman, R. M. Steinman, and W. A. Muller. A physiologic function for p-glycoprotein (MDR-1) during the migration of dendritic cells from skin via afferent lymphatic vessels. *Proc. Natl. Acad. Sci. USA* **95**:6924–6929 (1998).
37. W. Pfützner, A. Terunuma, C. L. Tock, E. K. Snead, T. M. Kolodka, M. M. Gottesman, L. Taichman, and J. C. Vogel. Topical colchicines selection of keratinocytes transduced with the multidrug resistance gene (MDR1) can sustain and enhance transgene expression *in vivo. Proc. Natl. Acad. Sci. USA* **99**: 13096–13101 (2002).
38. B. Laupèze, L. Amiot, N. Bertho, J. M. Grosset, G. Lehne, R. Fauchet, and O. Fardel. Differential expression of the efflux pumps p-glycoprotein and multidrug resistance-associated protein in human monocyte-derived dendritic cells. *Hum. Immunol.* **62**:1073–1080 (2001).
39. F. Vecchini, K. Mace, J. Magdalou, Y. Mahe, B. A. Bernard, and B. Shroot. Constitutive and inducible expression of drug metabolizing enzymes in cultured human keratinocytes. *Br. J. Dermatol.* **132**:14–21 (1995).
40. B. Hirel, C. Chesne, J. P. Pailheret, and A. Guillouzo. *In vitro* expression of drug metabolizing enzyme activities in human adult keratinocytes under various culture conditions and their response to inducers. *Toxicol. In Vitro* **9**:49–56 (1995).
41. B. Lehmann, O. Tiebel, and M. Meurer. Expression of vitamin D_3 25-hydroxylase (CYP27) mRNA after induction by vitamin D_3 or UVB radiation in keratinocytes of human skin equivalents – a preliminary study. *Arch. Dermatol. Res.* **291**:507–510 (1999).
42. B. Lehmann, T. Rudolph, J. Pietzsch, and M. Meurer. Conversion of vitamin D_3 to 1α,25-dihydroxyvitamin D_3 in human skin equivalents. *Exp. Dermatol.* **9**:97–103 (2000).
43. B. Lehmann, T. Genehr, P. Knuschke, J. Pietzsch, and M. Meurer. UVB-induced conversion of 7-dehydrocholesterol to 1α,25-dihydroxyvitamin D_3 in an *in vitro* human skin equivalent model. *J. Invest. Dermatol.* **117**:1179–1185 (2001).
44. F.-X. Bernard, C. Barrault, and A. Deguercy. Expression of type 1 5α-reductase and metabolism of testosterone in reconstructed human epidermis (SkinEthic®): a new model for screening skin-targeted androgen modulators. *Int. J. Cosmet. Sci.* **22**:397–407 (2000).
45. J. F. Zhao, Y. J. Zhang, J. Kubilus, X. H. Jin, R. M. Santella, M. Athar, Z. Y. Wang, and D. R. Bickers. Reconstituted 3-dimensional human skin as a novel *in vitro* model for studies of carcinogenesis. *Biochem. Biophys. Res. Commun.* **254**:49–53 (1999).
46. N. Chouinard, J. P. Therrien, D. L. Mitchell, M. Robert, R. Drouin, and M. Rouabhia. Repeated exposures of human skin equivalent to low doses of ultraviolet-B radiation lead to changes in cellular functions and accumulation of cyclobutane pyrimidine dimmers. *Biochem. Cell Biol.* **79**:507–515 (2001).
47. M. Robert, V. Bissonauth, G. Ross, and M. Rouabhia. Harmful effects of UVA on the structure and barrier function of engineered human cutaneous tissues. *Int. J. Radiat. Biol.* **75**:317–326 (1999).
48. M. Podda, M. G. Traber, C. Weber, L. J. Yan, and L. Packer. UV-irradiation depletes antioxidants and causes oxidative damage in a model of human skin. *Free Radical Biol. Med.* **24**:55–65 (1998).
49. K. Punnonen, T. Puustinen, and C. T. Jansén. The antipsoriatic drug metabolite etretin (Ro 10-1670) alters the metabolism of fatty acids in human keratinocytes in culture. *Arch. Dermatol. Res.* **280**:103–107 (1988).
50. A. A. Shvedova, J. Y. Tyurina, K. Kawai, V. A. Tyurin, C. Kommineni, V. Castranova, J. P. Fabisiak, and V. E. Kagan. Selective peroxidation and externalization of phosphatidylserine in normal human epidermal keratinocytes during oxidative stress induced by cumene hydroperoxide. *J. Invest. Dermatol.* **118**:1008–1018 (2002).
51. M. Rivier, I. Castiel, I. Safonova, G. Ailhaud, and S. Michel. Peroxisome proliferator-activated receptor-a enhances lipid metabolism in a skin equivalent model. *J. Invest. Dermatol.* **114**:681–687 (2000).
52. M. Ponec, A. Weerheim, J. Kempenaar, A. Mulder, G. S. Gooris, J. Bouwstra, and A. M. Mommaas. The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C. *J. Invest. Dermatol.* **109**:348–355 (1997).
53. M. Schaller, C. Schackert, H. C. Korting, E. Januschke, and B. Hube. Invasion of *Candida albicans* correlates with expression of secreted aspartic proteinases during experimental infection of human epidermis. *J. Invest. Dermatol.* **114**:712–717 (2000).
54. M. Schaller, R. Mailhammer, and H. C. Korting. Cytokine expression induced by *Candida albicans* in a model of cutaneous candidosis based on reconstituted human epidermis. *J. Med. Microbiol.* **51**:672–676 (2002).
55. R. Osborne and M. A. Perkins. An approach for development of alternative test methods based on mechanisms of skin irritation. *Fd Chem Toxic* **32**:133–142 (1994).
56. S. Gibbs, H. Vietsch, U. Meier, and M. Ponec. Effect of skin barrier competence on SLS and water-induced IL-1α expression. *Exp. Dermatol.* **11**:217–223 (2002).
57. V. K. Sieber, W. R. Otto, and D. J. Riches. Cytotoxicity of wound dressing materials assessed using cultured skin equivalents. *Burns* **21**:249–254 (1995).
58. J. P. Laugier, S. Shuster, and M. Rosdy, A. B. Csóka, R. Stern, and H. I. Maibach. Topical hyaluronidase decreases hyaluronic acid and CD44 in human skin and in reconstituted human epidermis: evidence that hyaluronidase can permeate the stratum corneum. *Br. J. Dermatol.* **142**:226–233 (2000).