Assessing the sensitivity of two TEWL measuring methods: open vs. closed chamber

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Introduction

The lipid matrix of the stratum corneum (SC), consisting primarily of ceramides, cholesterol and fatty acids, is crucial for the integrity of the skin barrier. Linoleic acid is an essential fatty acid, whose deficiency could lead to abnormal epidermal permeability barrier function (Feingold et al. 2000). It has been proposed that topical treatment with linoleic acid could repair defective barrier function in detergent-treated skin (Elias et al. 1980).

A range of commercial and newly developed products containing linoleic acid was used in this study, in order to assess both aspects of the proposed positive effects of the topical products on the skin barrier:
a) **The protection potential**, whereby the products are applied for a period of time before the insult to the skin barrier is carried out, in this case with sodium lauryl sulphate (SLS) solution;
b) **The repair potential**, whereby an insult with SLS to the skin barrier was followed by a period of product application.

Transepidermal water loss (TEWL) was used as the main indictor of the skin barrier impairment and recovery.

Aim

The aim of this study was to perform a comparative assessment of the two types of TEWL instruments, with specific emphasis on their sensitivity in detecting small differences in TEWL, while investigating the skin barrier protection and repair effects of topical products containing linoleic acid.

Materials and Methods

Materials

Sodium lauryl sulphate (SLS) (≥ 99.0% purity, Sigma-Aldrich, USA), diluted in distilled water, was used as anionic detergent, known to cause SC barrier impairment (e.g. Friebe, Effendy and Loffler, 2003). A range of commercially available and newly formulated products containing linoleic acid was used to treat relevant test sites. The 18 mm-Finn chambers and 18 mm filter paper discs were used as occlusive patches; both were supplied by Smart Practice (USA).

Methods

Both studies were carried out after obtaining the Ethics approval from the relevant Ethics committee, following the guidelines of the good laboratory practice. Before the start of the study, each participant had signed an informed consent form.

Open chamber method was represented by **Tewameter TM300** (C&K, Germany), while **AquaFlux AF200** (Biox Systems Ltd., London) was used as an example of a condenser-chamber version of the **closed chamber method.**

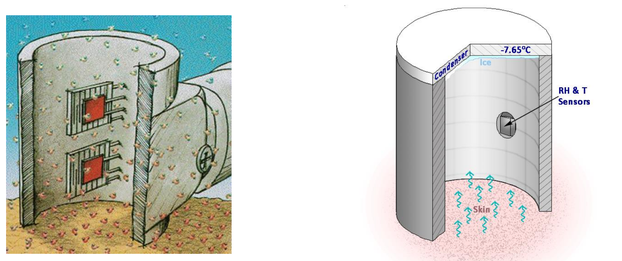

Figure 1. Measuring principles of the two TEWL instruments: Tewameter TM300 (left) and AquaFlux AF200 (right)

Study 1: Exploring the protection potential of the test products

Fourteen healthy volunteers, aged 19 – 42, participated in this 14-day study. Three test sites of 3 x 3cm, were allocated symmetrically on both inner forearms of each participant. Baseline measurements were obtained after a 30-min acclimatisation at 21^{0}C. A 14-day supply of the 5 test products was handed out to each participant, with the application instructions and a customised template, with site C as an untreated control. Products were applied twice a day and tests carried after 7 and 14 days. Next, all test sites were treated with 200 µl of 5% w/v SLS under occlusion, using a 18 mm-Finn chamber for 30 min. After the removal of SLS patches, the test areas were rinsed under running water and gently blotted with tissue. The sites remained exposed to air for an hour prior to the final set of measurements.

Study 2: Exploring the repair potential of the test products

Thirteen healthy volunteers (aged 20 – 42) participated in this study. Four test sites of 3 x 3cm were allocated symmetrically on both dorsal forearms. The study duration was 16 days. After obtaining the baseline measurements, all sites were fitted with 18 mm Finn chambers. Seven of them contained 200µl of 1.25%w/v SLS, while test site C was covered by a filter disc soaked with 200 µl of distilled water. The occlusion lasted 24 hours and the skin measurements were carried out 24 h after the patch removal, as suggested by Friebe et al. (2003). Following the measurements after the SLS damage, all sites were treated with test products, except two: site C as a positive control (undamaged with SLS) and site H as a negative control (damaged with SLS). Participants were given a 2-week supply and customised templates, as in Study 1, and asked to use test products twice daily for 2 weeks. Final measurements were completed at day 16 of the study.

Statistical analysis

The results were tested using analysis of variance (ANOVA), followed by Tukey HSD test for paired differences, using a 95% family-wise confidence level. A significance level of $p<0.05$ was chosen.

Results and Discussion

Study 1

The analysis of the results obtained by the **open chamber method** (Figure 2) revealed a statistically significant increase in TEWL on week 2, after exposure to 5% SLS for 30 min, compared to baseline values on all test sites. However, no statistically significant differences were found in TEWL among different test sites ($p = 0.99$), and with the control site.

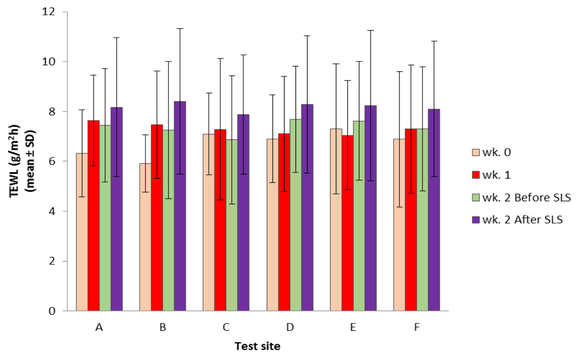

Figure 2. Mean TEWL values obtained in Study 1 using an open chamber instrument. Error bars represent standard deviations of the data (n=14)

Figure 3 shows the TEWL values obtained by the **closed chamber method.** While standard deviations were of the same order as with the open chamber, ANOVA analysis has shown three sets of statistically significant data: TEWL values at baseline, week 1 and week 2 before the irritation with SLS, compared to week 2 after SLS. Again, no statistically significant differences were found in TEWL between different test sites, showing that not only there were no difference among the test products, but that none of the test products differed from the non-treated control. Therefore, the Study 1 TEWL measurements failed to show the protection potential of the test products.

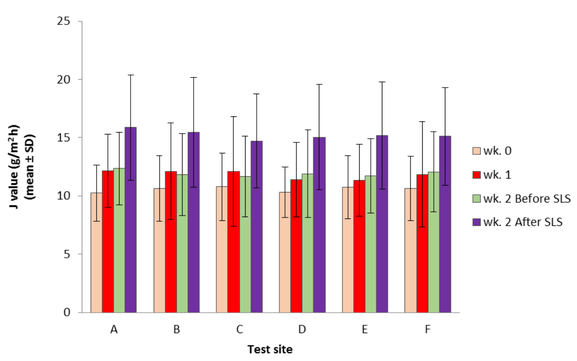

Figure 3. . Mean TEWL values obtained in Study 1 using a closed chamber instrument (n=14)

Study 2

Figure 4 shows the TEWL values measured on dorsal forearms using the **open chamber method**. After exposure to 1.25% SLS for 24h and subsequent stabilisation for another 24h, a highly statistically significant increase was found in TEWL on day 2 compared to baseline values, as expected. No statistically significant differences were found in TEWL on day 16 compared to the baseline values ($p= 0.360$), indicating successful barrier recovery. The negative control site H, which was not treated with any product after the SLS damage, has performed similarly to the treated sites, demonstrating the power of natural skin barrier recovery. No statistically significant differences were found on site C (treated with the water patch) throughout the study.

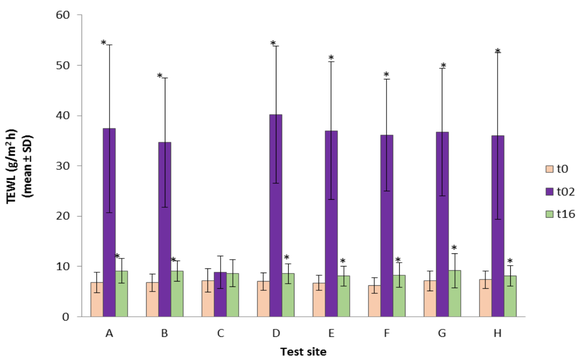

Figure 4. Mean TEWL values obtained in Study 2 using an open chamber instrument. The measurements marked t02 were conducted 24h after the removal of SLS patches. Symbol * indicates significant difference to the preceding set of values ($p<0.05$).

Figure 5 shows the mean TEWL values measured using a **closed chamber (condenser-chamber) method**. It has yielded the same statistical conclusions as the open chamber method between day 2 and baseline values ($p < 0.05$), and between day 2 and day 16 in all SLS-damaged test sites ($p < 0.05$). No statistically significant differences were found in TEWL on day 16 compared to the baseline values ($p= 0.23$). The results obtained in Study 2 indicate that the two methods used possess similar sensitivity when measuring relatively large differences in TEWL.

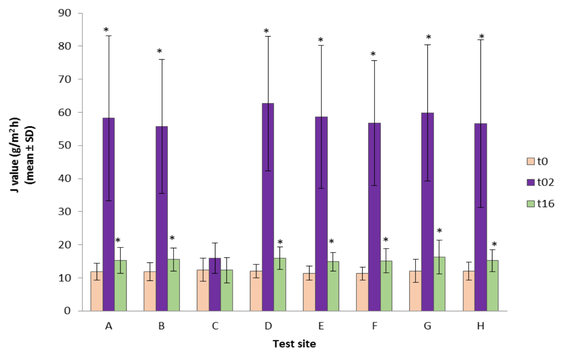

Figure 5. Mean TEWL values obtained in Study 2 using a closed chamber instrument

Being an open cylinder, the probe of the open chamber instrument possesses 'natural ventilation', which enables it to measure the condensed water flux with good accuracy (Nuutinen, 2006), but makes it extremely sensitive to the ambient air movements. In contrast to the above, the closed chamber method eliminates the effect of air movements, but requires the removal of water vapour from the microenvironment. In the condenser-chamber method used here, this is achieved by trapping water molecules as ice on an electronically cooled condenser (Imhof et al, 2002). The condenser provides the added benefit of maintaining constant and defined humidity at the skin surface, independent on the external environment. This feature is probably the reason for the higher sensitivity shown by the closed chamber method in the **Study 1**, when measured TEWL differences were very small.

Conclusion

The results of this study have shown that the closed chamber method possesses a higher sensitivity than the open chamber method when dealing with smaller differences in TEWL, producing a higher number of statistically significant findings under the same experimental conditions. When larger variations in TEWL were detected between the test sites, the findings obtained by the open chamber method were consistent with the closed chamber ones.

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