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In vivo investigations on the penetration of various oils and their influence on the skin barrier

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Background: The skin represents a potent barrier to the environment, which can be enhanced by the topical application of skin care products, such as oil and oil-based formulations by moisturizing the skin.

Methods: The aim of this study was the investigation of the penetration behaviour of four vegetable oils and of paraffin oil into the stratum corneum by laser scanning microscopy. In addition, the occlusion capacity of these substances was assessed by transepidermal water loss (TEWL) measurements. Petrolatum served as a positive control for skin occlusion. The study was conducted in vivo and included six healthy volunteers.

Results: Paraffin oil, as well as the vegetable oils, penetrated only into the first upper layers of the stratum corneum. TEWL measurements indicated that the application of the vegetable oils (except jojoba oil) as well as paraffin oil, led to a similar occlusion of the skin surface. The most effective occlusion was found for petrolatum.

Conclusion: For the investigated oils, a deeper penetration than into the first upper layers of the stratum corneum could be excluded. The decreased TEWL values indicate that the application of the oils leads to a semi-occlusion of the skin surface as it is intended by the use of oils to retain moisture in skin.

Key words: vegetable oils – paraffin oil – penetration – occlusion – laser scanning microscopy – TEWL

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The skin represents a potent barrier towards the environment representing a challenge to cosmetic manufacturers and pharmaceutical companies in overcoming this barrier by means of cosmetic products. Whereas, sunscreens, barrier creams and most skin care products are topically applied to protect the skin and to enhance the barrier function, respectively, and are therefore intended to accumulate in the uppermost layers of the stratum corneum or to remain on the skin surface, various other cosmetic and especially pharmaceutical products are obliged to conquer the barrier, to reach their designated target structures, which are partially located below the skin barrier. The frequently used topically applied corticosteroids, e.g. act via a number of pathways to reduce inflammation targeting the dendritic cells, the leucocytes and eosinophils, collagen, etc (1). Also, several cosmetic products rely on vanquishing the skin barrier. Target structures of retinol, for example, which is frequently applied in anti-ageing products, are the keratinocytes as well as dermal collagen and elastin fibres (2). It is commonly established that the penetration properties of topically applied products are clearly related to the type of vehicle used (3). Therefore, appropriate in vivo methods are essential to characterize the penetration behaviour of vehicles and molecules aiming at continuous optimization and adaptation to special requirements. However, conventional in vitro measuring methods always require the removal of skin biopsies or the utilization of skin models, not displaying the real in vivo situation. In the meantime, a variety of optical and spectroscopic procedures are available permitting in vivo and online penetration investigations.

Skin care products with a high lipid content are recommended for the care of dry skin and inflammatory skin conditions (4). Pure oils, as well as oils integrated in diverse formulations
are frequently applied for skin care being known for their capacity to moisturize the skin by supporting the native lipids of the stratum corneum to provide a better barrier function (5). Therefore, a deep penetration through the skin barrier is not compulsory necessary. Nevertheless, to our knowledge, comprehensive in vivo investigations concerning the penetration behaviour of oils are still lacking, whereas the assumption that paraffin oil clogs the pores and prevents the skin from breathing, while vegetable oils penetrate deeper is commonly spread in non-scientific literature.

Recently, Stamatas et al. (5) used in vivo confocal Raman microspectroscopy to test the efficacy of paraffin oil and two vegetable oils concerning skin penetration and occlusion. They observed that the oils penetrated only into the top layers of the stratum corneum and discovered no difference in terms of occlusion between vegetable oils and paraffin oil.

Another very promising procedure is in vivo laser scanning microscopy. Laser scanning microscopic systems operate in the reflection (6) or fluorescence mode (7). Furthermore, laser scanning microscopic fluorescence measurements are highly selective and sensitive. They permit the clear detection of topically applied substances in the dermal barrier and in the living epidermis (8, 9). In particular, in vivo fluorescence microscopy is well suited to analyse the penetration into the cutaneous barrier (10, 11). The stratum corneum consists of flat dead horny cells, the corneocytes, which are well permeable for most wavelengths in the visible spectral range. This permits the analysis of the intercellular penetration of fluorescent or fluorescence-labelled substances within the lipid layers. In this study, in vivo laser scanning microscopy (LSM) and transepidermal water loss (TEWL) measurements were used to investigate and compare the penetration of paraffin oil (mineral oil) and several vegetable oils into the skin barrier and their occlusion capacity, respectively. Petrolatum served as a positive control for skin occlusion.

Materials and Methods

In this study, in vivo LSM was utilized to compare the penetration and distribution of various oils into and through the skin barrier. For a positive control, petrolatum was applied, which is known for its occlusion capacity of skin surfaces (5). In addition, TEWL measurements were performed to evaluate the quality of the skin barrier before and after the application of the oils.

Volunteers

Six healthy volunteers were involved in the conducted study (three female, three male) aged between 25 and 50 years. Approval for the measurements had been obtained from the Ethics Committee of the Charité-Universitätsmedizin Berlin. The volunteers participating in the study had given their informed written consent. The volunteers were instructed not to utilize any skin care products for at least 24 h and not to take a bath or shower for at least 4 h previous to the beginning of the experiments. Pre-treatment of the volunteers included washing of the skin with hand-warm water and careful drying with paper towels. After an acclimation time of 30 min, six skin areas of 4 × 4 cm each were marked on the volar forearms (three areas on each arm) using a permanent marker. To avoid lateral spreading of the topically applied substances, a silicone barrier was applied around the skin areas.

Substances

The following substances were used in the investigations:

- Jojoba oil (cold-pressed; Henry Lamotte, Bremen, Germany)
- Soybean oil (refined; Textron Technica SL, Barcelona, Spain)
- Avocado oil (refined, Cropure Avocado, Croda Chemicals Ltd., North Humberside, UK)
- Paraffin oil (Marcol 82 ™; Esso SAF, Rueil-Malmaison, France)
- Almond oil (refined; Afruse SL, Tarragona, Spain)
- Petrolatum (Vaselinum album, Bombastus-Werke AG, Freital, Germany)

Pre-investigations excluded any emanation of autofluorescence from the oils and the petrolatum after excitation with the laser scanning microscope at 488 nm. Therefore, the food dye, curcumin, was added to the oils and the petrolatum for visualization of the penetration and
distribution. Curcumin exhibits an intensive fluorescence after excitation at 488 nm. Previous investigations confirmed that curcumin spreads on and in the skin the same as the formulation, to which it had been added. Curcumin was added to the aforementioned substances above saturation level. Subsequently, the oils were vortexed (Vortex Genie 2, Scientific Industries, New York, USA) for 10 min before they were additionally treated in an ultrasonic bath (Sonorex Super RK 102 H, Bandelin, Berlin, Germany). Afterwards, the oils were filtered to remove the non-dissolved curcumin from the samples.

Application protocol
An amount of 2 mg/cm² of each curcumin labelled substance was applied to the corresponding skin area marked on the volar forearms of the volunteers. After a contact time of 30 min, a homogeneous film had formed on the skin surface, which exhibited an intensive fluorescence. To analyse to what extent the substances had penetrated into deeper layers of the stratum corneum or even deeper dermal layers, the film on the skin surface was wiped off with filter paper. Afterwards, TEWL and LSM measurements were conducted.

Measuring systems
TEWL measurements
The TEWL measurements were carried out with the Tewameter TM 210 (Courage + Khazaka electronic GmbH, Cologne, Germany) on each skin area before and 30 min after application, respectively. Each set of measurements was comprised of three individual measurements. From these measurements, the mean value was calculated. Our own experiences demonstrated that a difference in TEWL values before and after treatment of \( \leq 8\% \) could be neglected.

In vivo laser scanning microscopy
The in vivo laser scanning microscope Stratum (Optilas Ltd., Melbourne, Australia) was utilized to investigate the potential distribution and penetration of the topically applied substances. Pre-investigations excluded any autofluorescence deriving from the untreated skin, thus ensuring that the detected signals exclusively originate from the fluorescent marker curcumin. The laser scanning microscope consists of a handpiece, accommodating the optical imaging system. The handpiece is connected by optical fibres to the base station, which contains the excitation laser (argon system, 488 nm) and the spectrometer with the electronic evaluation system. The laser focus can be manually shifted into various depths of the human skin. The excitation radiation of 488 nm penetrates approximately with a maximum of 150 \( \mu m \) deep into the human skin, whilst the spot size of the system is 250 \( \times 250 \mu m \).

Statistical evaluation
Statistical evaluation was performed utilizing the software programme SPSS\textsuperscript{®} (IBM, New York, USA). Mean values and standard deviations were calculated. Descriptive statistic revealed that the data were not normally distributed. Therefore, the non-parametric Wilcoxon-test was utilized to calculate the differences. The significance level was determined at \( P < 0.05 \).

Results and Discussion
Representative images of skin areas treated with the various substances (paraffin oil, petrolatum and four vegetable oils) obtained by LSM are depicted in Fig. 1. All images were recorded at the same scanning depth. The substances investigated in the frame of this study exhibited only a very weak fluorescence signal after the excessive amounts had been wiped off, suggesting that a bulk of the substances remained on the skin surface and had thus already been removed during the wiping off procedure. In spite of the relatively low fluorescence signals, it can be clearly established that the substances after having been wiped off are located only on the skin surface and not deeper than in the two uppermost layers of corneocytes, respectively. It is clearly visible that the dye is located on the surface of the corneocytes, only. If the laser focus was shifted into deeper cell layers of the stratum corneum, no fluorescence signal could be detected. From all samples investigated, soybean oil and almond oil were established to penetrate the deepest into the stratum corneum. While curcumin in these two oils was detectable only in volunteers 1, 2, 3 and 6 on the skin surface and in the lipid layers surrounding the
first layer of the corneocytes, a penetration into the second and third corneocyte layers was observed in the case of volunteers 4 and 5 (Figs 1a and b), which was obviously due to the somewhat dry skin of these two volunteers.

Referring to jojoba oil, avocado oil and paraffin oil (Figs 1c–e), in all volunteers fluorescence was detectable only on the skin surface and in the lipid layers around the first cell layers of the corneocytes. Consequently, the investigations clearly show that the applied oils form an efficient protective film on the skin surface. A penetration into deeper layers of the stratum corneum, let alone through the skin barrier, could not be detected. These results are in concordance with the observations in previous studies. Stamatas et al. (5) revealed that paraffin oil, jojoba oil and almond oil penetrate equally only into the outermost layers of stratum corneum. Agero et al. (12) showed that coconut oil is as effective and safe as paraffin oil when used as a moisturizer.

Figure 1f also shows that petrolatum, although it had been wiped off, still formed a homogeneous protective film on the surface preventing sweat from penetrating into the stratum corneum. In the right corner at the bottom of Fig. 1f, a long dark sweat drop can be recognized, which is located on the protective film formed by petrolatum. Petrolatum was detected on the skin surface, only. No detection into the upper layers of the corneocytes could be observed.

The amount of drug or cosmetic active ingredient that can be delivered into or through the skin depends on the integrity of the skin barrier, the physicochemical properties of the permeant and the vehicle (13). Jacobi et al., for example, applied vanillin in ethanol and in a w/o emulsion and found a significantly deeper penetration of vanillin through the complete stratum corneum when applied in ethanol. (14). This can be explained by the ability of ethanol to extract the lipids from the stratum corneum, wherefore ethanol is also known as a penetration enhancer (3). Corresponding observations were not made in this study; therefore, a relevant penetration of the oils can be excluded.

In addition to the penetration investigations with laser scanning microscopy, TEWL measurements were performed pre- and post treatment, to evaluate potential changes in skin barrier properties. The mean values and stan-
TABLE 1. Mean values and standard deviations of TEWL values obtained previous to treatment and 30-min post treatment for all applied substances

<table>
<thead>
<tr>
<th>Applied Substance</th>
<th>TEWL values (g/hm²)</th>
<th>Significance</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Previous to treatment</td>
<td>30-min post treatment</td>
<td></td>
</tr>
<tr>
<td>Jojoba oil</td>
<td>11.82 ± 2.18</td>
<td>11.82 ± 2.68</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.78 ± 2.03</td>
<td>9.88 ± 2.06</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Avocado oil</td>
<td>11.70 ± 1.61</td>
<td>9.93 ± 2.22</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Paraffin oil</td>
<td>11.95 ± 1.54</td>
<td>10.70 ± 1.78</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Almond oil</td>
<td>11.82 ± 1.35</td>
<td>10.67 ± 1.54</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>10.95 ± 2.1</td>
<td>5.08 ± 1.78</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

standard deviations, pre- and post- application of the various substances are demonstrated in Table 1.

The TEWL measurement has become an important non-invasive tool in dermatology and cosmetology and is generally appreciated as a device for the monitoring of changes in the barrier function of the stratum corneum (15). In intact skin, TEWL values are rather low, whereas impaired skin provides increased values leading to decreasing values when the barrier recovers (9). Our own experiences demonstrated that a difference in TEWL values before and after the treatments of ≤8% could be neglected. Except for jojoba oil, differences in TEWL were calculated to be higher than 8%, which is in concordance with the statistical evaluation, revealing significant differences in pre- and post-treatment for all applied substances apart from jojoba oil. The TEWL decreased in all investigated substances, except for jojoba oil, the TEWL value remained stable. The decrease in the TEWL values was already expected as the applied substances form a protection film on the skin surface, which not only reduces the penetration of further topically applied substances but also decreases the water loss via the skin. In the case of petrolatum, which is known to have increased occlusion properties, the highest decrease in TEWL values was observed. Increased TEWL values were not observed within the previous study, suggesting that the application of none of the applied substances had led to barrier impairment. Therefore, also the TEWL values did not deliver any hint of a penetration of the applied substances into the skin.

Conclusion

The study revealed that LSM and TEWL measurements represent appropriate methods to investigate the penetration and the effect of occlusion of various topically applied oils into the stratum corneum. Paraffin oil, as well as the vegetable oils, penetrated only into the first upper layers of the stratum corneum. Thus, a deeper penetration can be excluded. The decreased TEWL values indicate that the application of the oils leads to a semi-occlusion of the skin surface as it is intended by the use of oils to retain moisture in skin. Similar results were obtained for mineral oil and the vegetable oils. The highest decrease was found for petrolatum, which is known on account of its increased occlusion properties and was, therefore, utilized as a positive control. While the maximum effect in terms of occlusion of skin by using raw oils in this study was shown, further investigations to understand the penetration of oils in combination with other cosmetic ingredients need to be undertaken.

Acknowledgments

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References

Penetration of oils


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