Evaluation of a Human Bio-Engineered Skin Equivalent for Drug Permeation Studies

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Purpose. To test the barrier function of a bio-engineered human skin (BHS) using three model drugs (caffeine, hydrocortisone, and tamoxifen) *in vitro*. To investigate the lipid composition and microscopic structure of the BHS.

Methods. The human skin substitute was composed of both epidermal and dermal layers, the latter having a bovine collagen matrix. The permeability of the BHS to three model drugs was compared to that obtained in other percutaneous testing models (human cadaver skin, hairless mouse skin, and EpiDerm™). Lipid analysis of the BHS was performed by high performance thin layered chromatrography. Histological evalulation of the BHS was performed using routine H&E staining.

Results. The BHS mimicked human skin in terms of lipid composition, gross ultrastructure, and the formation of a stratum corneum. However, the permeability of the BHS to caffeine, hydrocortisone, and tamoxifen was 3–4 fold higher than that of human cadaver skin.

Conclusions. In summary, the results indicate that the BHS may be an acceptable *in vitro* model for drug permeability testing.

KEY WORDS: drug delivery systems; skin alternatives; transdermal drug delivery; permeability.

INTRODUCTION

In the past decade, many controlled drug release investigations have concentrated on novel designs and improvements of existing transdermal drug delivery systems (TDDS). This can be attributed to the many advantages offered, including, 1) superior patient compliance, 2) avoidance of first pass metabolism, 3) the potential for decreased side effects resulting from the ability to give lower doses with high efficacy (1). Generally, evaluations of TDDS are conducted *in vitro* using excised skins from laboratory animals such as the hairless mouse, rat, and guinea pig (2). These studies usually yield results with much higher permeability data compared to human and this has made it difficult to predict the clinical outcomes of the TDDS. As a result, several other *in vitro* models have been developed to study percutaneous absorption which include shed snake skin, novel polymeric membranes, epidermal lipidic bilayers and dermal skin substitutes, and full-thickness skin substitutes. Recently, there has been an increase in the use of these tissue cultured skin substitutes for permeability and toxicological testing of TDDS and topical formulations (3,4). Traditionally, these substitutes focused on providing skin grafts for individuals with either severe burns or venous ulcers (5). Since the initial testing of skin alternatives in the 1980s a rapid advancement has occurred in development and testing due to the optimization of culture methods for skin cells. However, to date no model has been developed that fully mimics the skin in terms of cell type, numbers of cells, blood vessels, and appendages. At the present time three types of skin substitutes are available: 1) epidermal, 2) dermal, and 3) full-thickness (6–9). The epidermal substitutes were first produced in 1975 when Rheinwald and Green described a method allowing the *in vitro* culture of epidermal cells to produce viable epidermal sheets (10). More recently, MatTek patented an EpiDerm™ System consisting of normal, human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of human epidermis.

The full-thickness skin substitutes are composed of both dermal and epidermal equivalents. Most contain a dermal equivalent composed of fibroblasts and collagen. As the cultured dermal layer develops, the fibroblasts and collagen fibrils begin to interact and the dermal layer contracts uniformly. This contraction results in a gel that has the strength and pliability that mimics human dermis. The mechanism by which the fibroblasts are able to contract the collagen gel is not completely understood. However, the rate and extent of contraction varies directly with the cell and serum concentrations and inversely with the collagen concentration (11). After full-contraction of the collagen gel, keratinocytes are seeded on top and allowed to grow at the air-liquid interface in a culture dish. These keratinocytes differentiate and stratify in 7–10 days into an epidermis, which closely resembles the multilayered epithelium found *in vivo*. Although, these skin alternatives have been improved in recent years there remains a difference in barrier function as compared to native epidermis.

In the present study, bio-engineered human skin (BHS) was constructed and evaluated as an alternative *in vitro* model for transdermal drug delivery studies. This skin was cultured by using previously published methods of culturing artificial skin at the air liquid interface except for two modifications. In this study, the methods of culture were improved by a reduction in relative humidity and the use of serum free media. Separate studies published by Rosdy and Clauss and Mak *et al.* established the importance of humidity and the use of serum free media in improving the characteristics of skin alternatives (12,13). We examined the BHS in terms of its histology, lipid composition, and drug permeability using three model drugs of different lipophilicity. The percutaneous permeability parameters determined using the BHS were com-

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ABBREVIATIONS: BHS, bio-engineered human skin equivalent; DMEM, dulbeccos modified eagles medium; FBS, fetal bovine serum; HC, hydrocortisone; H&E, hematoxylin and eosin; HMS, hairless mouse skin; HPLC, high pressure liquid chromatography; NHEK, normal human-derived epidermal keratinocytes; PG, propylene glycol; SC, stratum corneum; Q_{24}, quantitative amount after 24 h; SKC, skin content.

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pared to those from hairless mouse skin, EpiDerm™, and human cadaver skin.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma Chemical Co. in the highest available purity. Baxter Diagnostics, Inc. (McGraw Park, IL) supplied reagent grade solvents, except for methanol and acetonitrile, which were HPLC grade.

Preparation of Novel Skin Alternative

Fibroblasts and Keratinocytes

Human neonatal foreskin specimens were incubated overnight, epidermal side up, in serum-free MCD153-LB medium containing 2% collagenase at 37°C in 5% CO^2, in a tissue incubator. Primary cultures of human fibroblasts were established from the dermal fragments of the collagenase-treated foreskins. Briefly, dermal fragments were gently minced to separate the cells from the residual stroma and the cells were collected by low speed centrifugation. The cells were then resuspended in Dulbecco's Modified Eagles Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, MO). Primary keratinocyte cultures were established from epidermal fragments of the collagenase treated foreskin. The fragments were placed in 5 ml of serum-free MCDB153-Lb media containing insulin (5 μg/ml), transferrin (10 μg/ml), triiodothyronine (10 nM), epidermal growth factor (5 ng/ml), bovine pituitary extract (35 μg/ml), and $CaCl_2$ (0.1 mM) and the mixture was pipetted up and down several times to disrupt any clumped cells. The cells were then collected by low speed centrifugation and re-suspended in the supplemented MCDB153-LB medium. The cells were reefed 24 h after plating and every 48 h thereafter with 10 ml of the supplemented MCDB153 medium. Confluence was reached in 10–14 days.

Final Preparation of Skin Alternative

The dermal layer was prepared by pouring into a 60-mm petri dish a solution containing 2-fold concentrated DMEM (1.6 ml), FBS (1.6 ml), 0.1 N NaOH (0.4 ml), Vitrogen (2.0 ml) (bovine dermal collagen type 1, Celtrix, Santa Clara, CA), and 150,000 fibroblasts in 1.0 ml of DMEM. The mixture was then incubated for 7 days in 10% CO_2 and 90% air at 37° C, during which time the fibroblasts contracted the hydrated collagen into a tissue like structure. The contracted collagen gel was then transferred to a Snapwell insert system (Corning Costar Corp., Cambridge, MA) and 150,000 keratinocytes in 30 μl of supplemented MCDB153-LB medium was added directly to the top of the collagen gel. The skin equivalent was then placed in a sterile hood for 2 h to allow keratinocytes to adhere to the dermal layer. The skin equivalents were then fed from the top and bottom of the insert daily for 3 days with DMEM (no serum) containing 10% CO_2 and 90% air. The relative humidity of the incubator was controlled at 75%. The skin equivalents where then fed only from the bottom (DMEM no serum) with the upper surface of the culture in contact with the gas phase. Culture at the air-liquid interface promoted the development of a full-thickness epithelium, which approximated epidermal morphogenesis *in vivo*. The skin equivalent was cultured at the air-liquid interface for 7–10 days at which time experimental testing began.

H & E Staining

Skin was fixed overnight in Carnoy's fixative (5% acetic acid in 100% ethanol). Skin was incubated with a mixture containing half toluene and half paraffin for 1-h. Samples were then embedded in 100% paraffin overnight and sectioned in 10 μm sections. The paraffinized section were placed on a microscope slide and incubated in toluene for 5 minutes followed by hydration in reduced ethanol concentration. Skin was stained in hematoxylin and eosin for 5 minutes followed by dehydration in increasing ethanol concentrations. Stained skin samples were treated with paramount and stored overnight before microscopy.

Extraction and Analysis of Lipids

The cultures were lyophylized, after which the dried tissue was extracted successively at 2 hour intervals with chloroform:methanol, 2:1, 1:1 and 1:2. Full skin was extracted rather than just stratum corneum to include both the lipids, acylceramide and acylglucosylceramide, which are potent indicators of differentiation. These lipids are found only in the living epidermal layers. The combined extracts from each set of cultures were dried under a gentle stream of nitrogen and redissolved in 100 μl of chloroform:methanol, 2:1. Twenty ×twenty cm glass plates coated with 0.25-mm-thick silica gel G (Adsorbosil-plus-1; Alltech Associates; Deerfield IL, USA) were washed with chloroform:methanol, 2:1, activated in a 110°C oven, and the adsorbent was scored into 6-mm-wide lanes. Calibrated glass capillaries were used to apply 5 or 10 μl samples 2–3 cm from the bottom edge of the plate, and the chromatogram was developed. Two development regimens were used. To resolve ceramides, glucosylceramides and phospholipids chromatograms were developed with chloroform:methanol:water, 40:10:1, to 6 cm; followed by chloroform:methanol:acetic acid, 190:9:1, to 20 cm; followed by hexane:ethyl ether:acetic acid, 70:30:1 to 20 cm. To resolve cholesterol, fatty acids, triglycerides and cholesterol esters chromatograms were developed with hexane to 20 cm; followed by toluene to 20 cm; followed by hexane:ethyl ether:acetic acid, 70:30:1, to 12 cm. After development, chromatograms were air dried, sprayed with 50% sulphuric acid, and slowly heated to 220°C on an aluminum slab on a hot plate. After 2 hrs, charring was complete, and the chromatogram was quantitated by photodensitometry.

Diffusion Studies

The *in vitro* diffusion studies were performed using human dermatomed cadaver skin (Female, Caucasian abdominal, 500 μm), EpiDerm™, and hairless mouse skin (HMS) (male, 8 weeks old, SKH1, Charles River Labs.). Dermatomed skin and HMS was stored in the freezer at –20°C for no more than two weeks. EpiDerm™ was purchased from MatTek Corp. (Ashland, MA) and used immediately upon arrival.

All diffusion experiments were performed as described by Kim *et al*., with the only exception that 1.0 % W/V polyoxyethylene-20-cetyl ether (solubilizer) was added to the isotonic phosphate buffer solution for the tamoxifen penetration study. The addition of 1% polyoxyethylene-20-cetylether (solubilizer) to the diffusion buffer was to overcome the low solubility of Tamoxifen in phosphate buffer solutions. The use of this solubilizer is well documented in the literature for *in vitro* diffusion studies. In addition our lab has performed studies in which the affect of 1% polyoxyethylene-20-cetylether on the permeation of caffeine (unpublished data). The results demonstrated that there is no significant affect on the permeation of caffeine between phosphate buffer without solubilizer and phosphate buffer with solubilizer.

Briefly, model skins were mounted on top of the modified Franz diffusion cells. Following one-hour hydration of skins, 8 μl of PG was applied to each of the control skins. After a 1 hr pretreatment period, a 16.2 μl saturated suspension of model drug in PG was applied per cell. Saturated suspensions of model drugs were added in order to insure maximum thermodynamic activity and maintain sink conditions. In all cases, a saturated drug solution with little excess of solute was used to ensure unit thermodynamic activity. Samples of 300 μl were withdrawn at predetermined intervals over 24 h. After 24 h of sampling, the skins were removed, washed briefly in methanol, and homogenized using a Kinematica GmbH tissue homogenizer and filtered through a C_{18} Sep pak® cartridge (15). All samples were analyzed using HPLC.

HPLC Methodology

Concentrations of model drug in the samples were determined using a Hewlett Packard 1050 HPLC system and C_{18}-Microsorb column (15 cm × 4.6 mm; 5μm) at ambient temperature. For hydrocortisone (HC), the mobile phase was a mixture of acetonitrile: water (40:60, V/V) and injection volume was 40 μl. HC was detected at $\lambda = 242$ nm with retention time of approximately 3.5 min. Calibration of HC in the samples was determined using an external standard technique (Michniak *et al*., 1993b). Caffeine was detected at $\lambda =$ 270 nm using methanol: water: acetonitrile (20:70:10, V/V) mixture as a mobile phase with flow rate 1 ml/min. Injection volume was 20 μl and approximated retention time was 3.5 min. Calibration curve was constructed ($R^2 = 0.999$) and validated for intra- and inter day variation (coefficient of variation, CV < 5 %). For tamoxifen, the mobile phase was triethylamine: methanol: water (92.8: 7: 0.2, V/V) and injection volume was 25 μl. Tamoxifen was detected at $\lambda = 277$ nm. The flow rate was 1 ml/min. The retention time of tamoxifen was approximately 5.0 min. The HPLC assay for tamoxifen was validated (linearity $R^2 = 0.9999$, CV for intra- and inter-day variation were < 4%).

Data Analysis

The permeation parameters of model drugs were calculated by plotting the cumulative corrected amounts ($\mu g/cm^2$) of drug permeated through the skin versus time (hours). The slope of the linear portion of the graph provided maximum flux values (J) at steady state ($\mu g\ cm^{-2}\ h^{-1}$). The lag time was determined by extrapolating the linear portion of the curve to the X-axis. Statistical analysis of the data were done using analysis of variance (ANOVA, $p = 0.05$). Log P values of the model drugs (caffeine, tamoxifen, and hydrocortisone) were determined using ACD program (Advanced Chemistry Inc., Ontario, Canada).

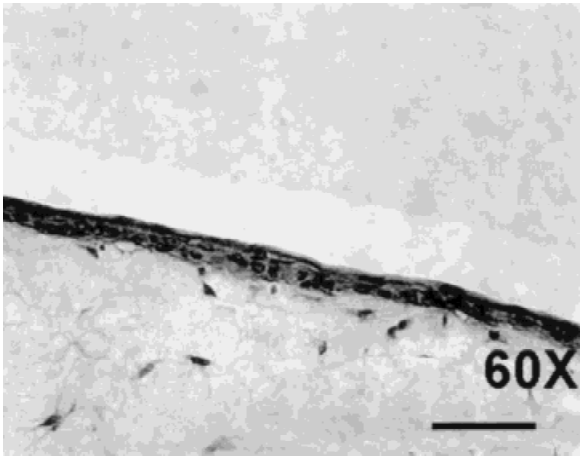

Fig. 1. H and E staining of skin equivalent after 1 week of culture. Original magnification x60. Scale bar = 10 μm.

RESULTS AND DISCUSSION

The fully-grown bio-engineered human skin (BHS) consisted of a dermal equivalent and an epidermis derived from human fibroblasts and keratinocytes respectively. Microscopic examination of hematoxylin and eosin (H & E) stained sections of BHS was performed after 1 and 2 weeks of culture. After one week of culture a dermis that was comprised of fibroblasts embedded in a collagen gel and an epidermis that consisted of 2–3 layered sheets of differentiated keratinocytes was clearly visible (Fig. 1). Interestingly, a more highly differentiated epithelium was observed in the culture after 2 weeks of incubation (Fig. 2). The outer layers of the multilayered epithelium appeared to closely resemble the stratum corneum found in human skin epidermis *in vivo* (Fig. 3). Also, after two weeks of culture the bio-engineered skin had several layers of nucleated keratinocytes that resembles the stratum basale found in native human skin (Fig. 2,3).

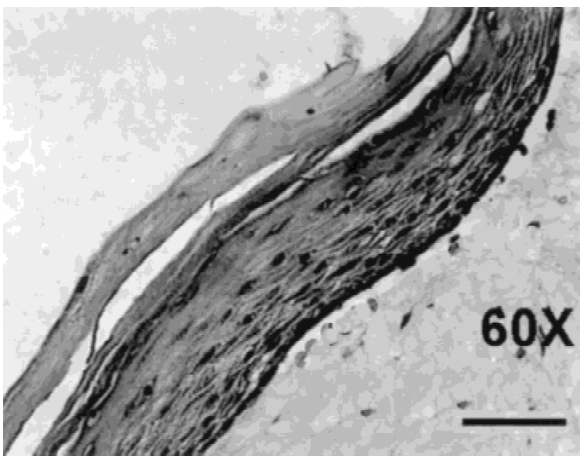

Fig. 2. H and E staining of skin equivalent after 2 weeks of culture. Original magnification x60. Scale bar = 10 μm.

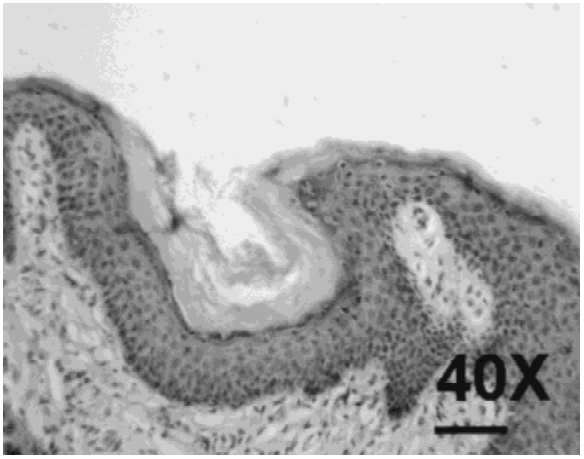

Fig. 3. H and E staining of a human foreskin. Original magnification x40. Scale bar = 10 μm.

In vitro Diffusion Studies

In order to investigate the potential of BHS as an *in vitro* model for transdermal permeation studies, the percutaneous permeation of three model drugs was evaluated and compared with other skin models, human cadaver skin, hairless mouse skin (HMS), and EpiDerm™. Caffeine is a highly hydrophilic drug with a log P of –0.07 ± 0.35. Tamoxifen is a highly lipophilic drug with a log P of 7.85 ± 0.75. HC is a widely examined model drug for transdermal studies and has a log P of 1.43 ± 0.47 (16). The results of model drug permeability through the skins were expressed as mean ± standard deviation and are presented in Table I. The Q_{24} (the cumulative amount of model drug that passed into the receptor per unit area of skin in 24 hours) showed the following trend: EpiDerm™ > HMS > BHS > human cadaver (for HC), HMS > EpiDerm™ > BHS > human cadaver (for caffeine), and HMS EpiDerm™ > human cadaver > BHS (for tamoxifen). The flux values obtained for both HMS and EpiDerm™ were 2 to 10 times higher than those obtained for human cadaver skin for all three drugs used ($p < 0.05$). However there was no statistically significant difference in flux values between human cadaver skin and BHS when using caffeine and tamoxifen ($p > 0.05$). Based on these results the permeation of all tested drugs through the BHS showed comparable Q_{24} and flux values with those obtained for human cadaver skin. The permeation profiles of each model drug through different skins are presented in Figures 4, 5, and 6. The trends for skin content (SKC) of HC, caffeine and tamoxifen are as follows: EpiDerm™ > HMS > BHS human cadaver skin; EpiDerm™ > HMS > human cadaver skin > BHS; and HMS > EpiDerm™ > human cadaver skin > BHS, respectively. With respect to SKC values, BHS produced the closest SKC values to human cadaver skin.

It is well documented that the dominant barrier for drug transport through skin is stratum corneum (SC), and non-polar and polar substances can diffuse through the SC via different diffusional pathways, a non-polar pathway and a polar pathway, respectively (17). Therefore drugs with different lipophilicities were chosen and tested permeation behavior through human skin, and then outcomes were compared with those obtained using BHS, mouse skin, and EpiDerm™. The penetration parameters (both Q_{24} and flux) indicated that more lipophilic drug (tamoxifen) penetrated readily through human cadaver than semi-polar (HC) and polar (caffeine) substances. These results were expected since lipophilic drug can easily pass through the SC intercellular lipid domain, which contributes significantly transdermal penetration of most of drugs. For BHS, HC penetrated highly than both tamoxifen and caffeine, but the degree of drug penetration was similar to that of human cadaver skin. For mouse skin and EpiDerm™, penetration of caffeine was higher than other model drugs and the extent of drug penetration was significantly higher than that of human cadaver skin. These results may reflect that each skin has different organization of intercellular lipid matrix, and/or different degree of barrier function.

Lipid Analysis of BHS

The goal in the development of human skin equivalents is to generate models, which have similar lipid compositions and permeability characteristics to those of human skin. It is well established that the lipid composition is an important factor regarding the barrier properties and permeability of skin (18). Since the BHS mimicked human cadaver skin in

Table I. Drug Permeability of BHS Compared with Three Skin Models ($n = 5$, means ± SD)[a]

Drugs	Permeation parameters	Human cadaver	Hairless mouse	EpiDerm™	BHS
Hydrocortisone	Lag time (h)	2.5 ± 0.3	3.1 ± 0.7	0	0
	Q_{24} (μg/cm^2)	27.4 ± 7.2	43.1 ± 5.2	93.0 ± 18.4	31.8 ± 7.5
	Flux (μg/cm^2/h)	1.8 ± 0.2	2.0 ± 0.2	4.8 ± 0.8	5.8 ± 1.0
	SKC (μg/g)	382 ± 117	1060 ± 79	7219 ± 2930	848 ± 315
Caffeine	Lag time (h)	5.5 ± 0.3	1.4 ± 0.1	0	4.1 ± 0.1
	Q_{24} (μg/cm^2)	11.0 ± 0.9	339.2 ± 6.8	210.2 ± 78.6	24.2 ± 2.5
	Flux (μg/cm^2/h)	0.7 ± 0.1	22.9 ± 3.7	11.0 ± 1.7	3.23 ± 1.1
	SKC (μg/g)	81 ± 12	245 ± 8	417 ± 275	42 ± 13
Tamoxifen	Lag time (h)	5.2 ± 2.7	2.1 ± 1.0	2.1 ± 1.4	1.2 ± 0.12
	Q_{24} (μg/cm^2)	49.1 ± 7.8	130.4 ± 52.5	69.4 ± 5.2	30.2 ± 3.3
	Flux (μg/cm^2/h)	2.8 ± 0.8	7.4 ± 1.6	6.3 ± 2.4	3.8 ± 0.3
	SKC (μg/g)	91 ± 30	18645 ± 705	151 ± 48.0	49 ± 11

[a] SKC: Skin content of model drug after 24 hr. Q_{24}: Cumulative amount of model drug after 24 hr in the receptor. BHS: Bio-engineered human skin.

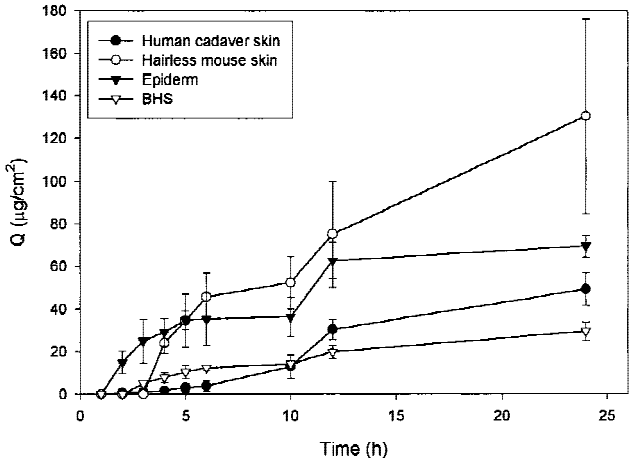

Fig. 4. Permeation profiles of tamoxifen in skin models. (n = 5, means ± S.D.)(Q is the cumulative amount of drug permeated during 24 h).

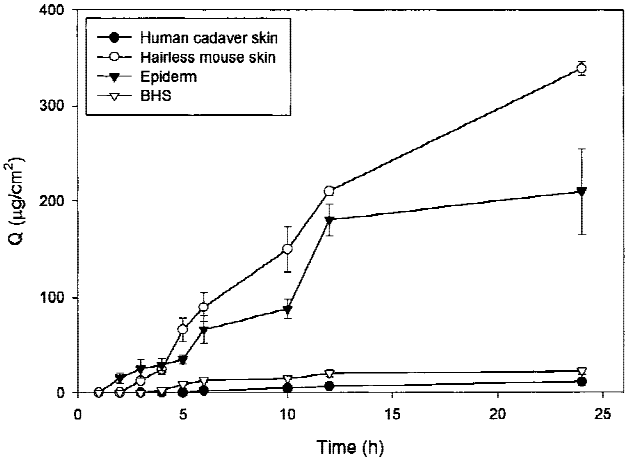

Fig. 6. Permeation profiles of caffeine in skin models. (n = 5, means ± S.D.))(Q is the cumulative amount of drug permeated during 24 h).

permeability, the lipid content of the BHS was examined in order to compare it to the published reports on human skin lipid compositions (18,19). Full thickness cultures of the BHS were extracted rather than isolated stratum corneum in order to include both acylceramide and acylglucosylceramide, both of which are sensitive differentiation markers. The latter is confined to the living layers of the epidermis and is completely absent from the stratum corneum. The lipid analysis of BHS is shown in Table II. Evaluation of the BHS indicated that the lipid composition is different from that of native human skin. However, all the lipid classes found in human skin are present in the BHS. The most significant difference was in the amount of triglyceride present: 55.9 ± 4.65 % (total lipid weight). It has been reported by Wertz *et al.*, (20) that the triglyceride content ranges from 0%–3% of total lipid weight in human stratum corneum. Similar results have been reported by Ponec *et al.,* (21) in which a large difference in triglyceride content was found in keratinoycte cultures grown at the air-liquid interface compared with that found *in vivo* (25–30% vs. 5%). It has been suggested that the elevated triglyceride content of cultured keratinocytes reflects, at least in part, aberrant triglyceride catabolism under culture conditions. It was shown that lowering the glucose content of the culture medium resulted in significant lowering of triglyceride content, but the triglyceride was still higher than in normal epidermis. It was suggested that high accumulation could be attributed to the culture conditions employed during the cultivation of skin equivalents. This is verified by growing fresh excised human epidermis in tissue culture air-exposed conditions results in significant increase in triglyceride levels when compared to normal epidermis (22). In terms of ceramide profiles, the more polar ceramides, such as ceramide fractions 6II (ceramides with α-hydroxy acid amide linked to 6-hyroxysphingosine) and 6I (ceramides with α-hydroxy acid amide linked to phytosphingosine) are underrepresented. The lack of polar ceramides in skin grown at the air liquid interface has been verified in other published papers. Unfortunately, it is not clear why the synthesis of these polar ceramides such as fractions 6I and 6II is inhibited in artificial skin. The relative proportions of both fractions 6I and 6II is significantly less than that observed in human skin (Based on a percentage of the total ceramide content: 2% vs. 10–18%). The less polar ceramides, fraction 4/5 (ceramides with normal fatty acids amide linked to 6-hydroxysphingosine and α-hydroxy acid amide linked to sphingosine), fraction 3 (ceramides with normal fatty acids amide linked to phytosphingosine and omega hydroxy acids ester linked to 6-hydroxysphingosine), fraction 2 (ceramides with normal fatty acids amide linked to sphingosine), and fraction 1 (ceramides with omega hydroxy acids amide linked to sphingosine and linoleate ester-linked to the ω-hydroxyl group, are correspondingly over-represented (Table II). It is noteworthy that trace amounts of acylglucosylceramide were found in the BHS (Table II). Acylglucosylceramide, the glucosylated analogue of ceramide 1, is a differentiation marker found in all of keratinizing epithelia and was proposed by Wertz *et al.*, (23,24) to be an important contributor to the assembly of lipid bilayers. It was suggested that the ω-hydroxyacyl chain of acylglucosylceramide overlaps one bilayer while the linoleate inserts into an adjacent lipid layer. This action most likely promotes the aggregation and progressive flattening of membrane vesicles, thereby insuring proper lipid organization.

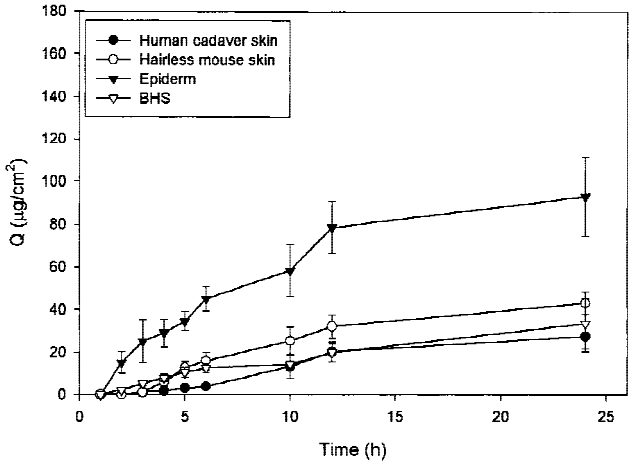

Fig. 5. Permeation profiles of hydrocortisone in skin models. (n =5, means ± S.D.) (Q is the cumulative amount of drug permeated during 24 h).

Table II. Lipid Composition of Bio-Engineered Human Skin (BHS). Values are Expressed as the Percentage Total Lipid Weight ($n = 6$, means ± SD)

Lipid type	Total weight (%)
Phospholipids	11.9 ± 4.50
Glucosylceramides	0.95 ± 0.25
Acylglucosylceramide	0.16 ± 0.05
Ceramide 6II	0.10 ± 0.01
Ceramide 6I	0.10 ± 0.01
Ceramide 4/5	0.23 ± 0.15
Ceramide 3	0.13 ± 0.05
Ceramide 2	1.47 ± 0.55
Ceramide 1	0.33 ± 0.06
Cholesterol	8.73 ± 2.00
Fatty acids	3.50 ± 1.21
Triglycerides	55.9 ± 4.65
Cholesterol esters	6.63 ± 0.66

CONCLUSIONS

The purpose of this study was to examine the permeability and lipid composition of a novel BHS in order to access its utility as an *in vitro* model for transdermal/topical formulation testing. The permeability of the BHS was similar to that found in human cadaver skin (3–4 fold more permeable). In terms of lipid composition, the BHS contained all of the lipids present in human skin. However, the composition was different with triglycerides making up the majority of lipids found in the BHS. The polar ceramides were under-represented in the BHS while the non-polar ceramides where over-represented. The difference in permeability of the BHS may be attributed to the differences in the lipid profile. The findings in this study suggest that the BHS is a more acceptable model for *in vitro* percutaneous permeability testing than EpiDerm™ or mouse skin. Future studies will focus on improving the barrier properties of the BHS through modification of culture conditions.

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