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Research paper

Effect of abrasion induced by a rotating brush on the skin permeation of solutes with varying physicochemical properties

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Abstract

A transient reduction in the barrier nature of the skin can be a pre-requisite for successful (trans)dermal delivery of some drugs. The aim of this present study was to investigate and effect of a dermal abrading "rotating brush" device on percutaneous absorption and skin integrity. *In vitro* experiments were conducted using excised human epidermal membrane. The effect of device parameters (bristle type, treatment duration and applied pressure) on skin permeability of model solutes (methyl paraben, butyl paraben, caffeine, acyclovir and angiotensin II) with varying physicochemical properties was examined and compared to established methods of skin penetration enhancement (positive controls). The device parameter which was found to have the most marked effect on permeability of the compounds was bristle type. Profound changes (2- to 100-fold increase) were observed in the epidermal permeability of the hydrophilic penetrants (caffeine, acyclovir and angiotensin II), when the brush device was employed compared to positive controls (ethanol enhancement, delipidisation, iontophoresis and tape-stripping). Findings from this present study support the effectiveness of a rotating brush applied to the skin in enhancing epidermal permeability. Further optimization of operational parameters is required to exploit this simple and effective delivery device.

Keywords: Abrasion; Brush; Human skin; Transdermal delivery; *In vitro* diffusion

1. Introduction

(Trans)dermal drug delivery offers several advantages over conventional methods of drug therapy (oral and parenteral). However, the barrier nature of human skin which confers its key protective function also imposes physicochemical limitations on the type of permeant that can traverse the barrier [1]. For a drug to be delivered passively via the skin it needs to have adequate lipophilicity (partition coefficient, *P*; 10–1000) and also a molecular weight <400 Da [2,3]. The physicochemical and physiological restrictions addressed above limit the number of drugs that can be delivered via this route especially those of biotechnological origin.

Mechanical disruption (abrasion) of the skin barrier provides an alternative method of potentially enhancing the skin transport of topically applied solutes. Abrasive techniques are mostly employed by dermatologists for superficial skin resurfacing e.g. treatment of acne, scars, hyperpigmentation and other skin blemishes [4]. The use of abrasion to enhance and control the delivery of penetrants namely vitamin C [5], 5-aminolevunic acid [6], vaccines and biopharmaceuticals [7,8] has been reported in the skin absorption literature. The abrasion methods reported in the literature include the use of adhesive strips [9,10], abrasive pads [7], dermabrasion [4] (motor-driven wire brushes, diamond fraises, serrated wheels) and microdermabrasion [5,6,11]. The latter method is by far the costliest as it requires a relatively costly device [5,6,12] to

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produce a stream of aluminium oxide crystals [13]. In addition such microdermabrasion devices can be operated only by trained personnel, thereby limiting the applicability of the technique as a patient-operated device. However conversely, the method offers better process control and an apparent reduction in non-biological variability compared to simpler abrasion methods.

Unlike microdermabrasion, conventional dermabrasion via a wire brush (or diamond fraises and serrated wheels) itself can be a painful surgical procedure, and local refrigerant anaesthesia is thus routinely administered to the skin region prior to its use [4]. As a result of this, the use of this technique as a means of enhancing topical delivery has not been extensively reported in the literature. Thus if the latter mode of abrasion is to have a practical application in enhancing percutaneous absorption, a technique is required which causes minimal associated discomfort to the patient and which can be effected without the need for anaesthesia. As such the aim of this study was to investigate the feasibility of using a "rotating brush" device to induce possible mild mechanical abrasion to the skin and therefore temporarily reduce the barrier nature of skin. The objectives of the study were as follows: (i) investigate changes in the structural properties of the epidermal membrane after using the device, (ii) investigate the effect of the device parameters on the skin delivery of three standard model penetrants of differing lipophilicity (methyl paraben, butyl paraben and caffeine) as well as a currently marketed drug (acyclovir formulated as a commercial cream (Zovirax®)) and a model peptide (Ang II) across the skin and (iv) compare the efficiency of the rotating brush device with other established modes of skin penetration enhancement such as iontophoresis [14,15], tape-stripping [9,10], delipidisation [16,17] and the use of a chemical (ethanol) enhancer [18–20]. A pseudo-finite dose study, which best simulates the clinical use of penetrant and device, is also employed in this present investigation, although previous studies [5,6,12] examining the effect of microdermabrasion on skin absorption were conducted using infinite doses.

2. Materials and methods

2.1. Materials

Methyl paraben, MP (MW; 152.15, log octanol/water (P); 1.96), butyl paraben, BP (MW; 194.23, log P; 3.57), caffeine, CF (MW 194.19, log P; −0.07), ^{3}H-acyclovir ACV (MW; 225.21, log P; −1.56) in aqueous ethanol solution (radiochemical purity ⩾95%, activity) and angiotensin (Ang) II (MW; 1046) and triethylamine were purchased from Sigma Chemical Co. (Dorset, UK). Orthophosphoric acid and potassium dihydrogen orthophosphate (KH_2PO_4) were obtained from BDH laboratories supplies (Loughborough, UK). Phosphate buffered saline (PBS, pH 7.4) tablets were supplied by Oxoid Ltd. (Basingstoke, UK). Zovirax (5% ACV cream) was produced by GlaxoSmithKline, Middlesex, UK. Deionised water was prepared using an Elgstat water purifier, Option 3A, Elga Ltd., (Elga, Buckinghamshire, UK). All solvents involved in this study were of HPLC grade and purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Bristle type A (Soft bristles) was the brush (refill) head of an electric toothbrush (Superdrug brand) purchased from Superdrug stores Plc, London, UK. Bristle type B (Hard bristles) was the brush head of a Roberson grade 8, hog hair bristle brush purchased from L. Cornelissen & Son Ltd., London, UK.

2.2. Preparation of human epidermal sheets

Fresh, surgically excised samples of human skin were obtained directly after abdominoplastic surgery with informed consent and approval from the Research Ethics Committee of King's College London. Subcutaneous fat was carefully removed from the skin sample using forceps and a scalpel. Following removal of the fat, individual portions of skin were immersed in water at 60 °C for 45 s [21]. The skin was then pinned, dermis side down, on a corkboard and the epidermis (comprising stratum corneum and epidermal layer) gently removed from the underlying dermis by rolling the membrane using a gloved thumb or middle finger. The dermis was then discarded and the epidermal membrane floated onto the surface of water and taken up onto a Whatman No. 1 filter paper (Whatman international, Maidstone, UK). The resultant epidermal sheets were blotted dry with tissue paper and stored flat, wrapped in aluminium foil at 4–8 °C until use.

2.3. Brush device description and mode of operation

A rectangular section (∼3 × 2 cm) of epidermal sheet was selected and a circular region (∼1 cm^2) demarcated. The demarcation was performed by applying pressure to the sharp end of an appropriately sized cork borer in contact with skin. Brush treatment of the skin was performed as previously described [22]. In brief, the sample of epidermal sheet was inserted into the device clamp ensuring the demarcated region was exposed. The clamp was tightened and gently raised by means of the latch (lift) until the demarcated region of the epidermis was in slight contact with bristles (surface area of ∼1 cm^2) of the brush. Predefined operational parameters (speed, applied pressure, treatment duration) of interest (pre-defined) were then set on the control box for the abrasion process to occur.

2.4. Microscopy studies

The effect of bristle perturbation on the epidermal membrane was assessed by scanning (SEM) and transmission electron microscopy (TEM), where the integrity of brush-treated and untreated (control) epidermal samples was compared. Brush-treated and untreated (control) samples were fixed in 2% formaldehyde/2.5% glutaraldehyde in PBS (pH 7.4) overnight. Samples were immersed for 20 min sequentially in solutions comprising 30%, 50%,

70%, 95%, 100%, 100%, 100% v/v acetone in water, then dried using liquid carbon dioxide in a Polaron E3000 critical point drier. The samples were then mounted on 12.5 mm aluminium pin stubs using double-sided adhesive carbon pads and sputter coated with approximately 20 nm of gold in a Polaron E5100 sputter coater. These were subsequently examined and photographed using a Philips SEM501B scanning electron microscope. For TEM, a 1×2 mm rectangular strip was cut from the fixed skin, and this was then dehydrated by sequential immersion in acetone at increasing concentrations. The dehydrated sections were then embedded in spur resin and polymerised for 48 h at 60 °C. Ultra-thin sections were cut using a diamond knife installed on a Reichert-Jung OMU4 ultramicrotome and these were taken up on to 200 mesh hexagonal copper grids. Sections were stained for 15 min by immersion in 1% w/w uranyl acetate in 50% v/v ethanol in water followed by 5 min in Reynolds lead citrate [23]. The prepared sections were examined and photographed using a JEOL JEM100CX II transmission electron microscope.

2.5. Infinite dose study for BP, MP and CF

The skin was treated with the device (as described above) using different bristle types (A soft and B hard) at different pressures and contact times, whilst maintaining a constant device speed (Table 1) since a single (fixed) speed motor was used.

An infinite dose study using human epidermal sheets was conducted to determine the flux of MP, BP and CF across the membrane. Calibrated Franz cells of known surface area (~0.65 cm^2) and receptor volume (~2 mL) were used. The receptor chamber was filled with PBS (pH 7.4) and stirred throughout the duration of the experiment using a PTFE-coated magnetic flea (5×2 mm). The skin was treated as described using the device parameters shown in Table 1. The treated membrane (using brush or positive controls) or untreated control was then clamped between the donor and receptor chambers of the Franz cell (with the stratum corneum (SC) facing upwards). A saturated solution (250 μL) of the model penetrant in PBS was then directly introduced into the donor chamber of cell. Saturated solutions were prepared as described previously [24,25]. All experiments were conducted in a water bath at 37 °C over a minimum period of 4 h for MP and BP and 6 h for CF, with sink conditions being maintained throughout [25]. The human skin samples used in the experiments were from the same donor. Sample analysis of penetrant concentration in the receptor chamber was conducted using the respective assay method described for each penetrant. Infinite dose methodology was employed to allow the calculation of skin permeation parameters such as flux, permeability coefficient and lag time, which cannot be assessed under finite "in use" conditions.

Table 1
Device parameters used during optimisation studies

Speed (rpm)	80
Brush (bristle type)	Soft (A) and hard (B)
Pressure applied on skin (Nm^{-2})	300–1200
Treatment duration (s)	15–45

2.6. Pseudo-finite dose study for CF and ACV

Pseudo-finite "in use" dose permeation experiments were performed using similar experimental conditions as described in the infinite dose study. Independent studies were conducted for ACV (model drug) and CF (selected penetrant). The skin was treated with the rotating brush device using ideal parameters obtained from experiments conducted in the previous studies (Section 2.5). Formulations with a target dose of either approximately 9 ± 1 mg cm^{-2} (ACV cream) or 20 ± 2 mg cm^{-2} (CF in PBS) were applied to the epidermal membrane surface using a previously calibrated positive displacement pipette (Gilson Pipetman®, P20 Anachem UK Ltd.) and carefully spread to cover the effective surface area by means of a tared syringe plunger. Selected control experiments were performed as described earlier. At certain time intervals 200 μL of the receiver fluid was carefully withdrawn from the receiver fluid. The maximum duration of each experiment was 4 h except for iontophoretic studies, where the experiment was conducted for only 2 h. For studies involving ACV, approximately 4 mL of scintillation cocktail was added to each 200 μL sample. Analysis of ACV and CF was conducted using either scintillation counting or HPLC, respectively.

2.7. Angiotensin II permeation

An infinite dose study, as described above, was conducted using Ang II, where the diffusion of the peptide across skin following brush treatment, using optimized fixed parameters, was investigated. Selected positive controls were incorporated in the study. A 250 μL solution of Ang II in PBS (1 mg mL^{-1}) was introduced into the donor well. The receptor chamber was filled with enzyme immunoassay (EIA) buffer (a standard reagent in EIA kit). Two samples were removed from the receptor chamber after 4 and 24 h, respectively, and these were analysed using the EIA method.

2.8. Positive controls

Established methods of skin penetration enhancement were incorporated within the study design as described:

Tape-stripped skin sections used were prepared by repeated stripping of skin [9,10] with D-squame® adhesive disc. The disc was gently placed on the skin after which a set weight (100 g) was placed on the adhesive disc-skin

composite for 20 s. The weight was then lifted and the adhesive disc removed. This was then repeated eight times.

Skin delipidisation was conducted as described previously [16,17]. Delipidised skin was prepared by immersing skin sections in chloroform and methanol (2:1) for 40 min. After this the skin was removed, blotted dry with tissue and dried via vacuum drier at 760 mm Hg, 25 °C for 1 h to remove any remaining organic solvent. The delipidised skin was then used for Franz cell studies.

The iontophoretic (anodal) treatment protocol employed was as described previously [26,27]. A 0.4 mA current limit and 10 min treatment duration were maintained to simulate "in use" conditions. A shorter iontophoretic treatment (0.4 mA for 10 min) was also employed, so as to reduce the likelihood of potential damage to the skin as a result of prolonged current exposure.

The chemical enhancement procedure used involved the application of ethanol [18,19]. The experiments were conducted by forming saturated solutions of each penetrant containing 50%v/v ethanol in PBS, which were produced by stirring penetrant with solvent overnight. The resulting solution was then introduced into the donor well of Franz cells containing untreated skin.

2.9. Analytical methods

2.9.1. HPLC analysis of MP, BP and CF

High performance liquid chromatographic (HPLC) measurements were carried out as described previously [24].

2.9.2. Analytical studies for ACV

The radiochemical purity of the ^{3}H-ACV was determined by accurately spiking a 10 μL aliquot of the radiolabelled drug (ethanolic solution) into 1 mL of PBS and injecting a sample of this solution onto an HPLC column. Eluted fractions, containing the analyte (ACV), were collected at 1 min intervals. At completion of the permeation studies the radiochemical purity of ^{3}H-ACV samples taken from the receptor solution was determined by direct injection onto the HPLC.

Chromatographic measurements were carried out using a Waters 2487 Dual absorbance detector, Waters 717 Plus Autosampler and Waters 600 Controller pump. ACV chromatographic conditions were as follows; Phenomenex® Prodigy™ 5μ ODS 2, C18 (150 × 4.6 mm, 5 μm), mobile phase comprising 90% acetonitrile: 10% phosphate buffer (0.02 M KH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid). Flow rate, injection volumes and wavelength of detection were set at 1 mL min^{-1}, 10 μL and 254 nm, respectively. Each eluted fraction was mixed with scintillation fluid (4 mL) and analysed using a scintillation counter (Beckman Liquid scintillation counter, LSC 3801). Controls (blanks) involved a mixture of PBS and scintillation fluid (4 mL). A radiochemical purity (>95%) was confirmed and deemed satisfactory for the purpose of this study.

Zovirax® spiked with ^{3}H-ACV (ethanolic solution) was used as the formulation for the entire study. Briefly, 300 μL of ^{3}H ACV was placed into a 1.5 mL centrifuge tube and the solvent evaporated using a stream of air. Zovirax® was weighed (target weight of 1 g ± 1%) into the centrifuge tube and this was carefully mixed in the tube with the radiolabelled drug using a fine spatula, in order to achieve a homogeneous mix. To test for homogeneity, three samples (target weight of 1 mg ± 10% per sample) were taken (top, middle and bottom) using a previously calibrated positive displacement pipette (Gilson Pipetman®, Anachem UK Ltd.). The sample was then added to 4 mL of scintillation fluid and the radioactivity determined by scintillation counting (Beckman Liquid scintillation counter, LSC 3801). Homogeneity of the mixture was confirmed when the % coefficient of variation (CV) was below 2.5%.

2.9.3. Analytical method for Ang II

Analysis of the peptide (Ang II) was carried out using an EIA in accordance with the method described in the EIA booklet provided by the manufacturer and present in the commercial kit (Peninsula Laboratories Inc., San Carlos, California, USA). The extent of binding of peptide with the antibody in the analytical kit was determined by measurement of absorbance using a microplate spectrophotometer (uQuant™, Witec AG, Switzerland). The EIA kit is specifically designed to only detect Ang II (EIA booklet) with cross reactivity due to derivatives of Ang II being reported as negligible. The analytical method was shown to be 'fit for purpose' by determining the absorbances associated with different standard concentrations of Ang II. In addition, the ability of the method to determine accurately different concentrations of the analyte when present in either PBS or EIA buffer was investigated and found to be suitable.

2.10. Data and statistical analysis

The concentrations of penetrants in the receptor solution were corrected for the removal of previous samples. The cumulative amount of penetrant permeating per unit skin surface area was plotted against time and steady state fluxes (J_S) were derived from the linear portion of the concentration–time profiles. The permeability coefficient (K_P) was calculated by normalising (J_S) with the saturated solubility of penetrant in donor [24]. The lag time (L_T) was determined from the intercept of the linear portions of the skin permeation profile with the x-axis. Experimental data presented represent mean values ($n \geqslant 3$) ± standard deviation (SD) unless otherwise stated. Enhancement factors (EF) were calculated as a ratio of flux of permeant through treated skin to that of untreated skin. The following measures were employed to ensure skin integrity and avoidance of erroneous permeability data: visual examination of all skin samples was conducted prior *in vitro* diffusion studies. The introduction of methylene blue solution (1% w/v in deionised water) in the Franz cell donor

compartment at the end of each experiment to confirm skin integrity [24] was only conducted for untreated controls. Any cells that failed these tests were immediately rejected. Permeability data were also rejected when a markedly higher permeability/flux than usual ($\geqslant$100-fold) was obtained (this includes treated skin). Statistical analysis was conducted using the analysis of variance method (ANOVA) and Student's *t*-test, the level of significance was taken at $p \leqslant 0.05$.

3. Results and discussion

3.1. Permeation of BP, MP and CF: effect of bristle type (hard and soft)

The selection of the three model penetrants was made on the basis of the different routes they are likely to employ to traverse the skin barrier via different routes [24]. Process or device parameters identified as being likely to affect permeation, on the basis of results from earlier studies which employed different mechanical devices, were the: (a) speed of bristles/frequency of movement [4,28], (b) duration of treatment [5,6,12], (c) force (pressure) exerted on membrane surface [29,30] and (d) bristle type [4,31].

An infinite dose study using excised human epidermis was conducted and the rotational speed of the brush was maintained at 80 rpm for each experiment since a single (fixed-speed) motor was used. A maximum treatment time of 45 s was utilised, as this speed was identified in preliminary experiments as being sufficient to ensure minor physical damage to skin but sufficient to ensure an effective change in percutaneous absorption [22]. A maximum pressure of 1200 Nm^{-2} signified the upper limit of sensitivity for the balance (pressure monitor) used. The skin was treated with the device using bristle type A (soft) and type B (hard) at different pressures and for different treatment times whilst maintaining a constant device speed. Studies using brush A (Fig. 1a–c) were found to result in an average enhancement factor of 2 in the order CF > BP $\geqslant$ MP at pressures of between 450 and 1200 Nm^{-2} for 45 s at 80 rpm. A further study was carried out replacing the softer bristled brush (type A) with one that had harder bristles (type B) and the data are summarised in Table 2. Minimum threshold pressure of 300 Nm^{-2} and a maximum treatment time of 25 s were employed when brush B was used. Significant differences ($p \leqslant 0.05$) were found in the resultant penetrant fluxes across human epidermal sheets which had been treated using brush B and brush A for MP, BP and CF. Significant reductions in the epidermal lag times of all penetrants were also observed after brush treatment. Dependent upon the treatment parameters employed, the device fitted with brush B generally produced higher EF values compared to the use of brush A.

The use of minimal pressure on skin is likely to be required when the device is used in an *in vivo* situation, where it may be necessary to minimize patient discomfort without compromising any potential resultant increase in

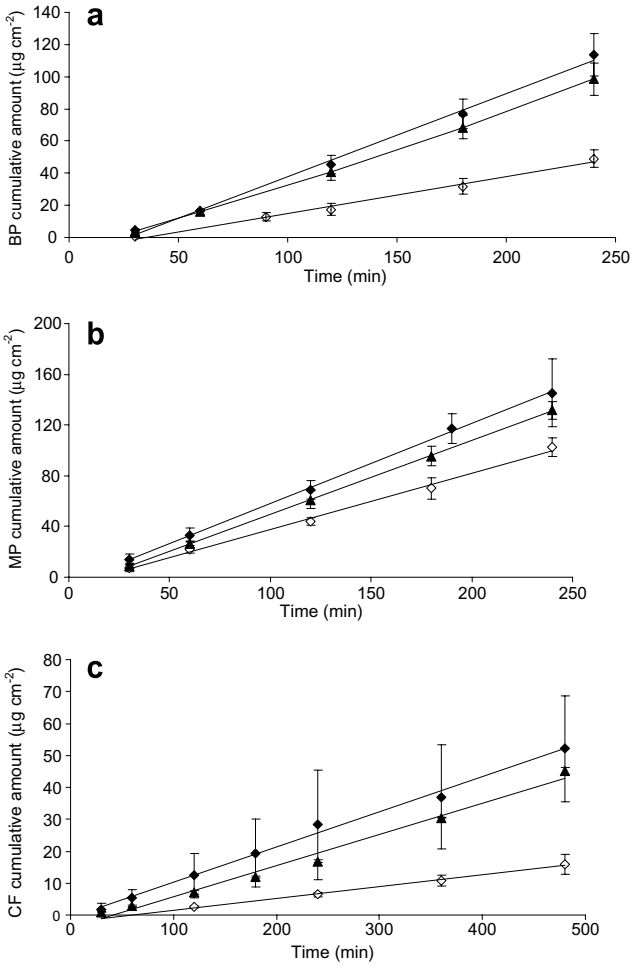

Fig. 1. Effect of rotating brush A, operated at 80 rpm on the skin permeation profile of (a) BP, (b) MP and (c) CF: (◇) untreated skin; (▲) 450 Nm^{-2}, 45 s; (◆) 1200 Nm^{-2}, 45 s. Data represent means ± SD ($n \geqslant 3$).

percutaneous absorption. It was found that an increase in applied pressure allows a shorter treatment duration to achieve an equivalent flux, since it is apparent from the data presented in Table 2 that a pressure of 450 Nm^{-2} applied for 15 s produced a comparable enhancement in the diffusion of MP and BP as a pressure of 300 Nm^{-2} for 25 s.

The rotating brush device significantly enhanced the skin permeation of the three model penetrants ($p \leqslant 0.05$), although the extent of the increase was dependent on the physicochemical properties of the compound and on the device parameters employed. The lower EF values recorded for the parabens (EF < 6), compared to CF (EF of 37–64), signified that the latter was transported markedly more easily when the upper layers of the skin were mechanically disrupted. This supports the suggestion that the potential or existing CF routes for permeation were made more accessible than those for the parabens when this abrasion device was employed. Skin absorption of hydrophilic penetrants such as CF has been reported to be very sensitive to differences in skin barrier integrity

Table 2
In vitro skin absorption parameters for MP, BP and CF across brush (type B) treated epidermis

Penetrant	Absorption parameters	Untreated	Brush (type B) treatment			
			450 Nm^{-2}, 45 s[a]	300 Nm^{-2}, 15 s	300 Nm^{-2}, 25 s	450 Nm^{-2}, 15 s
BP	J_S (10^{-3} μg cm^{-1} s^{-1})	4.93 ± 0.22	8.38 ± 1.09[c]	6.16 ± 0.89[c]	10.47 ± 0.96[c]	12.69 ± 2.07[c,d]
	K_P (10^{-5} cm s^{-1})	2.47 ± 0.11	4.19 ± 0.54	3.08 ± 0.44	5.23 ± 0.48	6.34 ± 1.03
	T_L (min)	35.84 ± 1.98	24.51 ± 5.50	22.65 ± 3.22	23.06 ± 2.19	14.55 ± 5.18
	EF[F]		1.69	1.25	2.12	2.57
MP	J_S (10^{-3} μg cm^{-1} s^{-1})	10.90 ± 1.28	13.53 ± 0.95[c]	nd	50.16 ± 9.25[c,d]	62.34 ± 12.75[c,d]
	K_P (10^{-6} cm s^{-1})	5.14 ± 0.61	6.38 ± 0.45	nd	23.66 ± 4.36	29.41 ± 6.01
	T_L (min)	15.02 ± 3.74	6.18 ± 1.34	nd	3.29 ± 2.09	6.73 ± 3.63
	EF[b]		1.24	nd	4.60	5.72
CF	J_S (10^{-4} μg cm^{-1} s^{-1})	1.87 ± 0.26	8.52 ± 0.29[c]	70.19 ± 34.87[c,d]	120.92 ± 42.55[c,d]	nd
	K_P (10^{-8} cm s^{-1})	9.15 ± 1.27	41.61 ± 14.19	343.44 ± 170.62	589.93 ± 207.58	nd
	T_L (min)	49.69 ± 3.54	16.74 ± 3.99	nd	nd	nd
	EF[b]		4.55	37.53	64.44	nd

All data represent means ± SD ($n \geqslant 3$).
The device speed maintained at 80 rpm. nd, not done.
[a] Data obtained using brush A.
[b] EF is the enhancement factor calculated as the ratio of the flux of permeant through treated skin to that of untreated skin.
[c] Flux significantly different from untreated skin ($p \leqslant 0.05$).
[d] Flux significantly different from skin treated at 450 Nm^{-2} for 45 s with brush A ($p \leqslant 0.05$).

compared to lipophilic penetrant [24,32]. Similar effects on the enhancement of the percutaneous absorption of CF have been reported previously for other physical/mechanical methods [33–35].

The device parameter which was found to have the most marked effect on permeability of the compounds was bristle type. Microscopical examination of skin after treatment with the device depicts perturbation of the epidermal tissue (Fig. 2a–c). Disruption of the SC surface (i.e. the superficial layers of the skin containing dead cells) using the device created a rough and uneven epidermal surface. The use of brush B was found to perturb the skin to a higher degree compared to brush A, with a much rougher, more disrupted skin surface being apparent after treatment with the stiffer bristled brush (Fig. 2c). This observation signified that the extent of barrier reduction achieved on using brush B was higher than brush A and accounts for the higher relative fluxes of all model penetrants obtained after the use of brush B compared to brush A (Table 2). Shorter treatment times and lower pressures were required when brush B was used in place of brush A to produce marked changes in the barrier nature of the SC as is apparent from the scanning electron micrographs (Figs. 2a–c). Transmission electron micrographs (data not shown) confirmed that a loosening of the upper skin layers occurred after treatment of the upper epidermis with the device, in comparison to (untreated) controls, where the cell layers clearly remained tightly packed. Such evidence suggested that the depth of the disruption is restricted to the upper layers of the SC whilst the remainder of the epidermis was unaffected. These observations are consistent with that reported by previous investigators [5,6] in which the morphology and thickness of the viable epidermis was also unaltered after treatment of skin using microdermabrasion (aluminium oxide crystals).

3.2. Comparison of established methods of penetration enhancement using BP, MP and CF

The use of reported methods of skin enhancement involving delipidisation, chemical enhancement (ethanol exposure) and tape-stripping as positive controls allowed a comparison with the effects of the rotating brush device. The effects of ethanol on skin-vehicle and/or solute-vehicle interactions have been discussed previously [20]. Ethanol can have a fluidizing effect upon the lipid bilayer of the SC and/or cause the delipidisation of the skin, which can result in dilation of the gaps between adherent cornified cells or induce a weakened cohesion of the keratinocytes thereby increasing the free volume available for diffusion of compounds across the SC [18,19]. Solute-vehicle interactions also involve a modification of the thermodynamic activity of the penetrant, which might consequently result in increased penetrant diffusivity across the SC. The ethanol-enhanced effect has been reported to be concentration dependent with the maximum fluxes occurring at an ethanol:buffer ratio of approximately 0.6 [20] since beyond such a ratio a plateau or retardation in drug diffusivity across the epidermis might be expected to occur due to membrane dehydration. Tape-stripping involves the complete or partial removal of the SC layers which can facilitate solute permeation by reducing the resistance associated with the SC barrier and/or diffusional pathlength depending on the nature of the solute [10,36].

Generally the degree of enhancement associated in permeant flux with each method was as follows; tape-stripping (CF > MP > BP), delipidisation (CF > BP ⩾ MP) and chemical enhancement (CF > MP ⩾ BP) (Table 3). These positive controls were found to enhance transport of the three model penetrants significantly compared to their transport properties across intact skin ($p \leqslant 0.05$). With

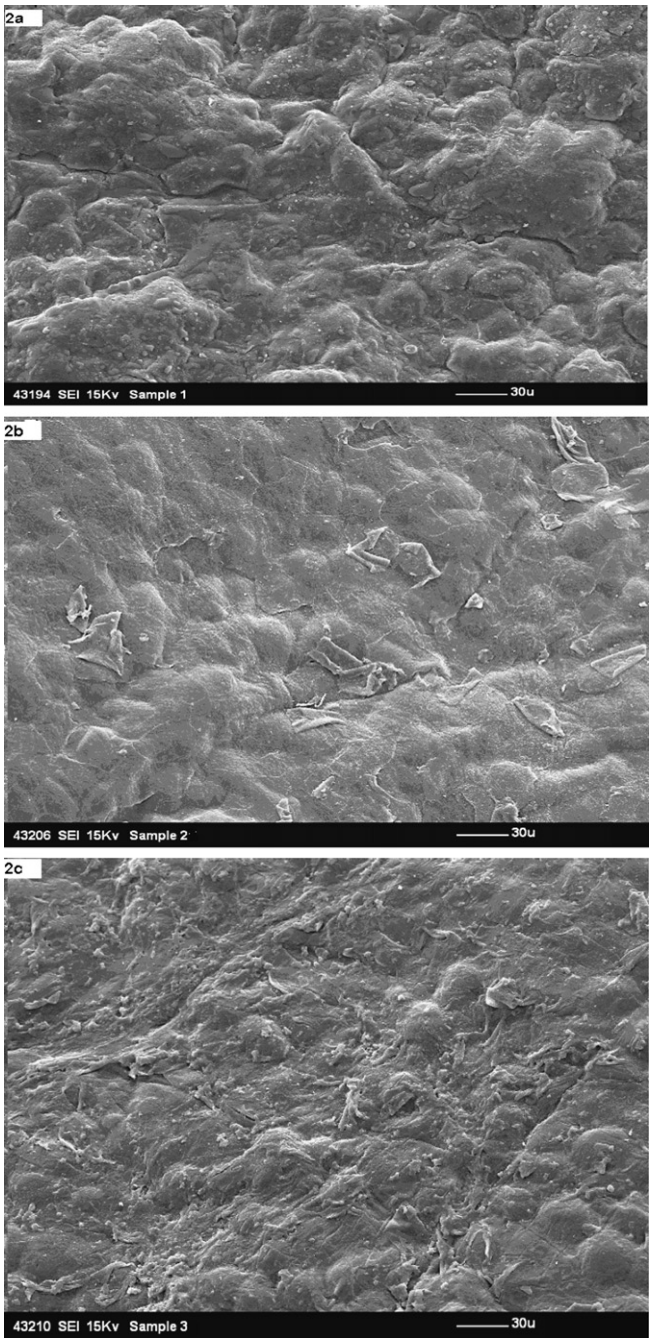

Fig. 2. Scanning electron micrograph of human epidermal sheet (a) untreated, (b) treated with brush A at 450 Nm^{-2}, 45 s, 80 rpm and (c) treated with brush B at 300 Nm^{-2}, 25 s, 80 rpm.

regard to the tape-stripped skin, the lowest EF was recorded for BP compared with the other permeants. This may signify the ease with which BP permeates the lipophilic SC and reflect the relative resistance provided by the more aqueous regions of the remaining epidermis. CF permeation through tape-stripped skin was 18-fold higher once a portion of the SC was removed, whilst there was only a 3- to 7-fold increase for either BP or MP. A similar trend was also observed when delipidisation or chemical enhancement was employed, the EF values obtained for BP and MP were ~4 in contrast to a value of 10 which was obtained for CF. The degrees of enhancement recorded for BP and MP when using the more recognized means of skin permeation enhancement were comparable to that observed for the device (brush B). However, with CF significantly higher fluxes were observed after the use of brush B on the skin (EF values up to 64) compared to those which resulted using the positive controls.

3.3. Permeation of CF (pseudo-finite dose study)

CF was selected as a suitable candidate for this part of the study due to its poor intrinsic permeability across intact skin and the large enhancement found when the abrasive device was employed in the infinite dose study (Table 2). The transdermal delivery properties of the device were further evaluated by applying CF in a smaller volume (Fig. 3) than used in the infinite dose experiments. The term 'finite' dose usually refers to an amount of $\leqslant 10\ mg\ cm^{-2}$ of the topical formulation, which is applied as a thin layer of formulation spread uniformly across the skin area of interest to mimic a clinically relevant situation. Under such conditions, dose depletion and drying occurs and steady state kinetics is not reached as in an infinite dose scenario. However, the limitations associated with the detection limit of the CF analytical method employed in this study and also the inability to uniformly spread the $10mg\ cm^{-2}$ target dose across the skin surface warranted the need for a higher dose. Therefore a dose corresponding to $20\ mg\ cm^{-2}$ (13 μL) of the saturated solution was introduced in the Franz cell donor, since this was found to be the minimum amount to sufficiently cover the skin surface. The administered dose was found to dry into a thin film after an hour. Such a phenomenon is usually observed under finite dose conditions, where an excess of the formulation is not applied. In this study the term pseudo-finite dose is used for CF not only to reflect the drying nature of the applied dose but also the inability to reach steady state levels (data not shown in this study). Second, even if a formulation dose of $10\ mg\ cm^{-2}$ or less were employed in this study, dose depletion normally associated with finite dose conditions would not have been observed due to the saturated nature of the formulation applied.

For untreated skin and chemical enhancement procedures the presence of CF in the Franz cell receptor was only determined after 120 min (limit of detection of HPLC method was $0.05\ \mu g\ mL^{-1}$), whereas for other methods of enhancement the amount of CF in the receptor compartment was determined after 30 min. The amount of CF accumulating in the receptor compartment of the Franz cell as a function of time after treatment of the skin using the chemical enhancement method was found to be not statistically different to that obtained after monitoring the diffusion across untreated skin ($p > 0.05$). Thus the application of ethanol failed to produce a significant enhancement in CF permeation, when applied as a pseudo-finite dose as opposed to when an infinite protocol

Table 3
Comparing effect of positive controls and brush treatment on skin absorption of BP, MP and CF

Penetrant	Absorption parameters[d]	Untreated	Chemical enhancer	Delipidised	Tape-stripped	Brush (type B) treated[a]
BP	J_S (10^{-3} μg cm^{-1} s^{-1})	4.93 ± 0.22	18.48 ± 1.61[c]	20.54 ± 2.85[c]	15.60 ± 3.69[c]	12.69 ± 2.07[c]
	K_P (10^{-5} cm s^{-1})	2.47 ± 0.11	9.24 ± 0.80	10.27 ± 1.43	7.80 ± 1.84	6.34 ± 1.03
	T_L (min)	35.84 ± 1.98	26.43 ± 5.05	8.17 ± 5.41	34.78 ± 4.44	14.55 ± 5.18
	EF[b]		3.75	4.16	3.16	2.57
MP	J_S (10^{-3} μg cm^{-1} s^{-1})	7.46 ± 0.67	44.45 ± 7.19[c]	41.98 ± 2.05[c]	53.86 ± 12.27[c]	62.34 ± 12.75[c]
	K_P (10^{-6} cm s^{-1})	3.83 ± 0.35	20.96 ± 3.39	19.81 ± 0.96	25.41 ± 5.79	29.41 ± 6.01
	T_L (min)	16.61 ± 5.15	7.76 ± 5.56	nd	2.71 ± 1.61	6.73 ± 3.63
	EF[b]		4.08	3.85	4.94	5.72
CF[x]	J_S (10^{-4} μg cm^{-1} s^{-1})	1.87 ± 0.26	28.98 ± 6.39[c]	20.43 ± 5.50[c]	35.55 ± 29.50[c]	120.92 ± 42.55[c]
	K_P (10^{-8} cm s^{-1})	9.15 ± 1.27	141.54 ± 31.21	99.68 ± 26.84	173.94 ± 144.34	589.93 ± 207.58
	T_L (min)	49.69 ± 3.54	26.71 ± 9.26	nd	17.16 ± 9.15	nd
	EF[b]		15.46	10.93	19.01	64.44

All data represent means ± SD ($n \geqslant 3$).
Device speed maintained at 80 rpm. nd, not done.
[a] Data shown in Table 2, obtained using brush B fat 450 Nm^{-2}, 15 s for BP and MP but at 300 Nm^{-2}, 25 s for CF.
[b] EF is the enhancement factor calculated as the ratio of the flux of permeant through treated skin to that of untreated skin.
[c] Flux significantly different from untreated skin ($p \leqslant 0.05$).
[d] Same skin donor(s) as in Table 2.

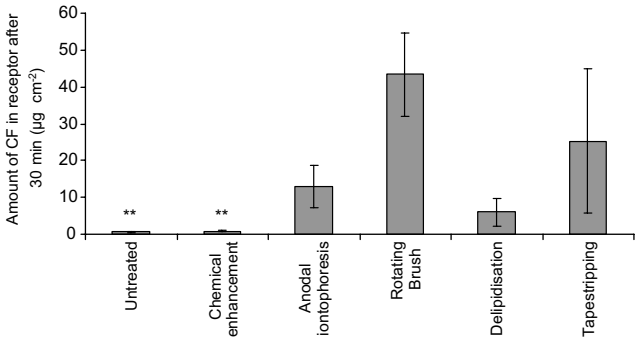

Fig. 3. Pseudo-finite dose study comparing the effect of the device with brush B (300 Nm^{-2}, 25 s, 80 rpm) to other established modes of skin permeation enhancement on CF absorption. **Represents the amount of CF in receptor after 120 min, since the concentration of CF at 30 min was below limit of detection. Data represent means ± SD ($n = \geqslant 3$).

was employed (Table 3). This may be as a result of the significant ethanol evaporation which occurred during the pseudo-finite dose study leading to drug immobility on the skin surface. This phenomenon however is not expected to occur under the infinite dose conditions implying that any perturbing effect of ethanol on the skin is likely to be short-lived when a finite dose is applied.

The effect of iontophoresis on the skin transport of hydrophilic solutes such as CF has been reported previously [35], and it was therefore considered to be a useful comparator method in this phase of the study. Anodal (+) iontophoresis was employed since at pH 7.4, 99% of the CF present in donor solution exists as positively charged ions (pK_a of CF is 10.4). A higher amount of CF was transported to the receptor chamber by the positive electrode in comparison to the untreated control. Such an increase in skin permeation using iontophoresis can be attributed to the mechanisms of electromigration and/or electroperturbation [27,35].

The amount of CF in receptor after 30 min using the brush device was found to be significantly higher ($p \leqslant 0.05$) than that recorded for the other methods of enhancement excluding the use of tape-stripping. The degree of enhancement recorded after 120 min was in the order; brush (B) device (EF = 70) > tape-stripping (EF = 8) > anodal iontophoresis (EF = 20) > delipidisation (EF = 15) > chemical (ethanol) enhancer (EF = 1.13). The EF rank order for the device (brush B), delipidisation and tape-stripping was similar to that found previously (Table 2) in the infinite dose study for CF.

3.4. Permeation of ACV (finite dose study)

The relatively poor efficacy of commercial ACV topical preparations has been attributed to the low permeability of the drug through the skin as a consequence of the hydrophilicity of ACV [37]. The latter results in a slow diffusion profile which prevents ACV from reaching the intended target site (basal epidermis) [38,39]. Such poor permeation characteristics ensure that it is an interesting candidate to use in the evaluation of any novel method or device intended to enhance percutaneous absorption. Iontophoresis currently serves as one of the most effective skin permeation strategies of enhancing the therapeutic profile of ACV [26,39–41,44].

The structure of the Zovirax formulation allowed a smaller volume (6 mg) to be spread evenly across skin surface compared to the aqueous formulation (13 μL) used previously for CF. The skin permeation of ACV (Zovirax cream) applied as a pseudo-finite dose was promoted to a greater extent as the duration of brush treatment was increased (Fig. 4). A significant increase in ACV transport was observed following brush treatment ($p \leqslant 0.05$). Hence EF values of 36–220 were obtained after 10–60 s of brush

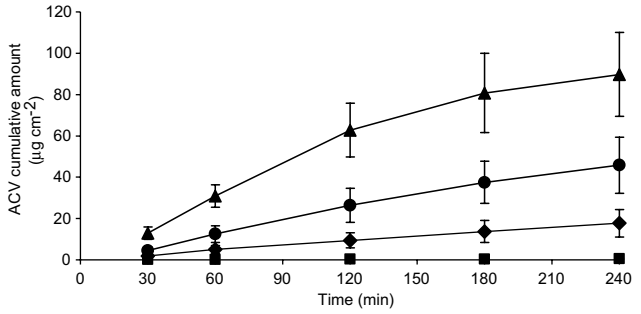

Fig. 4. Effect of treatment time on the skin permeation profile of ACV (finite dose) from a topical preparation using rotating device with brush B and constant device parameters (speed; 80 rpm, pressure; 300 Nm^{-2}) [(■) untreated (◆) 10 s (●) 30 s and (▲) 60 s]. Data represent means ± SE ($n \geqslant 4$) and error bars not shown are within size of symbol.

treatment. The use of iontophoresis proved generally less effective than employing the rotating brush in enhancing permeation. For example the effect of the 10 min anodal iontophoresis on the skin permeation of ACV proved to be comparable to that obtained after application of brush B at 300 Nm^{-2} for 10 s (Table 4). The iontophoretic method in this study employed optimum conditions (electrode type: anode, pH of buffer: 7.4, current intensity: ⩽0.5 mA) which have been previously shown to enhance ACV permeation *in vitro* [26,27,40].

3.5. Permeation of angiotensin II (Infinite dose study)

Remarkable results in skin permeation enhancement observed for, the hydrophilic solutes ACV and CF, prompted an investigation into the ability of the device to enhance the permeation of a model peptide, Ang II. The hydrophilic nature and large molecular weight of the peptide make it the less likely candidate to be absorbed across the skin compared to the other solutes already used in this study. Due to this potentially low permeability, the use of an infinite dosing technique was employed in an attempt to achieve adequate peptide permeation. An immunoassay was used in an attempt to quantify the potentially very low concentrations of the diffusing peptide. Whilst the EIA assay is not stability-indicating, conditions that would be expected to result in the enzymatic degradation of the peptide were not employed in this study. Non-viable skin was used and thus metabolic activity leading to peptide hydrolysis was negligible. The use of the heat separation method [21] to isolate epidermal sheets from the remaining skin tissue has been shown to result in the elimination of enzymatic activity within the skin [42]. The peptide was also chemically stable in the EIA buffer over the experimental period and temperature conditions used in the study (EIA booklet). It is thus safe to assume that peptide hydrolysis did not occur in the course of the study.

The ideal operational device parameters for the rotating brush recorded during the ACV study were employed to investigate the permeation of Ang II. The effects of using different enhancement strategies on the peptide diffusion are summarised by the data presented in Fig. 5. The amount of Ang II permeating untreated skin after 4 h and 24 h was significantly lower than that which had diffused across brush-treated, delipidised and tape-stripped skin ($p \leqslant 0.05$). At both time points (4 and 24 h) the highest amount of Ang II in the receptor was obtained when the rotating brush device was employed. Overall, the degree of enhancement associated with Ang II permeation across skin after both time periods was found to be in the order; brush (B) device (EF = 57–93) > delipidisation (EF = 44–75) > tape-stripping (EF = 25–69) untreated. The effect of tape-stripping was lower than that of delipidisation, with the number of strips (8) used in this study, only leading to partial removal of the SC. Recent reports using the same adhesive plates (D-Squame) as in this study reported the removal of 6–7 μm of the SC using 30 D-Squame plates [43], whereas the SC has been estimated to be approximately 10–20 μm thick [44]. Such a partial removal of SC is likely to account for the lower EF compared to

Table 4
A comparison of the effects of iontophoresis and treatment with brush B at various times on the *in vitro* skin permeation of ^{3}H-labelled ACV (finite dose)

	Amount in receptor (μg cm^{-2}) after 60 min	EF
Untreated brush (B) treatment[b]	0.14 ± 0.08	–
10 s	5.06 ± 1.88[a]	36.17
30 s	12.5 ± 4.02[a]	89.29
60 s	30.91 ± 5.45[a]	220.76
Iontophoresis (anodal)	4.95 ± 2.35[a]	35.42

Data represent means ± SE ($n \geqslant 4$) except otherwise stated.
[a] Significantly different from that of untreated skin ($p \leqslant 0.05$).
[b] Device parameters (speed; 80 rpm, pressure; 300 Nm^{-2}) were maintained constant.

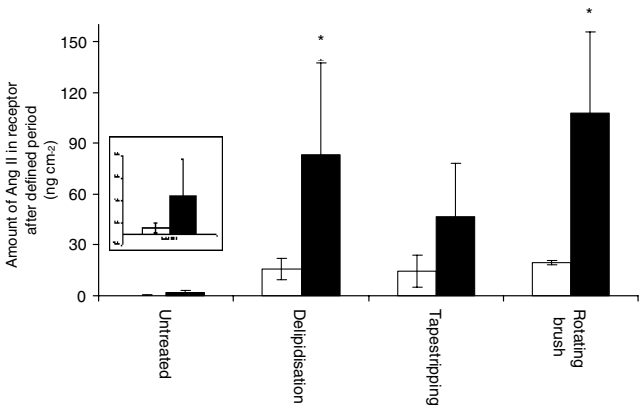

Fig. 5. Effect of brush (B) treatment and other percutaneous enhancement methods on the *in vitro* skin permeation of Ang II after (□) 4 h and (■) 24 h. *Significantly different from amount at 4 h using same enhancement method ($p \leqslant 0.05$). Brush device parameters (speed; 80 rpm, pressure; 300 Nm^{-2}, treatment duration; 30 s) were maintained constant. Data represent means ± SD ($n \geqslant 3$). Inset box shows amount of Ang II transported across untreated skin at 4 and 24 h.

delipidisation, due to differences in the level of resultant skin barrier perturbation attained using these methods. The rotating brush was however more effective in enhancing permeation of the peptide across the epidermis compared to the other established methods of penetration enhancement used (Fig. 5).

4. Conclusion

Findings from this present study support the effectiveness of a rotating brush applied to the skin, in enhancing the cutaneous permeability of subsequently applied model solutes of various physicochemical properties. Bristle type was found to have a marked effect on the permeability of the model compounds. The greatest penetration enhancement was obtained for hydrophilic solutes (CF, ACV and Ang II) in comparison to more lipophilic species (BP and MP). The former solutes normally permeate intact skin poorly due to the physicochemical properties of the latter. The observed enhancement in permeability was a result of the disruption of the cells of the SC which compromises the principal barrier that skin provides to the absorption of applied compounds. Abrasion devices which allow the controlled removal of only the upper layers of the skin are essential in attempting to generate a standardised skin treatment prior to the topical application of drugs. This pre-requisite is a limitation of previous research into this mode of skin penetration enhancement. The use of a rotating brush device, as described in this study, may serve as an efficient and simple means of overcoming such a limitation. Further *in vitro* studies are warranted using other solutes to optimize further device parameters, as is an *in vivo* delivery feasibility study using such a prototype device.

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References

[1] M.B. Brown, G.P. Martin, S.A. Jones, F.K. Akomeah, Dermal and transdermal drug delivery systems: current and future prospects, Drug Deliv. 13 (3) (2006) 175–187.
[2] C.K. Lee, T. Uchida, K. Kitagawa, A. Yagi, N.A. Kim, S. Goto, Relationship between lipophilicity and skin permeability of various drugs from an ethanol/water/lauric acid system, Biol. Pharm. Bull. 17 (10) (1994) 1421–1424.
[3] J.D. Bos, M.M. Meinardi, The 500 Dalton rule for skin penetration of chemical compounds and drugs, Exp. Dermatol. 9 (3) (2000) 165–169.
[4] M.H. Gold, Dermabrasion in dermatology, Am. J. Clin. Dermatol. 4 (7) (2003) 467–471.
[5] W.R. Lee, S.C. Shen, K.H. Wang, C.H. Hu, J.Y. Fang, Lasers and microdermabrasion enhance and control topical delivery of vitamin C, J. Invest. Dermatol. 121 (5) (2003) 1118–1125.
[6] J.Y. Fang, W.R. Lee, S.C. Shen, Y.P. Fang, C.H. Hu, Enhancement of topical 5-aminolaevulinic acid delivery by erbium:YAG laser and microdermabrasion: a comparison with iontophoresis and electroporation, Br. J. Dermatol. 151 (1) (2004) 132–140.
[7] J.A. Mikszta, J.M. Britingham, J. Alarcon, R.J. Pettis, J.P. Dekker, Topical delivery of vaccines, U.S. Patent 6,595,947, July 22, 2003.
[8] J.A. Mikszta, J. Alarcon, J.M. Britingham, D.E. Sutter, R.J. Pettis, G.H. Harvey, Improved genetic immunization via micromechanical disruption of skin barrier function and targeted epidermal delivery, Nat. Med. 8 (4) (2002) 415–419.
[9] J. Down, N.G. Harvey, in: R.H. Guy, J. Hadgraft (Eds.), Transdermal Drug Delivery, Marcel Dekker, New York, 2003, pp. 327–360.
[10] M.J. Choi, H.I. Maibach, Topical vaccination of DNA antigens: topical delivery of DNA antigens, Skin Pharmacol. Appl. Skin Physiol. 16 (5) (2003) 271–282.
[11] W.R. Lee, S.C. Shen, H.H. Lai, C.H. Hu, J.Y. Fang, Transdermal drug delivery enhanced and controlled by erbium: YAG laser: a comparative study of lipophilic and hydrophilic drugs, J. Control. Release 75 (1–2) (2001) 155–166.
[12] T. Fugimoto, K. Shirakami, K. Tojo, Effect of microdermabrasion on barrier capacity of stratum corneum, Chem. Pharm. Bull. 53 (8) (2005) 1014–1016.
[13] J.M. Spencer, Microdermabrasion, Am. J. Clin. Dermatol. 6 (2) (2005) 89–92.
[14] Y.N. Kalia, A. Naik, J. Garrison, R.H. Guy, Iontophoretic drug delivery, Adv. Drug Del. Rev. 56 (5) (2004) 619–658.
[15] Y. Wang, R. Taker, Q. Fan, B. Michniak, Transdermal iontophoresis: combination strategies to improve transdermal iontophoretic drug delivery, Eur. J. Pharm. Biopharm. 60 (2) (2005) 179–191.
[16] S.K. Rastogi, J. Singh, Lipid extraction and transport of hydrophilic solutes through porcine epidermis, Int. J. Pharm. 225 (1–2) (2001) 75–82.
[17] S.K. Rastogi, J. Singh, Lipid extraction and iontophoretic transport of leuprolide acetate through porcine epidermis, Int. J. Pharm. 215 (1–2) (2001) 241–249.
[18] A.H. Ghanem, H. Mahmound, W.I. Higuchi, U.D. Rohr, S. Borsadia, P. Liu, J.L. Fox, W.R. Good, The effects of ethanol on the transport of β-estradiol and other permeants in the hairless mouse skin (II): a new quantitative approach, J. Control. Release 6 (1) (1987) 75–83.
[19] A.H. Ghanem, H. Mahmoud, W.I. Higuchi, P. Liu, W.R. Good, The effects of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: methods/validation of a novel approach, lnt. J. Pharm. 78 (1–3) (1992) 137–156.
[20] A.C. Williams, B.W. Barry, Penetration enhancer, Adv. Drug Del. Rev. 56 (5) (2004) 603–618.
[21] A.M. Kligman, E. Chrstophers, Preparation of isolated sheets of human stratum corneum, Arch. Dermatol. 88 (1963) 702–705.
[22] M.B. Brown, G.P. Martin, Dermal drug delivery system, W.O. Patent 2005/058226 A1, June 30, 2005.
[23] E.S. Reynolds, The use of lead citrate at high pH as an electron opaque stain in electron microscopy, J. Cell Biol. 17 (1) (1963) 208–212.
[24] F.K. Akomeah, G.P. Martin, M.B. Brown, Variability in human skin permeability in vitro: comparing penetrants with different physicochemical properties, J. Pharm. Sci. 96 (4) (2007) 824–834.
[25] F. Akomeah, T. Nazir, G.P. Martin, M.B. Brown, Effect of heat on the percutaneous absorption and skin retention of 3 model penetrants, Eur. J. Pharm. Sci. 21 (3) (2004) 337–345.
[26] N.M. Volpato, S. Nicoli, C. Laureri, P. Colombo, P. Santi, In vitro acyclovir distribution in human skin layers after transdermal iontophoresis, J. Control. Release 50 (1–3) (1998) 291–296.
[27] P. Santi, S. Nicoli, G. Colombo, R. Bettini, M. Artusi, S. Rimondi, C. Padula, P. Rizzo, P. Colombo, Post iontophoresis transport of ibuprofen lysine across rabbit ear skin, Int. J. Pharm. 266 (1–2) (2003) 69–75.
[28] I. Niechajev, A. Ljungqvist, Peroral dermabrasion: clinical and experimental studies, Aesthetic Plast. Surg. 16 (1) (1992) 11–20.
[29] L.A. Ferrara, A.J. Fleischman, E.C. Benzel, S. Roy, Silicon dermabrasion tools for skin resurfacing applications, Med. Eng. Phys. 25 (6) (2003) 483–490.

[30] H. Löffler, F. Dreher, H.I. Maibach, Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal, Br. J. Dermatol. 151 (4) (2004) 146–152.
[31] J.M. Yarborough Jr., Dermabrasion by wire brush, J. Dermatol. Surg. Oncol. 13 (6) (1987) 610–615.
[32] G.M. Khan, Y. Frum, O. Sarheed, G.M. Eccleston, V.M. Meidan, Assessment of drug permeability distributions in two different model skins, Int. J. Pharm. 303 (1–2) (2005) 81–87.
[33] D. Monti, R. Giannelli, P. Chetoni, S. Burgalassi, Comparison of the effect of ultrasound and of chemical enhancers on transdermal permeation of caffeine and morphine through hairless mouse skin in vitro, Int. J. Pharm. 229 (1–2) (2001) 131–137.
[34] A. Boucaud, L. Machet, B. Arbeille, M.C. Machet, M. Sournac, A. Mavon, F. Patat, L. Vaillant, In vitro study of low-frequency ultrasound-enhanced transdermal transport of fentanyl and caffeine across human and hairless rat skin, Int. J. Pharm. 228 (1–2) (2001) 69–77.
[35] N. Sekkat, A. Naik, Y.N. Kalia, P. Glikfield, R.H. Guy, Reverse iontophoretic monitoring in premature neonates: feasibility and potential, J. Control. Release 81 (1–2) (2002) 83–89.
[36] C. Gunther, A. Kecskes, T. Staks, U. Tauber, Percutaneous absorption of methylprednisolone aceponate following topical application of Advantan lotion on intact, inflamed and stripped skin of male volunteers, Skin Pharmacol. Appl. Skin Physiol. 11 (1) (1998) 35–42.
[37] A.K. Seth, A. Misrad, D. Umrigar, N. Vora, Role of acyclovir gel in herpes simplex: clinical implications, Med. Sci. Monit. 9 (7) (2003) P193–P198.
[38] G.E. Parry, P. Dunn, V.P. Shah, L.K. Pershing, Acyclovir bioavailability in human skin, J. Invest. Dermatol. 98 (6) (1992) 856–863.
[39] G. Stagni, M.E. Ali, D. Weng, Pharmacokinetics of acyclovir in rabbit skin after i.v.-bolus, ointment and iontophoretic administrations, Int. J. Pharm. 274 (1–2) (2004) 201–211.
[40] N. Volpato, P. Santi, P. Colombo, Iontophoresis enhances the transport of acyclovir through nude mouse skin by electrorepulsion and electroosmosis, Pharm. Res. 12 (11) (1995) 1623–1627.
[41] D. Goldberg, Iontophoretic based drug delivery, Innov. Pharm. Technol. 16 (May edition) (2005) 68–72.
[42] R.C. Wester, J. Christoffel, T. Hartway, N. Poblete, H. Maibach, J. Forsell, Human cadaver skin viability for in vitro percutaneuos absorption: storage and detrimental effects heat separation and freezing, Pharm. Res. 15 (1) (1988) 82–84.
[43] S. Tinkle, J.M. Antonini, B.A. Rich, S.R. Roberts Jr., R. Salmen, K. DePree, E.J. Adkins, Skin as a route of exposure and sensitation in chronic beryllium disease, Environ. Health Perspect. 111 (9) (2003) 1202–1208.
[44] K.A. Walters, M.S. Roberts, in: K.A. Walters (Ed.), Dermatological and Transdermal Formulations, Marcel Dekker, New York, 2002, pp. 41–60.