*Skin Research and Technology 2010; **16**: 9–15*
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doi: 10.1111/j.1600-0846.2009.00406.x

Correlation of transepidermal water loss with skin barrier properties *in vitro*: comparison of three evaporimeters

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Aim: This study investigates the relationship between transepidermal water loss (TEWL) and skin permeability to tritiated water as a rapid assessment of the integrity of the barrier properties of skin as part of *in vitro* skin permeation studies.

Methods: TEWL values before and during the experimental period were measured using three evaporimeters (A, B, and C) representing different measuring principles and technologies. Single application of tritiated water was dosed on dermatomed human skin samples in a flow-through diffusion cell system. Radioactivity of the absorbed dose and the removable dose residues was counted to determine percent dose and flux rate. These data were further combined with TEWL values to analyze the correlation.

Results: Evaporimeter C, a closed chamber–condenser technology, had higher measurement capacity than other instruments, evaporimeter A, an open chamber, and evaporimeter B, a closed chamber ($P < 0.001$). The baseline TEWL value correlated with tritiated water flux ($r = 0.34$, $P = 0.04$). The pattern of tritiated water expressed as percent dose permeated into receptor fluid was similar to that of TEWL values.

Conclusion: These data indicate that TEWL can be ascribed to be a measure of skin water barrier function. Further work should be conducted to interpret the significance of measuring TEWL by evaporimetry.

Key words: transepidermal water loss (TEWL) – evaporimeter – skin barrier integrity – tritiated water permeation

Accepted for publication 30 June 2009

Percutaneous penetration is frequently measured by *in vitro* techniques due to the simplicity of the experimental design. A commonly used technique is the use of vertical diffusion cells; a challenge in their routine is assuring barrier integrity. OECD guideline 428 (1) recommends the measurement of barrier integrity because it is crucial for the experiment, as mishandling of skin could result in stratum corneum damage. Integrity is ascertained either by measuring the penetration of a marker molecule, e.g. tritiated water, caffeine or sucrose, or by physical methods, such as determination of transepidermal water loss (TEWL) or transcutaneous electrical resistance (TER) (2).

In comparison with other methods, TEWL measurements have an advantage that no solutions are added to perform the barrier integrity test other than those used in the permeation experiments, thus not resulting in a hydrated stratum corneum. In addition, it is not time consuming (3). Therefore, our investigation focused on the potential of TEWL measurements to be used for routine testing for the integrity of human skin prepared for *in vitro* permeation experiments.

Researchers have attempted to develop on the *in vitro* correlation between TEWL and skin permeation measurements, which might be a facile tool for assessment of skin barrier function and prediction of percutaneous absorption. Results, however, are contradictory: some yielded strong correlations, some were weak and a few concluded no quantitative correlation (4, 5).

Here, we evaluated specific measures of TEWL, comparing TEWL levels obtained with commercially available evaporimeters (open chamber and closed chamber). These devices were used to compare water flux rate following tritiated water application.

All these devices measure water evaporation from the skin surface, i.e. across the epidermis, thus quantifying epidermal permeability barrier function. Moreover, as SI units for these instruments are provided in g/m^2h, their recorded values should be comparable. Yet, differences could still result from varying measure-

ment principles or measurement time frames. We utilized one open-chamber evaporimeter, evaporimeter A (Tewameter® TM 210), and two closed-chamber evaporimeters, evaporimeter B (VapoMeter™) and evaporimeter C (Aqua Flux AF200) systems, in order to comprehensively compare commercially available instruments intended to measure TEWL. Our results validate both TEWL as a measure of barrier function and the utility of all these instruments to assess permeability status *in vitro* when utilizing diffusion cells.

Measurements Devices

Open-chamber instruments

Tewameter® TM 210 (evaporimeter A) is an open cylinder system that measures water evaporation rate based on diffusion principles. The vapor density gradient is measured indirectly by two pairs of sensors (temperature and relative humidity) inside a hollow cylinder, and the resulting data are analyzed by a microprocessor. Measurement values are given in $g/m^2 h$. The standard version of the instrument shows the following experimental parameters: TEWL (0–$90\,g/m^2 h$), ambient relative humidity (0–100%) and temperature (0–50 °C) at the level of the sensors (6, 7). One major concern with the open-chamber system is that they require a special environment in which air turblulance does not interfere with their measurements (8).

Closed-chamber devices

The VapoMeter™ (evaporimeter B) is a small portable closed-chamber system that calculates water evaporation rates. Increases in relative humidity correlate with TEWL, as the instrument compares the ambient humidity with the humidity within the chamber after a filling time of 10 s (9). Compared with open-chamber techniques, measurement times are standardized and relatively short (10 s). However, continuous measurements are not possible, because the water vapor captured during measurements needs to be allowed to evaporate before starting the next measurement. The values of the different measurements are expressed in $g/m^2 h$, with a maximum obtainable value of $300\,g/m^2 h$ (6, 7).

Aqua Flux AF200 (evaporimeter C) uses the patented condenser–chamber method to measure water vapor flux density (10). The measurement chamber is a hollow cylinder, whose lower end acts as a measurement orifice that is placed into contact with the test surface. Its upper end is closed with an aluminum condenser that is maintained below the freezing temperature of water by means of an electronic peltier cooler. When in contact with the test surface, the chamber is closed and the air within it is protected from disturbance from ambient air movements. The condenser controls the humidity in the chamber independently of ambient conditions. It acts as a vapor sink by forming ice on its surface, thus creating a zone of low humidity in its immediate vicinity. By contrast, the test surface acts as a vapor source, creating a zone of higher humidity in its immediate vicinity. This humidity difference causes water vapor to migrate from the source to the sink by passive diffusion, leading to a linear distribution of humidity parallel to the axis of the chamber under steady conditions. The water vapor flux is calculated from measurements of this humidity gradient and Fick's first law of diffusion (11). An advantage over conventional closed-chamber is that continuous flux vs. time measurements can be recorded for many hours, as the water vapor entering the measurement chamber is continuously removed by the condenser.

An in-depth overview of evaporimetry technology is provided by Wilson and Maibach (12, 13).

Materials and Methods

Materials

[3H] Tritiated water (lot no. 097K9614), with a specific activity of 5.0 Ci/g, was purchased from Sigma (St Louis, MO, USA). Soluene 350 Tissue Solubilizer and Ultima Gold scintillation cocktails were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA, USA) and HPLC-grade water was from Fisher Scientific (FairLawn, NJ, USA). Phosphate-buffered saline (PBS) tablet and polyethylene glycol-400 were purchased from Sigma (St Louis, MO, USA).

Methods

Skin preparation

Dermatomed human cadaver thigh skin without obvious signs of skin disease was obtained during an autopsy procedure from the Department of Pathology at the University of California San Francisco. Skin was dermatomed using Padgett Electrodermatome (Padgett Instruments Inc., Kansas City, MO, USA) to target a thickness of 300–500 μM. The

TABLE 1. Correlation analyses of TEWL and skin thickness at 0, 1, 2, 4 and 24 h

	TEWL measurement*				
	0 h	1 h	2 h	4 h	24 h
Skin thickness (mm)					
Correlation coefficient	− 0.44	− 0.04	− 0.33	− 0.64	− 0.54
P value	0.01	0.83	0.05	0.01	0.00

*TEWL measured with evaporimeter C.
Negative correlation between the test skin thickness and baseline TEWL (measured before dose application) with correlation coefficient = − 0.44 and $P = 0.01$).
TEWL, transepidermal water loss.

TABLE 2. Analysis of skin donor variation of 3H-water flux through dermatomed skin

Group	Flux (% dose/cm^2/h)*
1	0.23 ± 0.07
2	0.31 ± 0.17
3	0.26 ± 0.08
4	0.36 ± 0.02
5	0.35 ± 0.10
6	0.35 ± 0.10
7	0.28 ± 0.10
8	0.37 ± 0.12
9	0.29 ± 0.18
10	0.41 ± 0.17
11	1.13 ± 0.40†
12	0.78 ± 0.46

*Values of each group are the mean of three samples ± SD.
†Values obtained from the 11th group was significantly higher than other groups ($P < 0.05$).
Correlation coefficient (r^2) of each skin donor group was ≥ 0.97.

skin was stored at − 25 °C in a vacuumed bag until use. Two hours before the experiment, the frozen skin samples were thawed at room temperature. Skin was placed in PBS, pH 7.4. The samples were inspected under microscope and against daylight for any damage or irregularities (scar, holes or birthmarks) and then cut into small square sections large enough to fit onto diffusion cells. The thickness was measured and recorded using an electronic digital caliper (Fisher Scientific).

Skin permeation experimental design

Square skin pieces were cut (using a scalpel) and mounted between the donor and the receptor compartments of flow-through glass diffusion cells (item no. LG-1084: Laboratory Glass Apparatus, Berkeley, CA, USA) and placed in a flow-through diffusion apparatus (Crown Glass Laboratory Inc., Somerville, NJ. USA). The exposed skin area was 1.0 cm^2. The receptor fluid was PBS, 0.01 M, pH 7.4, with 6% v/v polyethylene glycol and was perfused through the chamber with a multichannel peristaltic pump (Pump Pro MPL: Watson-Marlow Inc., Wilmington, MA, USA) at a rate of 4 ml/h and stirred with a Teflon-coated magnetic bar at 600 r.p.m. The chamber temperature was thermostat regulated (Lauda Heating Circulator-ecoline 019: Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany) to maintain the skin surface temperature at 32 °C. Receptor fluid samples were collected every 4 h up to a total duration of 24 h using Retriever IV Fraction Collector (ISCO Inc., Lincoln, NE, USA). A brief equilibrium period of skin and PBS was taken before TEWL measurement.

Tritiated water permeation

Ten microliters of tritiated water (1 μCi) was applied to the epidermal skin surface of the donor compartment to examine skin water barrier function. For the next 24 h, perfusate samples were collected every 4 h on a fraction collector and assayed for radioactivity with a liquid scintillation counter (Perkin Elmer life and Analytical Sciences, Downer Grove, IL, USA; Tri-Carb 2900 TR, Packard Instrument, Downers Grove, IL, USA). All experiments were performed in triplicate. The amount of radioactivity in each sample was related to the amount of tritiated water by reference to standards prepared and measured simultaneously.

TEWL measurements

To assess the skin barrier functions, water loss through the skin was measured with a open-chamber evaporimeter A (Tewameter® TM 210: Courage & Khazaka, Cologne, Germany; Acaderm Inc., Menlo Park, CA, USA) and two closed-chamber systems [evaporimeter B (VapoMeter™: Delfin Technologies Ltd, Kuopio, Finland) and evaporimeter C (Aqua Flux AF200: Biox Systems Ltd, London, UK)]. TEWL measurements were carried out by placing the collared probe over the specially designed top of the donor compartment 1 cm away from the surface of the skin, and was fitted using a surrounding rubber ring. Measurements were conducted at 0 times (before 3H-water dosing) and 1, 2, 4 and 24 h after dosing. For each time point, three measurements with evaporimeters B and C were performed. As with evaporimeter A, TEWL was recorded continuously and mean readings were taken after 45 s.

Data analysis

Statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). The

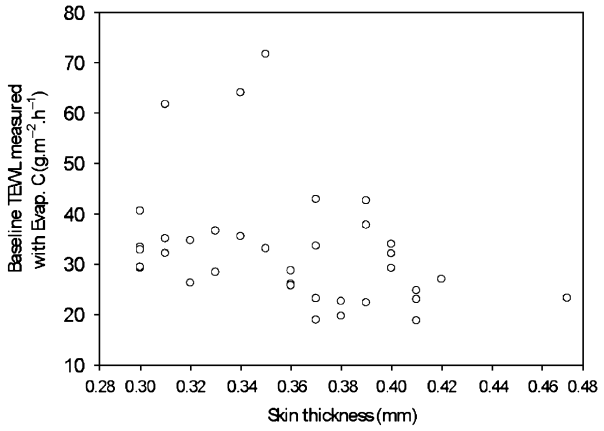

Fig. 1. Comparison of baseline transepidermal water loss (TEWL) values measured with Evaporimeter C as a function of thickness of dermatomed skin. Each symbol represents individual observed (n = 36). Negative correlation between the test skin thickness and baseline TEWL (measured before dose application) with correlation coefficient = −0.44 and P = 0.01).

linear correlation between TEWL and the fraction of tritiated water absorbed was examined for each treatment. Relations between groups were determined with Pearson's or Spearman's correlation coefficient for normal or non-equal variances distributions, respectively, at $P < 0.05$.

Results

Dermatomed demographics

Twelve dermatomed human skin donor sources were used. The average age of the donor was 57 ± 15 years with a range of 24–75. The average skin thickness was 0.36 ± 0.04 mm, with a total range of 0.30–0.47 mm ($N = 36$). Inter-group comparison of the thickness was tested, and no significant difference was observed among 12 donors ($P > 0.05$).

Correlation of TEWL and skin thickness

A correlation between TEWL value and thickness was first evaluated. A negative correlation between the test skin thickness and baseline TEWL (measured before dose application) with correlation coefficient = −0.44 and $P = 0.01$). This correlation was perturbed approximately within 2 h after topical dose application and returned as the skin surface dried (Table 1, Fig. 1).

Comparison of three TEWL measurement instruments

Treatment with water resulted in hydrated skin and showed a higher TEWL, i.e. the TEWL increased to 138%, 144% and 119% of baseline

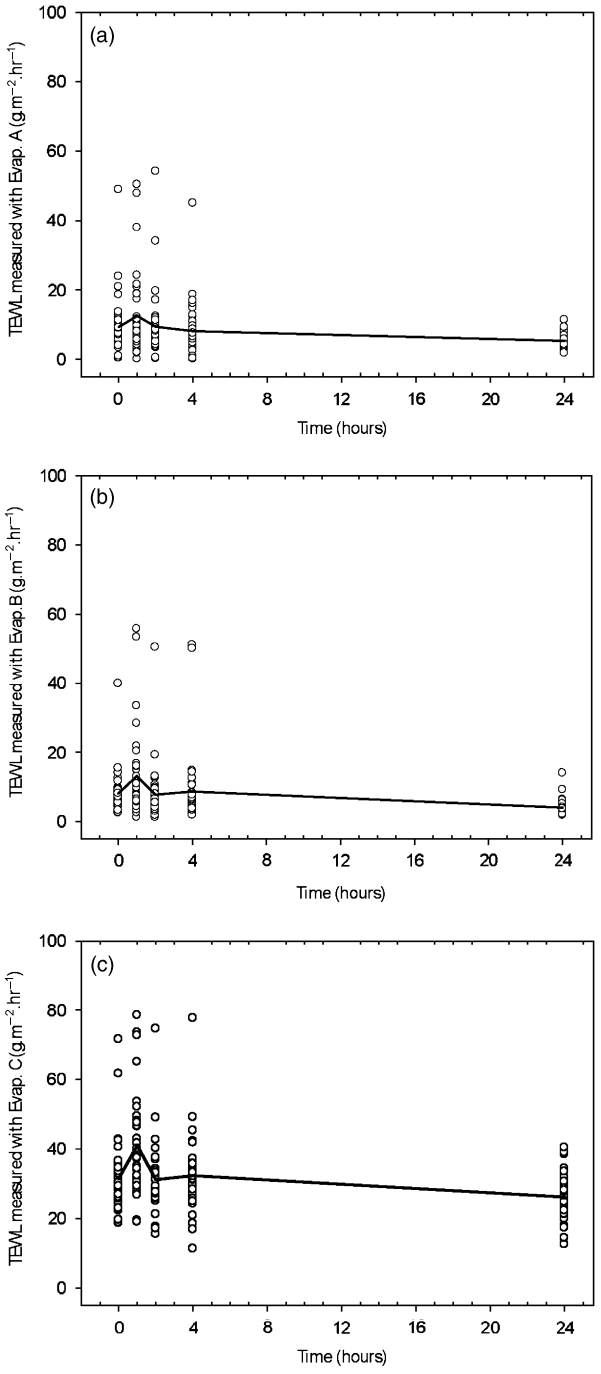

Fig. 2. (a) Transepidermal water loss (TEWL) values measured with evaporimeter A over time. Symbol represents each individual observation. The line is the mean of 36 samples. Evaporimeter A had similar measuring capacity to B, yet significantly lower than C. (b) TEWL values measured with evaporimeter B over time. Symbol represents each individual observation. The line is the mean of 36 samples. Evaporimeter B had similar measuring capacity to A yet significantly lower than C. (c) TEWL values measured with evaporimeter C over time. Symbol represents each individual observation. The line is the mean of 36 samples. Evaporimeter C, a closed chamber – condenser technology had higher measurement capacity than other two TEWL instruments, evaporimeter A and B.

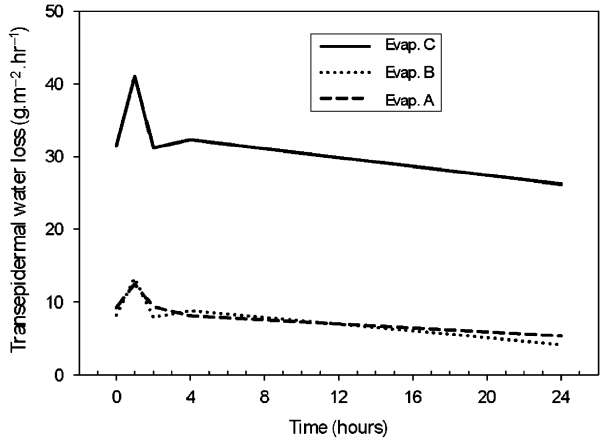

Fig. 3. Comparison of transepidermal water loss (TEWL) values measure with three instruments: A, B, and C over time. Each line represents the mean of 36 samples. Evaporimeter C is significantly higher than those of B or A ($P < 0.001$).

TEWL for evaporimeters A, B and C, respectively, when measured initially, and then declined sharply to the baseline value in approximately 2 h (Fig. 2a–c). Our results show that all instruments were useful for the assessment of epidermal barrier function. The patterns of TEWL profiles from the three instruments were similar (Fig. 3). However, as shown in Fig. 3, the measurement capacity of evaporimeter C is significantly higher than those of A or B ($P < 0.001$).

Permeability of tritiated water

The flux rate of tritiated water in the receptor is in Table 2. Following linear regression analysis (of percent administered dose of tritiated water over skin surface area penetrated against time), the correlation coefficient (r^2) of each skin donor group was ≥ 0.97.

Discussion

This study's goal is to determine the ability of TEWL measurement to assess skin integrity compared with the use of tritiated water.

Measurement of the flux of tritiated water across human skin *in vitro* has been a conventional method to assess membrane integrity and permeability of chemicals (14). However, this method is time consuming, and results in a hydrated stratum corneum. It might damage the skin barrier during the *in vitro* experiment if researchers attempt to completely remove the residual tritiated water and clean the dosed area. In addition, the use of radioactivity is costly and has environmental implications.

TABLE 3. Correlation analysis of TEWL and 3H-water flux

TEWL	Time (h)	*P* value	Correlation coefficient (r^2)
Evaporimeter C TEWL measurement	0	0.04	0.34
	24	0.16	0.24
Evaporimeter A TEWL measurement	0	0.00	0.50
	24	0.20	0.22
Evaporimeter B TEWL measurement	0	0.07	0.31
	24	0.06	0.32

Both baseline TEWL measured by evaporimeters C and A correlated well with the tritiated water flux.

In another study, a test of the correlation of *in vitro* measurement of TEWL and tritiated water penetration with an unventilated evaporimeter was performed (3). Nangia et al found that TEWL strongly correlated with tritiated water skin permeation within 4 h whether or not the skin was pretreated. They suggested that TEWL is a rapid, convenient measurement method that provides a clear indication of the time dependence of barrier integrity. However, another study observed a contradictory result. Chilcott et al. (5) measured the TEWL rate in human epidermal membrane, in full-layer pig skin, and in physically damaged skin, such as tape-stripped or needle-punctured skin, with a calibrated, ServoMed EP-3 Evaporimeter. The values were than compared with the permeability of tritiated water through each membrane. Based on the results, they concluded that there was no correlation between the TEWL rate and permeability of tritiated water ($P > 0.5$) *in vitro*.

Here, three TEWL instruments show different degrees of correlation between TEWL values and permeability of skin to tritiated water (Table 3). Baseline TEWL measured with evaporimeter C at zero time is correlated with the flux rate of tritiated water ($P = 0.04$, $r^2 = 0.34$). Baseline TEWL measured with evaporimeter A, similar to that of C, correlates with the flux of tritiated water ($P = 0.00$, $r^2 = 0.50$). Evaporimeter B, however, shows no statistically significant correlation with the flux rate of tritiated water ($P = 0.07$, $r^2 = 0.31$). The results suggest caution when evaluating these results; one should consider the variation of TEWL values due to various instruments that are designed according to sensing principles or manufactured with different technologies. For example, as shown in Figs 2a–c and 3, evaporimeters C and B use the closed-chamber method, but the measurement capacity of C is significantly higher than that of

TABLE 4. Correlation analysis of TEWL and 3H-water flux at 0, 1, 2, 4, 24 h

TEWL measured by evaporimeter C (mean ± SD, g/m/h)	32.8 ± 9.0	39.1 ± 11	30.2 ± 6.8	31.8 ± 6.6	26.2 ± 6.3
Time point for measurement (h)	0	1	2	4	24
3H-water flux (% cumulative dose/cm^2/h)					
Correlation coefficient	0.34	0.05	0.48	0.37	0.24
P value	0.04	0.80	0.00	0.03	0.16

TEWL, transepidermal water loss. Baseline TEWL values correlated with fluxes of tritiated water ($r = 0.34$, $P = 0.04$).

B ($P < 0.001$). However, evaporimeter A uses the open-chamber method and the measurement capacity resembles that of evaporimeter B ($P > 0.05$), non-significant, but significantly lower than that of C ($P < 0.001$).

TEWL was measured not only before topical treatment (as baseline) but also at 1, 2, 4, and 24 h after dosing (Fig. 3, Table 4). As observed from the three TEWL instruments, after the peak value at 1 h, TEWL values decreased continuously with time until the experiment was terminated. Interestingly, the pattern of changing TEWL values vs. time is similar to that of the rate of tritiated water penetrating through the skin membrane over the experimental period. We know some variables affecting TEWL measurements during *in vitro* study such as skin thickness, environmental temperature and relative humidity, or air circulation for open-chamber instrument. We also observed changing internal pressure of the receiving chamber, such as to eliminate air bubbles from the chamber in flow-through diffusion cells or to replace the receptor fluid in static Franz-type diffusion cells, which may increase TEWL values.

Conclusion

In conclusion, the baseline TEWL value was correlated with the thickness of dermatomed skin ($r = -0.44$, $P = 0.007$). It also correlated with tritiated water fluxes ($r = 0.34$, $P = 0.04$). When TEWL and permeation data were compared in parallel throughout the experiments, the pattern of tritiated water expressed as percent dose permeated into receptor fluid was similar to that of TEWL values. These data indicate that TEWL can be ascribed to be a measure of skin water barrier function.

TEWL has long been used as an indicator of skin water barrier integrity. Recent studies have validated the use of both open and closed chambers in the measurement of TEWL.

Here, we have validated the use of a new model of closed-chamber instrument based on the use of the condenser–chamber method. Evaporimeter C has proven to be simple, easy to use and a sensitive method of screening the barrier integrity *in vitro* and can be used as an alternative method to tritiated water.

Taken together, measuring tritiated water *per se* or by instrumentation provides an index of skin integrity. Yet, the data shown here delineates complexity and the need for careful description of how the measurements are obtained.

Acknowledgements

The authors gratefully acknowledge the technical support and helpful discussions of Dr Robert E. Imhof and Biox Systems Ltd and the review and feedback of Dr Gert Nilsson.

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