

Skin barrier response to occlusion of healthy and irritated skin: Differences in trans-epidermal water loss, erythema and stratum corneum lipids

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Background: Occlusion of the skin is a risk factor for development of irritant contact dermatitis. Occlusion may, however, have a positive effect on skin healing. No consensus on the effect of occlusion has been reached.

Objectives: To investigate skin barrier response to occlusion on intact and damaged skin.

Methods: In study A, the response to occlusion (nitrile glove material) for either 8 hr daily for 7 days or for 72 consecutive hours, respectively, was determined and compared with that of non-occluded skin. In study B, the response to occlusion of for 72 consecutive hours of skin that had been damaged by either sodium lauryl sulfate (SLS) or tape stripping, respectively, was determined and compared with that of non-occluded pre-damaged skin. Skin barrier function was assessed by measurements of trans-epidermal water loss (TEWL) and erythema. In study A, stratum corneum lipids were analysed.

Results: Occlusion of healthy skin did not significantly influence skin barrier function, ceramide profile or the ceramide/cholesterol ratio. Occlusion of the skin after SLS irritation resulted in higher TEWL than in the control ($P = 0.049$). Occlusion of the skin after tape stripping resulted in lower TEWL than in control skin ($P = 0.007$).

Conclusions: A week of occlusion did not significantly affect healthy skin, but was found to decrease healing of SLS-damaged skin, and to improve healing of tape-stripped skin.

Key words: ceramides; irritant contact dermatitis; occlusion; skin barrier. © John Wiley & Sons A/S, 2010.

Conflicts of interest: The authors have no conflict of interest to disclose.

Accepted for publication 13 May 2010

Wet work and occlusion of the skin are major risk factors in the development of irritant contact dermatitis. In daily life, the problem arises when occlusive gloves are used for protection of the hands in work settings (1–3). On the other hand, occlusive treatment has a positive effect on skin healing (4–6). The effect of occlusion on damaged skin may, however, depend on the nature of the trauma, and no consensus on the effect of occlusion has been reached in the literature.

Experimental studies concerning irritant contact dermatitis show that the barrier function of the stratum corneum (SC) is compromised, and is affected by both protein and lipid composition, as well as the

lipid content of this layer (7). However, it should be noted that knowledge of the barrier function for non-experimental irritant contact dermatitis is very limited.

Occlusion affects the skin through many factors, such as temperature, sweating and increased pH. Increased skin pH is thought to influence the skin barrier by affecting lipid synthesis, as the pH optimum for many of the enzymes involved is low (8). To our knowledge, no *in-vivo* human studies have yet analysed SC lipids after occlusion.

The objectives of the present study were to investigate the skin barrier response to occlusion on intact skin, as well as the response to occlusion on

the skin barrier damaged by either tape stripping or sodium lauryl sulfate (SLS). This was assessed by non-invasive barrier measurements and SC lipid analysis.

Methods

Two independent studies were performed: study A investigated the response to occlusion on intact skin, and study B investigated the response to occlusion on skin with a damaged skin barrier. Both studies were performed after approval from the local ethical committee (SJ-7, 17583 and 19052), and participants were enrolled after responding to posters at the local library and educational centre. The participants had no major previous or current skin diseases, and did not suffer from atopic eczema.

Study A (Fig. 1)

Nineteen healthy participants were enrolled in study A, 14 females and 5 males (mean age 29 years, range 20–52 years). Healthy skin was occluded on the same individual in two different ways.

The volar aspects of the forearms on each participant were divided into three areas ($8 \times 6 \text{ cm}^2$) and randomized on each participant for the different occlusion regimens. All participants were asked not to use any moisturizers or soaps on their forearms throughout the study period.

Area 1 was occluded for 8 hr a day for 7 consecutive days. The volunteers were carefully instructed in the procedure of the occlusion, to enable them to change the occlusion material themselves. They received a text message (SMS) at an agreed time of the day as a reminder to put on the occlusion material. After 4 days, the participants were seen as a control measure and to encourage continued compliance, and then continued as before. The time duration was chosen to mimic a normal week in a wet work setting.

Area 2 was occluded for 72 consecutive hours. The occlusion material was placed on the skin at the clinic.

Area 3 on the forearms served as the non-occluded control.

Four hours after the occlusion material was removed, trans-epidermal water loss (TEWL), erythema and capacitance were measured, and an SLS patch was placed on each of the three areas for 24 hr. Adjacent to the three SLS patches, a cyanoacrylate SC sample was taken for lipid analysis (Fig. 1). One hour after removal of the SLS patch, measurements of TEWL, electrical capacitance and erythema were made.

Study B (Fig. 1)

The subjects were 19 healthy volunteers, 14 females and 5 males (mean age 38 years, range 19–52 years). The volar forearms were divided into four areas ($8 \times 6 \text{ cm}$). The areas were randomized for each participant. All participants were asked not to use any moisturizers or soaps on the forearms for the period of the experiment.

Areas 1 and 2. On day 1, an SLS patch was placed on the two areas for 24 hr. On day 2, the SLS patches were removed, and the forearm was gently washed in lukewarm water. After 1 hr, area 1 was occluded for 72 hr, and area 2 was left as the control.

Areas 3 and 4. These were left untouched for 1 day. On day 2, tape-stripping was performed on both areas. The stripped skin was then occluded for 72 hr on area 3, and area 4 served as the control.

Areas 1, 2, 3 and 4. On day 5, the occlusion material was removed 4 hr before any measurements were made, to ensure that the TEWL value had stabilized (1). The skin was evaluated by TEWL, erythema and electrical capacitance (Fig. 1).

Even though most cases of irritant contact dermatitis are on the hands, it has been shown that the forearm provides reliable and reproducible results and is therefore a good practical alternative location for experimental testing (9).

Techniques

For tape stripping, pieces of Transpore™ tape were placed on the forearm 30 times on each participant, using a glass slide to even the pressure. It has been shown that TEWL rises significantly after tape stripping has been performed 20 times, when using Transpore™ (10).

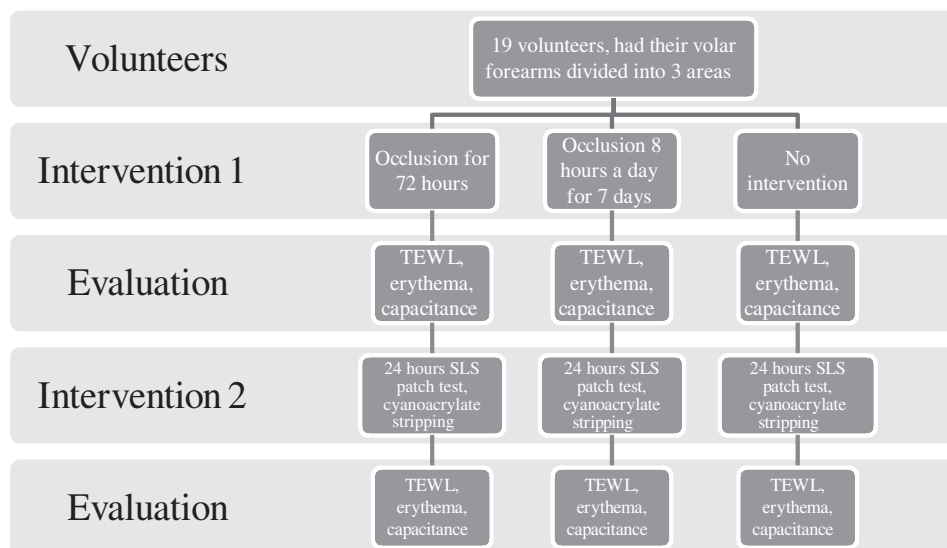
SLS patch testing was performed using large Finn Chambers® (diameter of 12 mm) with 60 μl of SLS (11) (>99% purity; Sigma Chemical Co., St Louis, MO, USA) in a 1% aqueous solution on filter discs, kept on the skin for 24 hr, and evaluated 1 hr after removal.

Occlusion was performed with $7 \times 5 \text{ cm}^2$ pieces of nitrile gloves (Touch N Tuff®; Ansell Healthcare Europe, Brussels, Belgium), placed on a film of adhesive dressing (3M™ Tegaderm™; 3M a/s, Glostrup, Denmark), an adhesive dressing, and then placed on the skin.

Evaluation was performed by TEWL ($\text{g/m}^2/\text{h}$) measurements, using a TEWA-Meter (Cortex Technology, Hadsund, Denmark) in accordance with guidelines (12). Erythema measurements were performed with a Chroma Meter (Minolta CR-300; Minolta Camera, Osaka, Japan). The colour is expressed in the L^*a^*b system, where erythema of the skin is indicated by a^* (13). Electrical

Flowchart–Study A

Occlusion on healthy skin



Flowchart–Study B

Occlusion on irritated skin

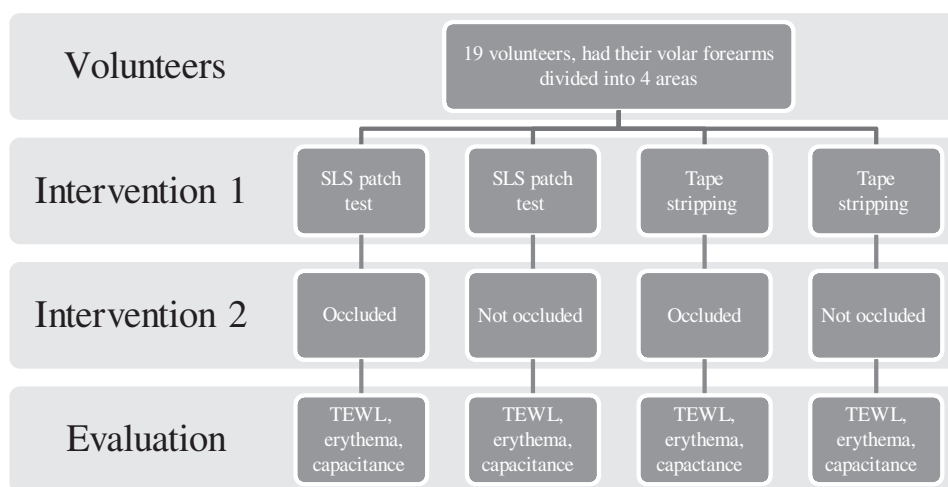


Fig. 1. Flowchart for study A and study B.

capacitance was measured with a Corneometer (CM825PC; Courage + Khazaka, Cologne, Germany), and gives information concerning the hydration of SC in arbitrary units (14). All of the non-invasive measurements were performed twice, and the mean value was used for further calculations.

For lipid analysis, we used the cyanoacrylate method for collection of SC samples (15). The area

of interest was first wiped with acetone to eliminate contamination from surface lipids, a glass slide with a drop of Liquiband® (Medlogix, Plymouth, UK) tissue glue was held against the skin for 1 min before removal, and the sample was kept frozen until further analysis. The lipids were extracted from the glass slide by sonication in n-hexane/ethanol [95:5 (v/v)]. Lipids were separated by high-performance thin-layer chromatography (HPTLC), in a solvent

mixture of chloroform/methanol/acetic acid [190:9:1 (v/v/v)]. The silica-coated HPTLC plates were dried, and then stained using primuline; the lipids were quantified through determination of the fluorescence intensity (Desaga TLC Densitometer CD; Desaga GmbH, Wiesloch, Germany), compared with standards of ceramide 5 and cholesterol (Matreya LLC, Pleasant Gap, PA, USA) (15).

Statistical analyses were performed with the non-parametric Friedman test as an omnibus test when more than two parameters were compared. The Wilcoxon matched pairs test was used for comparison of two values. $P < 0.05$ was considered to be statistically significant. SPSS 17.0 was used for all calculations.

Results

Study A consisted of two different occlusion regimens, followed by SLS patch testing and lipid analysis (Fig. 1 and Table 1).

No statistically significant differences were found for any of the non-invasive measurements between the two occlusion regimens and control (Table 1).

The results of the lipid analyses are illustrated in Fig. 2, and no statistically significant differences were found for the ceramide subspecies or the ceramide/cholesterol ratio.

Study B consisted of SLS patch irritation and tape stripping followed by occlusion for 72 hr as compared to no occlusion (Table 2).

The results of the non-invasive measurements from study B are presented in Table 2. Occlusion of the skin resulted in significantly higher levels of TEWL after SLS irritation than in the non-occluded area ($P = 0.049$). The opposite applied to the tape-stripped skin, where the occluded skin had a significantly lower value for TEWL than the non-occluded skin ($P = 0.007$). These findings only apply to TEWL, and we found no differences for erythema or electrical capacitance.

Discussion

The use of occlusion has been acknowledged for its healing effect on wounds, and for its ability to increase the effect of different topical treatments. However, a minimal use of protective occlusive gloves for healthcare workers is recommended, to reduce the risk of irritant contact dermatitis. These seemingly contradictory effects of occlusion were examined in this double study, where the effects of occlusion on healthy and damaged skin were examined.

People with atopic eczema were excluded from the study population; however, it should be noted that even within this healthy group, some people will be more prone to the negative effects of occlusion than others. In the present study, no attempt was made to identify the characteristics of these more susceptible individuals.

The results from study A, where healthy skin was occluded before SLS irritation, show that healthy skin copes well with occlusion. Even 8 hr of occlusion daily for 1 week does not affect the skin significantly; in fact, occlusion for 8 hr daily for 1 week and occlusion for 72 consecutive hours both resulted in less susceptibility to SLS irritation than in the non-occluded control area, even though the difference was not statistically significant (Table 1). A previous study, in which hands were occluded for 6 hr daily for 14 days, found that, after 14 days, there was more TEWL in the occluded skin than in the non-occluded skin (2). However, they found no statistically significant difference when measuring TEWL at day 8 or day 11. Nevertheless, together with clinical experience, it suggests that occlusion for durations longer than 1 week does influence the skin barrier negatively.

Previous studies with durations of occlusion of 3 days, 5 days and 4 hr daily for 1 week have shown that occlusion increases the skin pH (12, 17–19). As the enzymes involved in the formation

Table 1. Results of study A

	Occlusion for 72 hr	Occlusion for 8 hr a day, for 7 days	Control area, no intervention
TEWL (g/m ² /hr) after occlusion	5.0 (3.8–6.9)	5.6 (4.2–8.2)	5.1 (4.5–6.6)
Delta TEWL (g/m ² /hr)	9.8 (9.3–17.5)	14.4 (8.0–21.7)	15.8 (9.4–24.5)
Erythema after occlusion	8.7 (7.4–9.7)	8.9 (8.0–10.3)	8.7 (7.5–10.4)
Delta erythema	2.1 (0.6–4.1)	2.57 (1.5–4.1)	3.2 (0.9–4.5)
Baseline capacitance	31.0 (26.5–38.3)	30.0 (27.8–35.5)	27.0 (24.5–33.3)

Median values; first and second quartiles are in parentheses.

TEWL, trans-epidermal water loss.

TEWL after occlusion: TEWL values 4 hr after the occlusion material was removed.

Delta TEWL: (TEWL values 1 hr after SLS patch was taken off) – (TEWL after occlusion).

Erythema after occlusion: a* values 4 hr after the occlusion material was removed.

Delta erythema: (a* values 1 hr after SLS patch was taken off) – (erythema after occlusion).

Baseline capacitance: electrical capacitance (arbitrary units) 4 hr after the occlusion material was removed.

No statistically significant differences in Table 1.

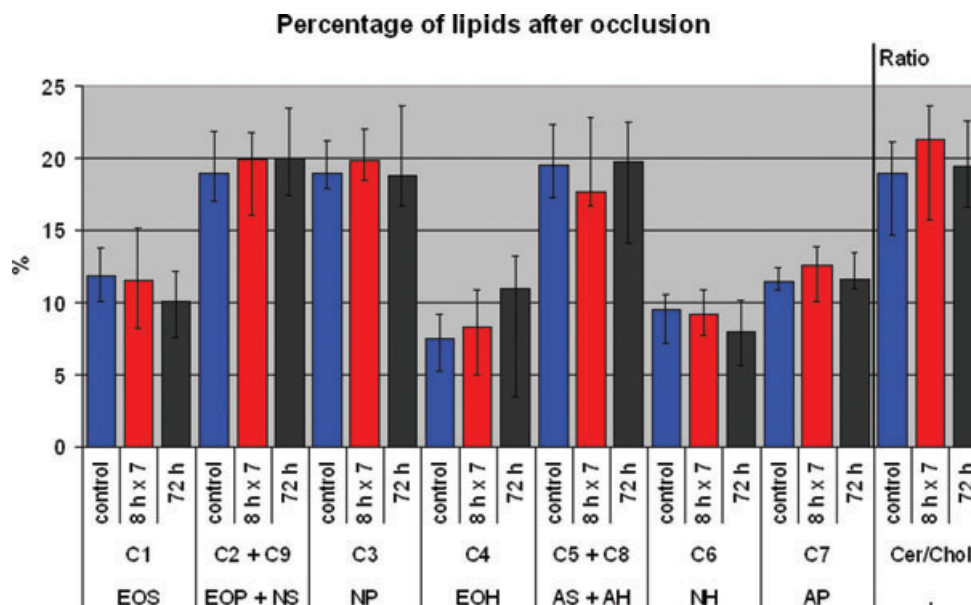


Fig. 2. Percentage distribution of ceramides after different occlusion regimens. Note that Cer/Chol is not a percentage, but illustrates a ratio. Error bars illustrate the first and third quartiles. Control: no intervention. 8 hr $\times$ 7: occlusion 8 hr daily for 7 days. 72 hr: occlusion for 72 consecutive hours. C, ceramides; Chol, cholesterol; A, α -hydroxy fatty acid; EO, ester-linked ω -hydroxyl acid; N, nonhydroxy fatty acid; P, phytosphingosine; S, sphingosine; H, 6-hydrosphingosine (16).

Table 2. Results of study B

	Occluded skin	Control skin	Wilcoxon matched pairs test
SLS-irritated skin – day 5, TEWL (g/m ² /hr)	15.5 (12.5–19.0)	11.6 (10.2–15.2)	$P = 0.049^*$
Tape-stripped skin – day 5, TEWL (g/m ² /hr)	4.0 (3.3–5.1)	5.5 (3.9–8.1)	$P = 0.007^*$
SLS-irritated skin – day 5, erythema	12.5 (10.7–13.1)	12.4 (10.9–13.5)	$P = 0.629$
Tape-stripped skin – day 5, erythema	9.1 (8.5–10.0)	8.9 (8.0–10.3)	$P = 0.10$
Capacitance – day 5	37 (32–41)	37 (31.8–41.5)	$P = 0.727$

Median values; first and second quartile are in parentheses.

SLS, sodium lauryl sulfate; TEWL, trans-epidermal water loss.

of ceramide from cerebroside and sphingomyelin in the SC, β -glucocerebrosidase (pH optimum 5.6) and shingomyelinase (pH optimum 4.5) (20), have different pH optima that are close or below the skin surface pH, the higher pH would skew ceramide formation away from sphingomyelin hydrolysis. Because hydrolysis of sphingomyelin and hydrolysis of cerebroside give rise to different ceramide species (21), this would lead to alterations in the ratio between the different ceramide species in the SC. This could not be confirmed in our study, as no differences in ceramides between the different occlusion regimens were found as compared with non-occluded skin. It should be noted, however, that our lipid results describe relative changes and not the total amount, indicating that, if all lipids were affected in the same way, this would not be detected. However, as β -glucocerebrosidase and shingomyelinase (20) are the two most important enzymes in ceramide syntheses (7), and cholesterol sulfatase, which is involved in cholesterol synthesis, has a pH optimum at 8 (20), an influence from

the elevated pH could be expected to affect the ceramide/cholesterol ratio, which was not observed in this study. An explanation for this could be that the duration of the interventions was too short for the altered enzyme activities to manifest themselves as a decreased ceramide/cholesterol ratio. Hence, studies of more than 1 week in duration might be necessary to show the effect of increased pH on lipid composition.

The above-mentioned negative effect of occlusion resulting from an increase in pH is challenged by other studies that point towards positive effects of occlusion. In a study where human skin was occluded for 3 weeks with a semipermeable silicone membrane used for 23 hr a day, statistically significant decreased susceptibility to SLS was found (22). Different explanations for the improvement were given, such as mechanical protection by the occlusion material, which leads to reduced desquamation and thereby a thicker SC. It was also suggested that occlusion could lead to larger corneocytes, and thereby a longer penetration pathway. In summary,

findings have been inconclusive. Another possible bias could arise from the different occlusive materials used in different studies, as the results obtained with one occlusive material may not be representative for all other materials.

When the skin barrier was damaged before occlusion, as compared with no occlusion, as in study B, the differences in outcome were statistically significant for important parameters.

For skin affected by SLS, the occluded arm healed less well than the non-occluded arm, as evaluated by TEWL. For skin affected by tape stripping, the occluded arm healed better than the non-occluded arm, as evaluated by TEWL. Both of these results are significant, even though they seem contradictory.

A possible explanation for this must be found in the differences in skin barrier damage by SLS and tape stripping. The effect of SLS on the SC is not depletion of the lipids. On the contrary, it has been suggested that SLS might even accelerate the synthesis of new lipids (23). SLS increases intercellular oedema and alters the parallel arrangement of the lipid bilayers (23). From our knowledge of the effect of occlusion, it seems plausible that occlusion does not facilitate healing, because occlusion increases hydration during the occlusion, which is unfavourable, as the SC is already affected by intercellular oedema. Another explanation for the negative effect of occlusion on SLS-damaged skin is the reservoir of SLS or other soaps in the skin, after it appears to have been rinsed off. This has been shown in a study in which the skin was washed with different soaps before occlusion. It was found that SLS and other detergents had formed a reservoir in the skin, after normal rinsing with water, leading to irritation (24). From a clinical point of view, this might be an important factor in understanding the damaging effect on the skin of the wearing of occlusive gloves, especially in the healthcare sector, where frequent hand washing is normal. One study, which obtained similar results to our study, immersed the participants' hands in an SLS solution before occlusion. They found a significant worsening, as measured by TEWL, on the occluded skin as compared with the non-occluded skin (1). However, there is no information on whether any attempt to wash the SLS off was made before occlusion.

Whether increased use of alcohol disinfection, instead of hand washing with soap, may overcome the reservoir dilemma is not yet known and requires further study.

The effect of tape stripping seems simpler; it removes the SC, both corneocytes and the lipid matrix (10). The present study shows that occlusion results in faster healing of the skin after this barrier damage. This is in agreement with clinical practice; as far back as 1963, it was shown, on human

skin, that occlusion of superficial wounds further increased the healing process (25). No theoretical explanations quite cover this area. It has been proposed that it is the prevention of desiccation that enhances re-epithelialization (26), which is a similar mechanism to the previously described mechanical protection and consequent reduced desquamation (22).

Another group has also evaluated the effect of occlusion on SLS-irritated and tape-stripped skin. No statistical differences were found (27), but only 10 people participated in the experiment, and the occlusion lasted for just 46 hr. Our results indicate that the longer duration of occlusion seems to be of importance, both for the negative effect of occlusion on SLS-irritated skin, and for the healing effect on tape-stripped skin. In future studies, the importance of longer durations of occlusion should be taken into account.

In conclusion, this study has shown that 1 week of occlusion does not affect healthy skin, that occlusion decreases healing for SLS-treated skin, and that occlusion increases healing for tape-stripped skin. In addition, we point to a possibly negative effect of a soap reservoir in the skin, which is magnified by occlusion.

Acknowledgements

The authors gratefully acknowledge support of the study by a grant from the Aage Bangs Foundation and FSS (Danish Council for Independent Research).

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