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Development of an *in vitro* model for studying the penetration of chemicals through compromised skin

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ABSTRACT

The conventional safety approach that includes dermal absorption of pharmaceutical or consumer products uses models that are based on intact skin. However, when products are intended for application to skin with a less effective barrier, such as in new-born infants, or in cases where the skin is mildly damaged or diseased, there are instances where absorption through compromised skin is also important. A tape stripping procedure was investigated using dermatomed pig skin to assess if an *in vitro* model could replicate the typical changes in barrier function observed in humans with compromised skin. The relationship between Trans-Epidermal Water Loss (TEWL), Electrical Resistance (ER) and Tritiated Water Flux (TWF), markers of skin barrier function in OECD 428 studies was investigated. There was a step-wise reduction in ER from normal (control) skin following 5, 10, 15 or 20 tape strips. This was mirrored by increases in both TWF and TEWL. An *in vitro* experimental protocol using 5 tape strips, ER and dermatomed pig skin provided a rapid, robust and reproducible approach equivalent to the 3–4-fold increases in TEWL observed clinically in compromised skin.

1. Introduction

Skin that has a compromised *stratum corneum* is likely to provide a less effective barrier to topically applied chemicals when compared with normal skin. For example, skin that is impaired due to irritation, sensitisation or more chronic skin disease, such as psoriasis, is likely to be a less effective barrier to the entry of chemicals into the systemic circulation via the dermal route (Goon et al., 2004; Kim et al., 2006; Stamatas et al., 2011). The measurement of dermal absorption of chemicals for consumer products intended for application to the skin is an important part of risk assessment. However, the *in vitro* animal and human models that assess the dermal penetration of topically applied products in Franz-type diffusion cells utilise intact skin (Franz, 1975; OECD, 2004a, 2004b; SCCS, 2010). Since there is no standardised model for evaluating skin penetration in conditions where the barrier properties of the *stratum corneum* are impaired, the use of additional safety factors to accommodate this is arbitrary, despite the fact that many products are targeted for use on skin that has impaired barrier properties. Therefore, a simple and robust *in vitro* technique would be useful for studying the dermal absorption of chemicals in compromised skin.

The purpose of this study was, therefore, to explore whether the tape stripping procedure used to assess the distribution of chemicals in the skin in regulatory protocols could be adapted, *in vitro,* to mimic damage to the *stratum corneum* barrier. Dermatomed pig skin[1] was used in these investigations since the morphological and permeability characteristics of the skin of this species are very similar to humans (Dick and Scott, 1992; Scott and Clowes, 1992) and pig skin is an accepted model for the skin penetration assessment of cosmetic ingredients (SCCS, 2010). One of the requirements of these regulatory studies that involve resected human or animal skin is to establish that the permeability characteristics of each skin sample is normal prior to the application of a test article to the skin surface. The commonly used skin integrity tests in OECD 428 *in vitro* dermal penetration studies using Franz diffusion cells include the measurement of Electrical Resistance (ER), Tritiated Water Flux (TWF) and Trans-Epidermal Water Loss (TEWL). Historically, the TWF approach was the most common barrier function test, but this

Abbreviations: ER, Electrical Resistance; TEWL, Trans-Epidermal Water Loss; TWF, Tritiated Water Flux; LSC, Liquid Scintillation Counting.

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[1] Animals were sacrificed for non-cosmetic purposes before the skin was harvested.

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has been largely replaced by the ER approach which is more practical, since the establishment of a steady state for water permeation takes several hours (Dugard et al., 1984; Lawrence, 1997). TEWL is also a useful method since it is non-invasive and the same instrument can be used for *in vitro* and *in vivo* barrier function assessment (Imhof et al., 2009). A major drawback for TEWL assessment is that it requires a period of stabilisation in a controlled temperature and humidity environment around the probe and is therefore not a practical option for studies involving large numbers of diffusion cells.

Values for "normal" or acceptable skin barrier properties for the three skin integrity parameters (ER, TWF and TEWL) have been published for six species, including human (Heylings et al., 2001; Davies et al., 2004). Of these methods, the ER approach has been shown to be the most practical and robust (Davies et al., 2004). However, different laboratories utilise different Databridge equipment to measure this resistance or impedance parameter and sometimes use different direct current and frequency settings. In addition, there are many different types of diffusion cells where the skin surface area and cell design also has an impact on the technique. Therefore, care has to be taken in the interpretation of values between laboratories (White et al., 2011). Ideally, investigators undertaking such work should link their own impedance/ER methodology to in-house TWF data for the same skin samples, in order to demonstrate the reliability of integrity data that is based on electrical properties of the skin membrane.

In our investigation we have explored the usefulness of Electrical Resistance (ER), Tritiated Water Flux (TWF) and Trans-Epidermal Water Loss (TEWL), for predicting the degree of skin damage achieved through sequential tape stripping of the skin surface. We aimed to establish how the permeability properties of skin changes with varying degrees of skin stripping using dermatomed pig skin in our glass static diffusion cells.

2. Materials and methods

Skin was obtained from suckling pigs (aged 6–8 weeks) of the British White strain that were sacrificed for non-cosmetic purposes before the skin was harvested. Pig skin is a predictive model for human skin penetration as it has very similar morphology and permeability properties to human skin (Dick and Scott, 1992) and it is permitted in regulatory studies to assess the skin penetration of cosmetic ingredients (SCCS, 2010).

2.1. Preparation of dermatomed skin membranes

Samples of whole skin were excised from the trunk area. Excess hair was removed and strips of skin membranes (approximately 6 cm diameter) were cut at a thickness of 200–500 μm using an electric dermatome. Each membrane was given an identifying number and stored frozen, at −20 °C, on aluminium foil, until required for use. The dermatomed skin membranes were used within 6 months of preparation.

2.2. In vitro static diffusion cell equipment

Details of the approach used in these investigations are similar to those described in the OECD guidance document No. 28 (OECD, 2004a). Discs of dermatomed skin membranes approximately 3.3 cm diameter were mounted dermal side down in Franz-type static diffusion cells with an exposed area of 2.54 cm^2 (Dugard et al., 1984; Scott and Clowes, 1992). The receptor chambers were filled with a recorded volume of physiological saline and placed on a magnetic stirrer plate in a water bath maintained at 32 ± 1 °C. Diffusion cells containing membranes intended for TEWL measurement were placed in an incubator maintained at the same temperature.

2.3. Measurement of skin barrier function

Three measures of skin barrier function (ER, TEWL and TWF) were utilised in this study using methods and previously established cut-off values for the rejection of abnormal samples (Davies et al., 2004; Heylings et al., 2001; Imhof et al., 2009).

For ER, this was measured using a PRISM Electronics AIM6401 LCR data bridge connected to two stainless steel electrodes using a setting of 100 kHz and ER was expressed as kΩ for the exposed skin surface area (2.54 cm^2). Further details on the equipment used can be found in our previous publication (Davies et al., 2004). The diffusion chambers were allowed to equilibrate in a water bath at 32 °C for approximately 30 min. One electrode was inserted into the saline in the receptor chamber underneath the skin via the side arm and the other electrode immersed in 2 ml of saline in the donor chamber above the skin. When the resistance across the skin sample had stabilised, the ER reading was recorded.

TWF was determined by firstly allowing the membranes to equilibrate in a water bath at 32 °C for approximately 30 min after which a 2 ml aliquot of tritiated water (T_2O), containing a known amount of radioactivity, was applied to the surface of the membranes. Contact between the T_2O and the skin membrane was deemed to be the start of the experiment (time zero). Samples of the receptor fluid were taken 3, 4, 5 and 6 h after application and analysed for radioactivity content by LSC. The receptor fluid removed was not replaced. However, the receptor fluid and skin membranes were in good contact throughout the T_2O permeability measurement. A permeability coefficient (K_p) was calculated by dividing the steady state absorption rate by the radioactivity concentration of the T_2O applied to the membranes.

TEWL was measured by firstly placing the diffusion cells containing skin membranes in a humidity (40–60%) and temperature-controlled incubator at 32 °C. The cells were allowed to equilibrate for at least 30 min before taking a measurement using a calibrated, ServoMed EP-2 Evaporimeter (ServoMed, Varberg, Sweden) by placing the probe directly on to the dry skin surface. Once the TEWL value had stabilised the reading was recorded.

2.4. Experimental design

Part of the pre-selection criteria of the membranes was a conventional ER skin integrity test which was used to identify any damaged pig skin samples. Any skin sample, in our static diffusion cells, that did not meet the cut-off value of 3 kΩ was discarded and not used in these investigations. The criteria for barrier damage in dermatomed pig skin was as described previously (Davies et al., 2004). Normal skin samples from five different animals were then randomly assigned to groups to be left unstripped (control membranes) or to groups to be subjected to tape stripping 5, 10, 15 or 20 times, in order to remove different proportions of the *stratum corneum*. Further skin samples from the same animals that had passed the initial ER skin integrity test were also randomly assigned to groups to be subjected to individual tape stripping (5 tape strips then individual strips up to a maximum of 14 strips). The tape stripping method followed the standard approach described in the OECD 428 test guideline (Trebilcock et al., 1994), using 22 mm diameter Cuderm D-Squame stripping discs (CuDerm Corporation, Dallas, USA) which were applied to the dry skin surface at a constant pressure of 225 g/cm^2 for five seconds using a purpose-built applicator. The three measures of skin barrier function (ER, TEWL and TWF) were recorded before the tape stripping procedure. The three values were recorded again after removal of the specified number of tape strips of the *stratum corneum* and finally for a third time after 24 h following the tape stripping procedure. Initial and 24 h measurements were also performed for the unstripped control membranes. For comparative purposes,

a separate group of pig skin samples were subdivided into an unstripped control group and a group where the epidermis had been completely removed by heat-separation.

2.5. Statistical analyses

The pre- and post-values for the three measures of skin integrity were recorded for the control and each tape stripping procedure and expressed as mean ± SEM for each group. A comparison of the three skin integrity measurements (ER, TEWL and TWF) was made between the unstripped control skin and the tape stripped skin using Students *t*-test for unpaired variates, as appropriate. ER was expressed as kΩ and was based on our Laboratory's standard diffusion cell area (2.54 cm^2).

3. Results

3.1. Skin integrity measurements of unstripped control pig skin

The multiple skin samples from five different animals were assigned to the three measurement groups in order to minimise any effects from different animals. Fig. 1A–F shows the three skin integrity markers which were measured at both 0 h and 24 h and plotted against one another for at least five replicates from each animal. The individual skin samples in normal skin gave an ER distribution of the order of 1–23 kΩ, a TEWL distribution of 1–15 g/m^2/h and a T_2O (TWF) distribution of 0.2–6 × 10^{-3} cm/h. Measurements taken 24 h later, for the same skin diffusion cells, were similar; ER distribution was 1–22 kΩ, a TEWL distribution of 1–11 g/m^2/h and a T_2O (TWF) distribution of 0.4–6 × 10^{-3} cm/h. For reference, the cut-off values from previously published data for pig skin from our laboratory have been added as intersect lines on Fig. 1A–F. These lines represent cut-offs deemed as normal skin integrity for this species (Davies et al., 2004), and include the majority of individual values measured in the present study. Table 1 shows the distribution of the values across each group that were used to plot Fig. 1A–F.

3.2. Skin integrity measurements of tape stripped pig skin

The next stage of the investigation involved a direct comparison of normal pig skin with samples from the same animals that had been tape stripped 5, 10, 15 or 20 times to remove different amounts of the *stratum corneum*. TWF measurements were only taken following 10 and 15 tape strips due to the time consuming nature of this technique. As shown in Fig. 2A–C, the control levels of TEWL and TWF were both affected with the water flux into the skin increasing and water efflux out of the skin increasing in direct proportion to the degree of tape stripping. Similarly, the ER of the pig skin showed a progressive fall in response to the number of strips taken as the resistivity of the skin sample decreased. For example, the initial batch of 5 tape strips resulted in a highly significant ($p < 0.0001$) 1.7-fold decrease in ER, when compared with the "control" and a highly significant ($p < 0.0001$) 3.5-fold increase in TEWL. Following ten tape strips, TWF increased 3.5-fold ($p < 0.001$), ER decreased 2.4-fold ($p < 0.0001$) and TEWL increased 5-fold ($p < 0.0001$) when compared to the unstripped control group. The trend continued with 15 tape strips resulting in 5.8-fold increases ($p < 0.0001$) in TWF, 3.3-fold decreases in ER ($p < 0.0001$) and 5.8-fold increases in TEWL ($p < 0.0001$) above control. The final ER and TEWL measurements following 20 tape strips, which prob-

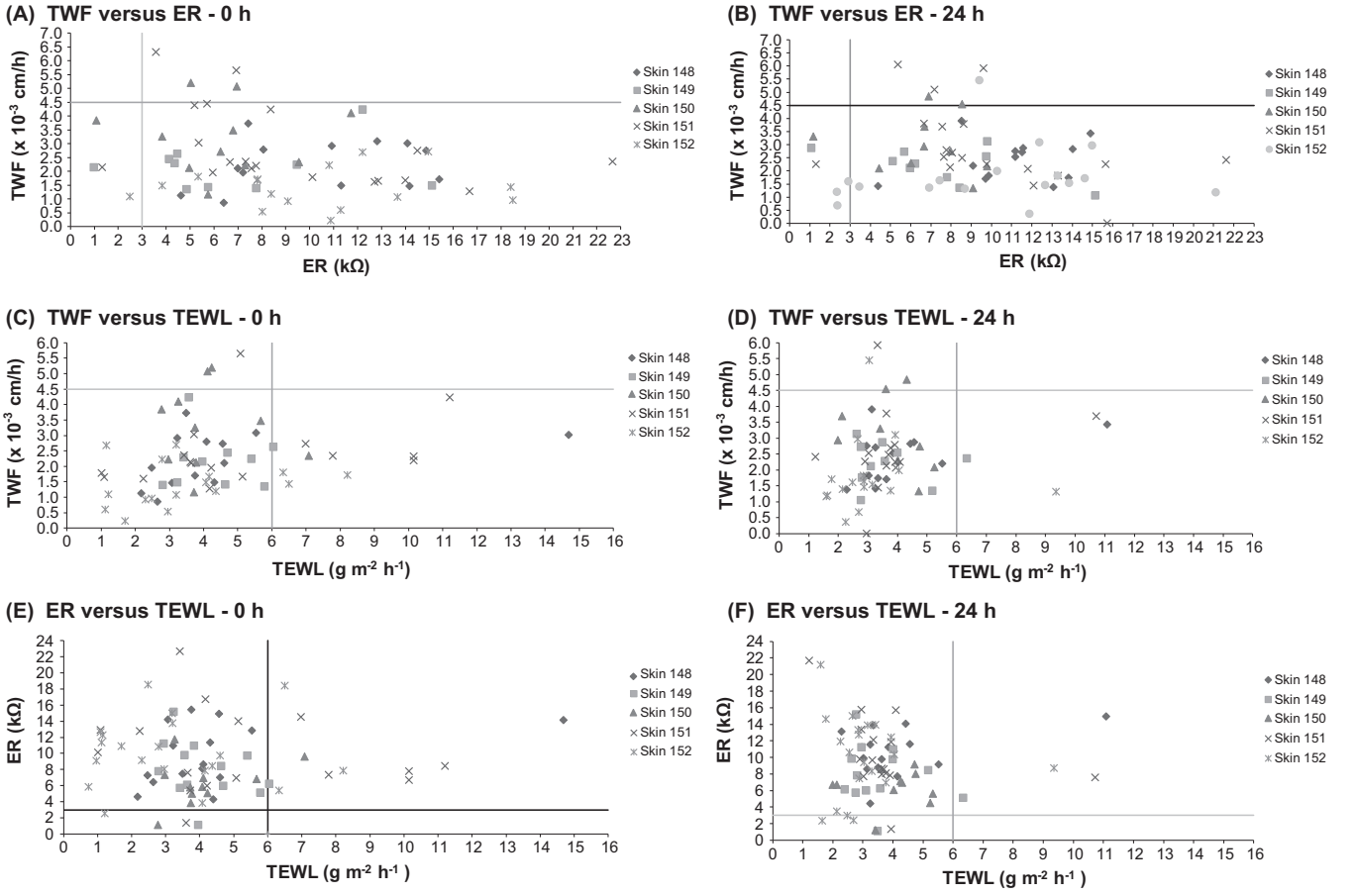

Fig. 1. (A–F) Skin integrity measurements of unstripped control pig skin at 0 h and 24 h using Trans-Epidermal Water Loss (TEWL), Electrical Resistance (ER) and Tritiated Water Flux (TWF).

Table 1
Distribution of values across each of the three skin integrity parameters (TEWL, ER and TWF) used in unstripped control pig skin.

	Trans-Epidermal Water Loss (g m^{-2} h^{-1}) TEWL		Electrical Resistance (kΩ)[a] ER		Tritiated Water Flux ($\times 10^{-3}$ cm/h) TWF	
	0 h	24 h	0 h	24 h	0 h	24 h
Mean ± SEM	4.18 ± 0.28	3.68 ± 0.19	8.94 ± 0.56	9.36 ± 0.48	2.34 ± 0.15	2.43 ± 0.14
n	75	75	75	75	68	68
Min	0.74	1.23	0.98	1.10	0.22	0.36
Max	14.7	11.1	22.7	21.6	6.30	6.05

n = number of determinations.
[a] DTL's standard diffusion cells – Skin area = 2.54 cm^2.

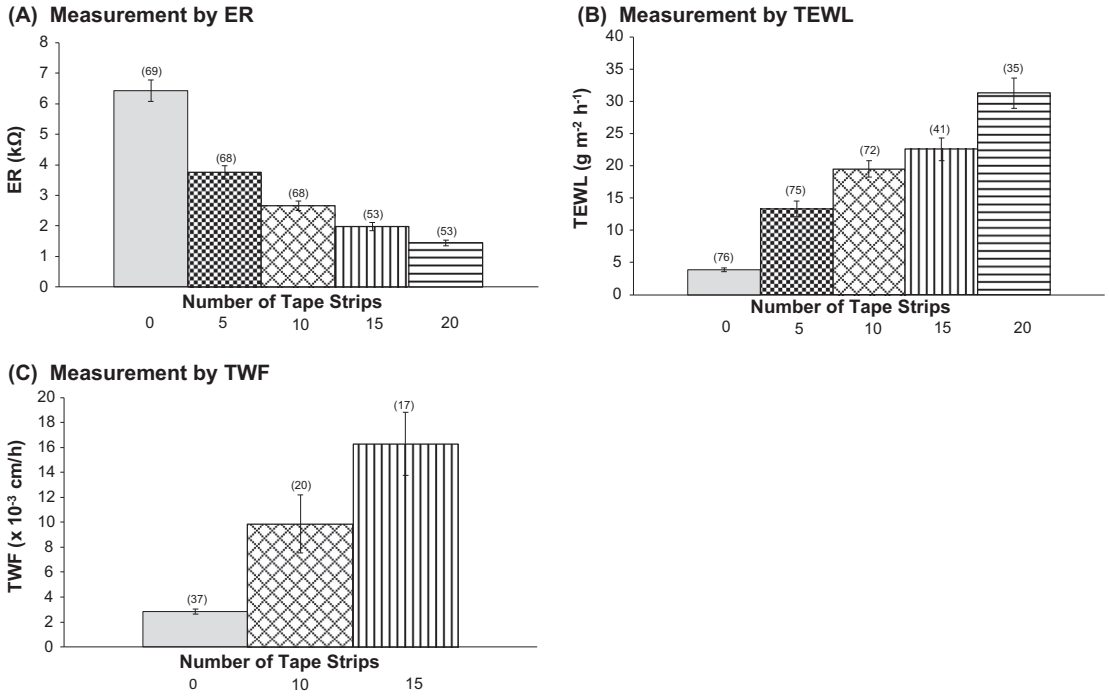

Fig. 2. (A–C) Skin integrity measurements of unstripped control (0 tape strips) pig skin versus skin tape stripped 5, 10, 15 or 20 times. Measurements were taken immediately post stripping. Mean ± SEM. Number of determinations in parenthesis.

ably results in the complete removal of the *stratum corneum*, gave 4.5-fold decreases ($p < 0.0001$) and 8.1-fold increases ($p < 0.0001$) compared with control, respectively. With the exception of TWF measurements following ten tape strips ($p < 0.001$), each batch of five tape strips resulted in a highly significant ($p < 0.0001$) change in the three integrity measurements when compared with the control (0 strips) value.

Further investigation into the effect of individual tape stripping after the first 5 strips reinforced the sensitivity of ER in detecting initial membrane damage following the 5 tape strips and then each subsequent individual tape strip thereafter. As shown in Fig. 3A, the ER value following 5 strips decreased 1.5-fold when compared to the "control" after which there was a small, but observable, further fall in ER of the skin membrane with each subsequent tape strip up to 14 strips. At this point there was an overall 3.4-fold decrease in ER ($p < 0.0001$) when compared to the "control". The individual strip data correlated well with the grouped 5 tape strip data for ER shown in Fig. 2A–C. TEWL measurements following 5 tape strips, as shown in Fig. 3B, demonstrated a 4.8-fold increase in water efflux from the compromised skin when compared to the 'control' which was broadly comparable to the batches of 5 strips. However, TEWL measurements following each subsequent individual tape strip did not show a uniform pattern of increased damage as assessed by water efflux.

3.3. Skin integrity measurements of heat-separated pig skin

In order to demonstrate what magnitude of change could be detected in our skin diffusion cell model of tape stripping, a separate group of pig skin samples were subdivided into a control group and a group where the epidermis had been completely removed by heat-separation, using the procedure described in Guidance documents for the assessment of the dermal penetration of consumer products (SCCS, 2010). As shown in Fig. 4A–C, complete removal of the epidermis leaving just the underlying dermal tissue reduced the ER from 8 kΩ to less than 1.0 kΩ. The TEWL in the same skin samples increased from about 5 g/m^2/h to 61 g/m^2/h. Both of these changes were highly significant ($p < 0.0001$) for $n = 20$ unpaired samples. Similarly, the Tritiated Water Flux (TWF) was much higher in the same heat-separated membranes ($p < 0.001$) with the skin permeability coefficient (K_p) rising from about 2 to 15 $\times$ 10^{-3} cm/h for $n = 10$. Essentially, these skin samples with "damaged" membranes had very little barrier to prevent water movement through the skin.

4. Discussion

This investigation has examined the potential of using the skin integrity methods used in OECD guidelines for *in vitro* dermal

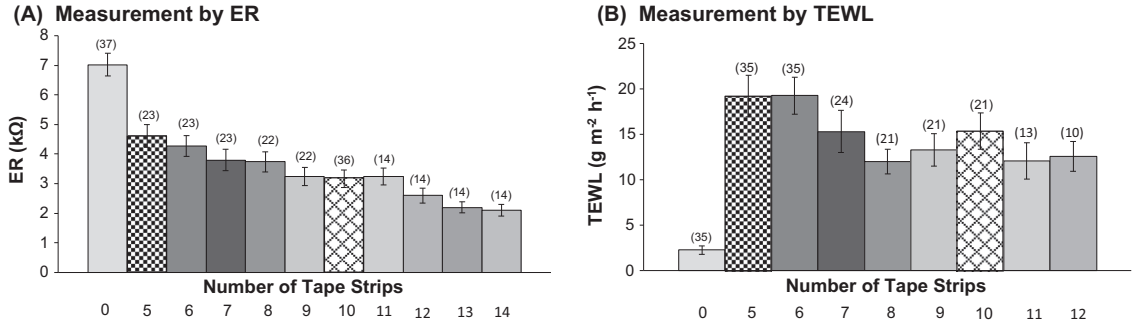

Fig. 3. (A and B) Skin integrity measurements of unstripped control (0 tape strips) pig skin versus skin individually tape stripped following the first five tape strips. Measurements were taken immediately post stripping. Mean ± SEM. Number of determinations shown in parenthesis.

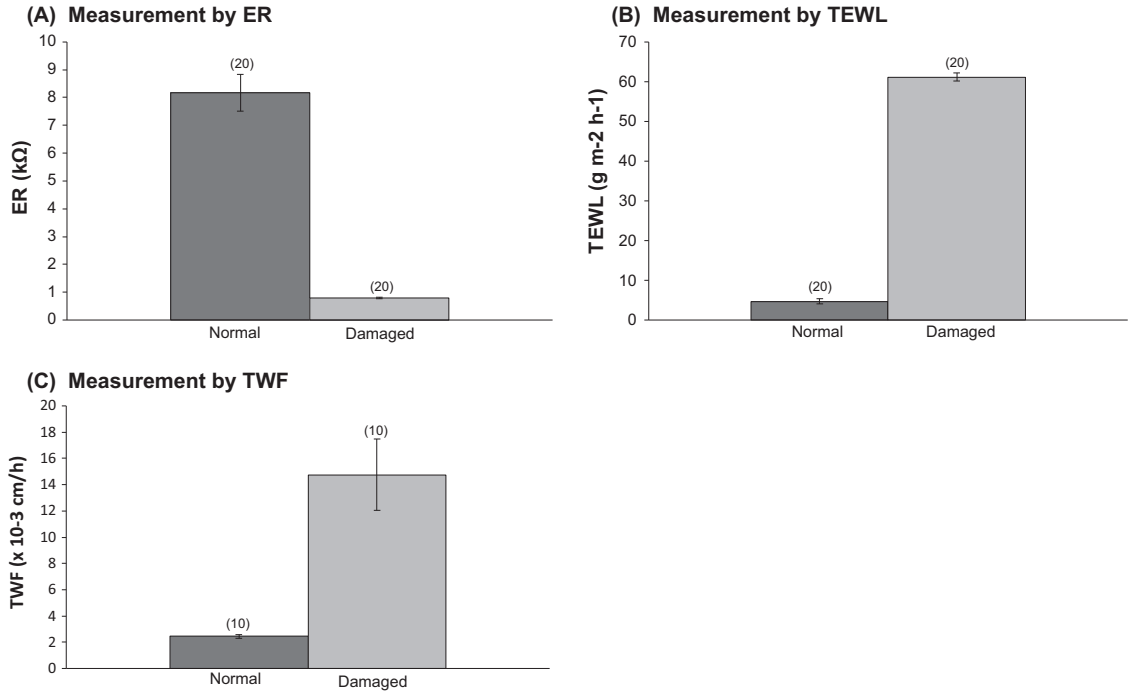

Fig. 4. (A–C) Skin integrity measurements of unstripped control pig skin versus skin where the epidermis had been completely removed by heat separation. Mean ± SEM. Number of determinations shown in parenthesis. Normal = Control untreated skin. Damaged = Heat separated skin.

absorption studies together with the tape stripping approach used to assess disposition of chemicals and drugs in the *stratum corneum*, in order to develop a new model for assessing skin penetration in situations where the skin barrier is abnormally permeable. We recognise that the use of ER measurements with tape stripped skin may be highly impractical when assessing the penetration of compounds through a compromised skin barrier *in vivo*. The ER method involves hydration of the anatomical face of the skin therefore, its applicability to disease situations involving changes in hydration of the *stratum corneum* may be limited. However, in this investigation, we have identified that tape stripping and use of ER, in particular, is a rapid, robust and practical *in vitro* approach that may be useful to study the absorption of chemicals and drugs through skin that has impaired barrier properties. Furthermore, being an *in vitro* model it avoids the ethical issues associated with creating surface damage to the organ in a living animal.

Our objective was to determine which is the most practical and robust *in vitro* method of barrier impairment using a step-wise approach of sequential tape stripping of dermatomed pig skin. Based on our own laboratory's equipment, which has been described in more detail in our previously published work in this journal, we have shown that of the three measures of skin integrity, only ER was robust enough to discriminate between the barrier property changes effected by sequential tape stripping (Davies et al., 2004). It is also a very rapid assessment and not prone to the effects of humidity stabilisation, air flow, temperature fluctuation and time-consuming instrument calibration. The measurement of water flux through the skin (TEWL or TWF) requires a long period of stabilisation, due to the equilibration of water in the spacial compartments of the skin layers and microenvironment immediately above the tissue (in the case of TEWL) taking time to reach stable readings between strips. These measures of water movement proved to be unsuitable as a rapid test for skin damage. This compared with the excellent performance of ER where a significant difference could be observed between control (normal) skin and each group of 5–20 tape strips. Importantly, the ER assessment is almost instantaneous and highly suited for static Franz-type diffusion cells. The magnitude of change per 5, 10 or greater number tape strips differed among the skin integrity indices measured. A further analysis of the data where the changes were compared with those observed for TEWL in clinical situations revealed that removal of 10 tape strips provided a loss of barrier

function approximately equivalent to a 3–4-fold increase in TEWL *in vivo*, while also providing a discernible decrease in ER. This 3–4-fold increase in TEWL approximates to the altered barrier function observed clinically in atopic dermatitis, psoriasis, and diaper dermatitis as described previously (Goon et al., 2004; Kim et al., 2006; Stamatas et al., 2011).

The experimental work presented here has shown that the removal of 10 tape strips is the most relevant procedure for this *in vitro* skin model in order to make realistic predictions of skin penetration in compromised skin. We recognise that all three measurements (TEWL, TWF and ER) can be utilised to determine whether skin barrier function has been compromised to a standardised level. Indeed, it may be appropriate to combine different measures depending on the circumstances being investigated. For example, if a skin application was designed to prevent water loss then the TEWL approach may be better to assess performance and this method, of course, can be run in parallel in clinical investigations.

One area where we think this *in vitro* methodology would be useful is for the safety assessment of new and existing consumer and pharmaceutical products. There is little information in the area of dermal penetration of topical drug and cosmetic formulations under conditions where the *stratum corneum* is damaged, diseased or even absent, such as following sunburn. The risk assessment process may incorrectly assume that the systemic exposure to a drug, for example, is perhaps ten times higher when the skin barrier is impaired. However, this may be a gross over-estimate for most compounds. It is obviously an area of safety assessment where the ethical considerations would not justify investigation of this effect in animals or humans. Therefore, the scientifically-based approach we have presented here using *ex vivo* skin and ER is a step forward in this area of dermatokinetics to aid the risk assessment process where exposure is to a compromised skin barrier. Clearly, further investigation is required to establish whether there is a clear link to the physicochemical properties of the compound in question or the vehicle and the formulation in which it is applied. This may lead eventually to mathematical prediction models similar to those used for dermal absorption through normal skin.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

References

Davies, D.J., Ward, R.J., Heylings, J.R., 2004. Multi-species assessment of electrical resistance as a skin integrity marker for *in vitro* percutaneous absorption studies. Toxicol. In Vitro 18, 351–358.

Dick, I.P., Scott, R.C., 1992. Pig ear as an *in vitro* model for human skin permeability. J. Pharm. Pharmacol. 44, 640–645.

Dugard, P.H., Walker, M., Mawdsley, S.J., Scott, R.C., 1984. Absorption of some glycol ethers through human skin *in vitro*. Environ. Health Perspect. 57, 193–197.

Franz, T.J., 1975. Percutaneous absorption on the relevance of *in vitro* data. J. Invest. Dermatol. 64, 190–195.

Goon, A.T.-J., Yosipovitch, G., Chan, Y.-H., Goh, C.-L., 2004. Barrier repair in chronic plaque-type psoriasis. Skin Res. Technol. 10, 10–13.

Heylings, J.R., Ward, R.J., Hughes, L., 2001. Comparison of tissue sources for the skin integrity function test (SIFT). Toxicol. In Vitro 15, 597–600.

Imhof, R.E., De Jesus, M.E., Xiao, P., Ciortea, L.I., Berg, E.P., 2009. Closed-chamber transepidermal water loss measurement: microclimate, calibration and performance. Int. J. Cosmetic Sci. 31 (2), 97–118.

Kim, D.-W., Park, J.-Y., Na, G.-Y., Lee, S.-J., Lee, W.-J., 2006. Correlation of clinical features and skin barrier function in adolescent and adult patients with atopic dermatitis. Int. J. Dermatol. 45, 698–701.

Lawrence, J.N., 1997. Electrical resistance and tritiated water permeability as indicators of barrier integrity of *in vitro* human skin. Toxicol. In Vitro 11, 241–249.

OECD, 2004a. Guidance Document No. 28. The Conduct of Skin Absorption Studies. Organisation for Economic Co-operation and Development, Paris.

OECD, 2004b. Test Guideline 428. Skin Absorption: In Vitro Method. Organisation for Economic Co-operation and Development, Paris.

The Scientific Committee on Consumer Safety. Opinion on Basic Criteria for the In Vitro Assessment of Dermal Absorption of Cosmetic Ingredients. Updated 22nd June 2010. SCCS/1358/10.

Scott, R.C., Clowes, H.M., 1992. In vitro percutaneous experiments: a guide to the technique for use in toxicology assessments. Toxicol. Meth. 2, 113–123.

Stamatas, G.N., Zerweck, C., Grove, G., Martin, K.M., 2011. Documentation of impaired epidermal barrier in mild and moderate diaper dermatitis in vivo using non-invasive methods. Pediatric Dermatol. 28 (2), 99–107.

Trebilcock, K.L., Heylings, J.R., Wilks, M.F., 1994. *In vitro* tape stripping as a model for *in vivo* skin stripping. Toxicol. In Vitro 8 (4), 665–667.

White, E.A., Horne, A., Runciman, J., Orazem, M.E., Navidi, W.C., Roper, C.S., Bunge, A.L., 2011. On the correlation between single-frequency impedance measurements and human skin permeability to water. Toxicol. In Vitro 25 (8), 2095–2104.