

Evaluation of barrier creams – introduction and comparison of 3 *in vivo* methods

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Often barrier creams (BC) do not fulfil their protecting behaviour, even when promised by the manufacturers. Therefore, it is of great importance to develop standardized *in vivo* techniques to prove the potency of BC in humans. In the present study, 3 promising techniques for the analysis of BC were evaluated: laser scanning microscopy, laser doppler flowmetry, and the tape-stripping procedure. Sodium fluorescein and glycerol trinitrate acted as hydrophilic model-penetrating substances. By means of these methods, 3 different BC were tested and compared for their potency. The investigations showed that primarily the tape-stripping procedure and also the laser scanning microscopy are promising tools for the evaluation of BC. In contrast, the laser doppler flowmetry represents a less feasible technique. In addition, the evaluation of BC showed that Vaseline enfolds a 100% barrier on the skin surface for the penetration of a hydrophilic dye, whereas BC1 only partially showed a protective effect and BC2 exhibited almost no potency. In conclusion, it can be referred that laser scanning microscopy and the tape-stripping procedure represent 2 non-invasive *in vivo* techniques, which enable a fast investigation concerning the potency of BC.

Key words: glycerol trinitrate; laser doppler flowmetry; laser scanning microscopy; skin protection; sodium fluorescein; tape stripping. © Blackwell Munksgaard, 2007.

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The skin as the outermost layer of the human body is exposed to several environmental influences like chemicals and microorganisms. Intensive wet work or frequent hand washing occurs in various professions: in industry, handcrafts, health care, and furthermore, in private households (1). If the skin is highly stressed because of such influences, this can cause irritative, allergic or cumulative-toxic contact dermatitis (1). A multiplicity of strategies is known to prevent dermatitis. A basic principle is the avoidance of unneeded contact with irritative substances. Furthermore, a soft cleaning of the skin with a gentle cleanser is recommended. In addition, regular using of barrier creams (BC) is approved, but up to now hotly debated (2). For the prevention of contact dermatitis, it is of interest to develop effective BC. For this purpose, methods for evaluation and ranking of BC are necessary, which are easy and fast in handling, non-invasive, standardized, and *in vivo* applicable. Currently, several *in vivo* and *in vitro* methods are in existence for the evaluation of BC. A commonly used *in vivo* method is the repetitive irritation test (3); in addition, diffusion cells and

in vitro skin models have been used (4, 5). Furthermore, the penetration of various dyes after pretreatment with a BC was measured by skin reflectance measurements, chromameter or laser-induced breakdown spectroscopy (6, 7, 8). All these investigations showed controversial results regarding the effectiveness of BC. Additionally, a number of these methods exhibit crucial limitations, being invasive, being applied *in vitro* or on animals. Here, the data cannot be transferred to *in vivo* or human conditions. Therefore, the aim of the present study was to introduce and compare 3 promising *in vivo* methods for the analysis of the effectiveness of BC: the laser scanning microscopy, the laser doppler flowmetry, and the tape-stripping procedure. Using these techniques, the potency of 3 different BC has been investigated. The laser scanning microscopy allows for online visualization of living human skin (9) and the penetration of topically applied fluorescing substances (10). With the help of the laser doppler flowmetry, it is possible to measure the blood microcirculation of the skin which can be influenced by vasoactive topically applied substances

(11) such as glycerol trinitrate. Previously, it has been shown that the tape-stripping procedure in combination with ultraviolet-visible (UV/VIS) spectroscopy represents a suitable method for the evaluation of BC (12).

Materials and Methods

Pretreatment of the volunteers

The experiments of the present study were carried out *in vivo* on 6 healthy men volunteers, aged between 22 and 27 years, on 3 different examination days. Ethics approval had been granted from the University Hospital Charité Ethics Committee, Berlin.

Each flexor forearm of the test persons was prepared by washing, drying with a soft tissue, and abscising the hairs. Next, 2 defined skin areas of 4×5 cm were marked on each forearm by using a permanent marker (reference area A, areas B, C, and D). To avoid lateral spreading of the topically applied substances, which were applied afterwards, a silicone barrier was built around the test areas (12). 4 mg/cm² of 3 different BC were applied to each dedicated test area.

Area A is reserved free as reference. Area B refers to Vaseline® (H. Hagner GmbH & Co., Freudensstadt, Germany). Area C refers to commercial BC1 containing dimethicone, H₂O, dicapryladipat, coconut oil, Polypropylene Glycol, PPG (15) stearyl ether, dipropylene glycol, isopropyl palmitate, mineral oil, ethylene/acrylic acid copolymer, acrylate terpolymer, paraffin, magnesium sulfate, propylene glycol, perfume, diazolidinyl urea, methyl parabene, and propyl parabene. Area D refers to commercial BC2 containing H₂O, talc, paraffin, stearic acid, glycerol, cetearyl alcohol, octyldecanol, cera alba, cellulose gum, sodium cetearyl sulphate, ascorbic acid, sodium hydroxide, methyl parabene, propyl parabene, polyethylene glycol-8, tocopherol, ascorbyl palmitate, perfume, amyl cinnamal, benzyl benzoate, benzyl salicylate, citronellol, coumarin, limonene, geraniol, hydroxyisohexyl-3-cyclohexenecarboxaldehyde, isoeugenol, linalool, cinnamyl alcohol, alpha-isomethyl ionone, and butylphenyl methylpropional.

The BC were applied for 15 min. The 3 different methods, laser scanning microscopy, laser doppler flowmetry, and the tape stripping were surveyed in this order with 1 week of recovery in between. With the help of laser scanning microscopy and the tape-stripping procedure, the penetration of sodium fluorescein was observed; by means of laser doppler flowmetry, the penetration of the vasoactive substance glycerol trinitrate (Aquo-Trinitrosan®; Merck KgaA, Darmstadt, Germany) was investigated.

The sodium fluorescein was applied in 1.5% solution by means of saturated filter papers. In the same manner, glycerol trinitrate was also applied. The filter papers were removed 5 min after application.

Method 1: laser scanning microscopy/first day of examination

The experiments were performed using the fibre-based laser scanning confocal microscope (LSM) 'Stratum' (OptiScan Ltd, Melbourne, Australia) (13). In our study, we used the fluorescent mode of confocal microscopy, as described previously (14).

After application of the BC, 1.5% sodium fluorescein-containing solutions were applied as described above. Then, the excess on the skin surface was removed with a soft tissue in one movement after the silicone barrier was abscised. To track the penetration of sodium fluorescein in the application areas, LSM was applied. Images of the different skin depths were taken and analysed visually.

Method 2: laser doppler flowmetry/second examination day, 1 week later

Using the laser doppler flowmeter developed at the LMTB GmbH (Berlin, Germany), the dermal microcirculation of blood was measured (11). To avoid occlusive effects (15, 16), the direct contact between the scanner of the flowmeter and the skin was prevented with the help of a metal ring (height 2 mm) fixed on the underside of the scanner. 1 cycle of gauge consisted of a 30-second measurement and a 30-second break in which the scanner was removed from the skin area to avoid occlusive effects. For every skin area, the basal blood microcirculation was detected by means of 15-gauge cycles. Afterwards, the BC, and 15 min later the glycerol trinitrate, were applied to their dedicated skin areas. Subsequently, the change of blood flow was examined by 20-gauge cycles.

Method 3: tape-stripping method/third day of examination, a further week later

After the application of the BC and the 1.5% sodium fluorescein-saturated filter papers, the silicone barriers were removed. To investigate the penetration of sodium fluorescein, the tape-stripping procedure was performed as described previously (12). By using an adhesive tape (tesa®, No. 5529; Beiersdorf, Hamburg, Germany), the stratum corneum of each pretreated skin area was stripped 20 times. With the help of a roller, the tapes were pressed onto the skin surface to avoid the influence of furrows and wrinkles (17).

By means of an UV/VIS spectrometer (Lambda 20; Perkin Elmer, Überlingen, Germany) with a quadratic beam of 10×10 mm, the amount of

corneocytes and sodium fluorescein was determined for every strip. The pseudo-absorption of corneocytes at 850 nm can be used to quantify the amount of corneocytes on the removed tapes. 24 hr later, the tapes were cut to a size of 3×1.5 cm, extracted in 4.5 ml ethanol (Uvasol; Merck) using ultrasound (Sonorex Super RK102; Bandelin Electronic, Berlin, Germany) and centrifugation (Centrifuge MR1812; Jouan GmbH, Unterhaching, Germany) followed by the determination of concentration of sodium fluorescein using spectroscopic measurement at the absorption maximum of 480 nm.

Data analysis

The analysis of the digital images of the laser scanning microscopy (method 1) was carried out by comparing the intensity of the fluorescence signal of the reference areas A (representing uninhibited penetration) with the BC-treated skin areas B, C, and D visually.

For a better comparison of the achieved data of methods 2 and 3, the results of the reference areas A of each volunteer were standardized to 100%. Provided that the reference skin areas represent the uninhibited penetration of substances (corresponding to a 100% penetration), these areas were compared with the pretreated skin areas B, C, and D. Results less than 100% for the pretreated skin

areas indicate a reduced penetration of the applied substances.

The data of the tape-stripping method (method 3) were presented by penetration profiles as described previously (12). The penetration profiles take into consideration the amount of corneocytes and the concentration of sodium fluorescein removed with each tape strip in a common figure and represent cuts through the skin.

Statistical analysis

For statistical analysis of the data, we calculated the mean values and standard deviations of methods 2 and 3. To show that the obtained data for the reference areas of both methods, compared to the other skin areas, were significantly different ($P < 0.05$); we used the Wilcoxon test (SPSS 12.0[®]; SPSS, Chicago, IL, USA).

Results

Method 1: laser scanning microscopy

The penetration of sodium fluorescein in pretreated and untreated skin was investigated by means of laser scanning microscopy, by comparing the fluorescence signal of the digital images of the reference area A with the 3 other application areas (B, C, and D). In Fig. 1, the results of the

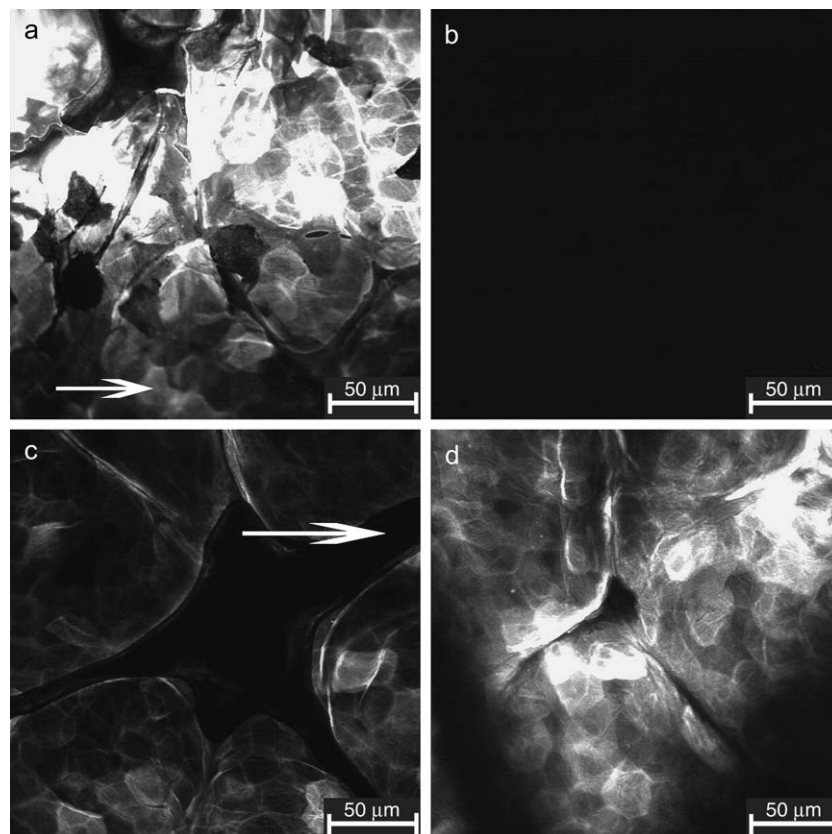


Fig. 1. Laser scanning microscopy, a reference area A; b area B pretreated with Vaseline; c area C (pretreated with BC1); d area D (pretreated with BC2).

skin areas A, B, C, and D are shown exemplarily for volunteer 4. Figure 1a represents the uninhibited penetration of sodium fluorescein in the reference area (area A). A strong fluorescent signal on and between the corneocytes can be seen and even single corneocytes can be distinguished (white arrow). Figure 1b shows the skin area pretreated with Vaseline. Here, no penetration of sodium fluorescein could be detected. This effect was seen in 4 out of 6 volunteers. In 2 volunteers, the superficial cell layers showed no fluorescent signals, but in the deeper layers a few single fluorescing corneocytes were detected. The BC1 pretreated test area already showed a weak fluorescent signal in the upper cell layers. Mostly, only the cell borders of the corneocytes showed a fluorescent signal, whereas the furrows (white arrow) are free of fluorescence. The results were obtained in 5 out of 6 volunteers. The sixth volunteer showed similar findings to those of the reference area. Figure 1d, representing the BC2-pretreated skin area, showed a strong fluorescent signal similar to the reference area.

Method 2: laser doppler flowmetry

With the help of the laser doppler flowmetry, the modification of the relative blood flow after the application of glycerol trinitrate on the different pretreated skin areas was measured. Figure 2 represents the mean percentage change of the relative blood flow of each pretreated test area. Provided that the reference area represents a 100% rise in blood flow, the blood flow in areas B (Vaseline pretreated) and C (BC1 pretreated) decreased, in the case of area B, below 0 (–24%) and in case of area C, down to 9%. In area D, the blood flow was raised up to 129%. For Vaseline (area B) and BC1 (area C) pretreated skin, a significant difference was seen compared to untreated skin

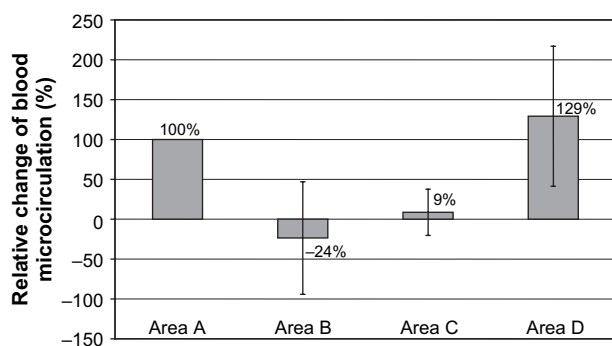


Fig. 2. Percentage modification of the relative superficial dermal blood flow after application of glycerol trinitrate (area A: uninhibited penetration, area B: pretreatment with Vaseline, area C: pretreatment with BC1, area D: pretreatment with BC2).

($P < 0.05$), whereas for the pretreatment with BC2, no significant differences were observed.

Method 3: tape-stripping method

By means of tape stripping, the amount of sodium fluorescein penetrated into the stratum corneum was investigated quantitatively in each skin area. In Fig. 3, the penetration of sodium fluorescein is shown for untreated and pretreated skin (volunteer 5). In the case of the reference skin area (area A, Fig. 3a) and the test area pretreated with BC2 (area D, Fig. 3d), a similar penetration behaviour was observed, although the penetration of sodium fluorescein seems to be increased even in area D. In the skin areas pretreated with BC1 (area C), sodium fluorescein was detected up to the fourth tape strip. No penetration of sodium fluorescein was observed in the deeper skin layers (Fig. 3d). Likewise, no penetration of sodium fluorescein was detected when Vaseline was applied (area B, Fig. 3b). Figure 4 represents the percentage amounts of recovered sodium fluorescein of volunteer 5. Preconditioned that the reference area (area A) represents a recovery of 100%, a percentage of 80% of sodium fluorescein was recovered from skin area C (BC1) and a percentage of 180% of sodium fluorescein was observed from skin area D (BC2). In the event of skin area B (pretreated with Vaseline), no sodium fluorescein was detected in the stratum corneum. Similar results were obtained for all 6 volunteers.

Discussion

The aim of the present study was the introduction and comparison of 3 *in vivo* methods: laser scanning microscopy, laser doppler flowmetry, and the tape-stripping procedure for the evaluation of BC. Therefore, 3 different BC were surveyed for potency against the penetration of hydrophilic substances. Reference area A, presenting uninhibited penetration of the topically applied substances served as control and were compared with areas B, C, and D. It has been shown that all 3 methods are suitable for the investigation of the effectiveness of BC.

By means of LSM, it is possible to track the penetration of a fluorescent topically applied substance and to detect even low differences by interpreting variations of the fluorescent signal. Therefore, a ranking of BC concerning their potency is highly feasible. Whereas the fluorescent signals in the reference skin area A and the BC2-pretreated skin areas D were very nearly identical, which indicates a low potency of BC2, in 5 out of 6 volunteers, the fluorescent signal was drastically

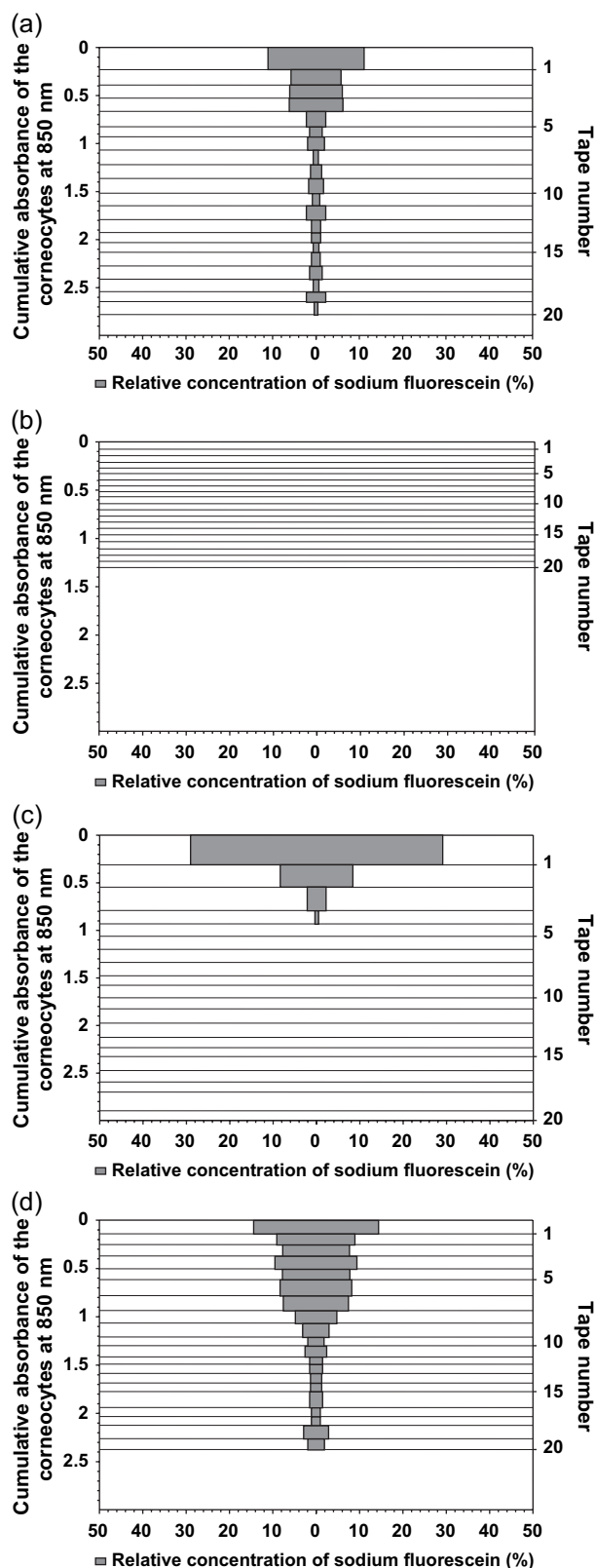


Fig. 3. Penetration profiles of the areas A a; B b; C c and D d.

reduced in the BC1 pretreated skin area C. The differential findings in the sixth volunteer must be considered as a technical mistake. Possible failings could be the spreading of the fluorescent dye after

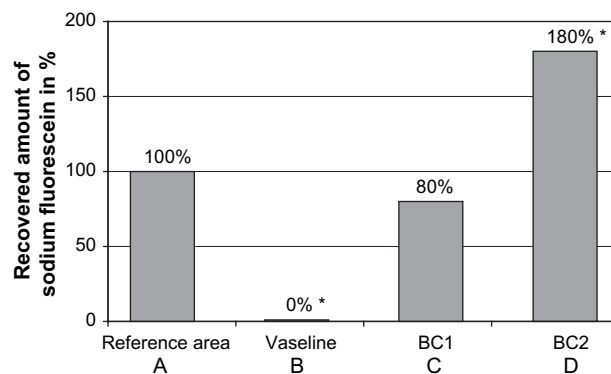


Fig. 4. Comparison of the percentage recovery of sodium fluorescein of the different skin areas. * $P < 0.05$.

removing the excess or even intrinsic differences of the skin between the test persons referable to the individual reservoir of the skin (18).

In 4 out of 6 volunteers, no fluorescent signal was detectable in the skin areas B pretreated with Vaseline. Here, no penetration of sodium fluorescein took place. However, in 2 volunteers, a low fluorescent signal was detectable, but only in the deeper cell layers, whereas the superficial layers were free of fluorescence, which indicates that no penetration from outside has occurred. This effect can be interpreted as an auto-fluorescent phenomenon. Alvarez-Roman et al (19) also showed the characteristic of auto-fluorescence, which can influence the results of LSM. Because of the online ability of laser scanning microscopy, it is possible to interpret the potency of BC immediately. Therefore, this *in vivo* method is a very quick technique with simple handling.

By means of laser doppler flowmetry, it is possible to detect variations of superficial dermal microcirculation after the application of vasoactive substances such as glycerol trinitrate. As BC are said to prevent the penetration of substances, the increase in blood flow should be reduced in BC-pretreated skin areas (areas B, C, and D) compared to untreated skin areas (area A). Also, Duval et al (20) showed the possibility of determining the effectiveness of BC by using the laser doppler flowmetry. They applied the BC for a period of 3 weeks and treated the skin afterwards with hexyl nicotinate. In the present study, the BC were applied only once for a period of 15 min and it could be shown that in the case of Vaseline (skin area B) and BC1 (skin area C), the penetration of glycerol trinitrate could be successfully inhibited as the blood flow was significantly lower in comparison to reference area A. Additionally, Vaseline-pretreated skin areas showed a decrease below 0 indicating a reduction of blood flow below the basal blood flow,

measured before the application of BC and glycerol trinitrate. Skin areas pretreated with BC2 showed no significant differences compared to the reference area. In principle, it is possible to use the laser doppler flowmetry for the evaluation of BC. However, this technique showed some disadvantages, as it is necessary to measure the blood flow several times to discover a mean value (21) because of immense fluctuation of blood microcirculation. Choi and Bennet (21) also alleged that it is not possible to calibrate the laser doppler flowmetry on the basis of a gold standard. The solution to this problem proves to be complicated because of an individual blood flow and, of course, individual response to the application of glycerol trinitrate. Furthermore, it must be considered that occlusive effects of the BC (22) as well as of the scanner, can influence the results of the laser scanning flowmetry. We endeavoured to keep the latter-named problem within a limit by removing the scanner between single measurements from the test areas. Another limiting factor of this method is the generated epidermal lipid film of the applied BC, which can cause a shift in the scanner. Additional influencing effects can be caused by the volunteer himself, such as movements, coughing, and speaking during the measurements (21), which were reduced by instructing the test persons to avoid such activities.

By the method last used, the tape-stripping procedure in combination with UV/VIS spectroscopy, it was also feasible to analyse the effectiveness of BC and even the objective depth of penetration and the dispensation of the fluorescent dye within the single tapes. This capability has been described previously by Teichmann et al (12) who used Patent Blue V instead of sodium fluorescein as penetrating substance. All 6 volunteers showed no penetration of sodium fluorescein in Vaseline-pretreated skin areas (area B) as described by Teichmann et al (12). If the skin was pretreated with BC1 (area C), no significant differences ($P > 0.05$) concerning the amount of recovered sodium fluorescein could be found compared to the reference area in all 6 test persons. However, with the help of the penetration profiles, it could be established that the fluorescent dye was only detectable in the upper 4 tapes. Indeed, we also observed that the amount of sodium fluorescein in these 4 tapes was much higher than in the comparable number of tapes of the reference area. We assumed that deeper parts of the horny layer were protected by BC1 against a deeper penetration of the fluorescent dye. The amount of sodium fluorescein, which penetrated uninhibited in deeper parts of the stratum corneum in area A, accumulated in area C probably in the upper

parts. Skin area D (pretreated with BC2), showed comparable penetration behaviour in all 6 volunteers. Nonetheless, a significantly higher amount of sodium fluorescein ($P < 0.05$) could be detected in this skin area. Here, obviously, an enhancing effect of the BC2 for sodium fluorescein has to be considered. With the aid of the tape-stripping procedure and the presentation of the results in the form of penetration profiles, it was possible to follow exactly the penetration of sodium fluorescein into the stratum corneum.

Considering the protective effect of the used BC, we can accede with several authors who described that not every BC keeps the promise, which is given by the manufacturers in case of prevention (12, 23). Frosch et al (23, 24) even described that 1 of their tested BC boosted the artificially created irritation instead of protecting the skin. The present study introduced and compared 3 *in vivo* methods to examine 3 different BC with the help of water-soluble-penetrating substances. The results of this study concerning the BC could support the spectrum of agency denoted by the manufacturers likewise only partially. Exclusively, Vaseline showed, in every tested method, a 100% protective effect against the applied hydrophilic substances. These outcomes suit well with the results shown by Wigger-Alberti et al (25) who analysed the protective effects of Vaseline in a cumulative irritation test. In conclusion, it seems possible to use Vaseline as a gold standard. Also Kucharekova et al (26) described that Vaseline can be used as gold standard for comparison of the potency of BC. Regarding BC1, the 3 methods showed that the barrier was increased in comparison to the reference skin area. BC1 appeared to be very protective against glycerol trinitrate by reducing the blood flow significantly compared to the reference area; it showed a slightly minor effect against sodium fluorescein in the case of laser scanning microscopy and the tape-stripping procedure. After pretreatment of the skin with BC1, the laser scanning microscopy detected a light fluorescent signal of the stratum corneum compared to the reference area. The tape-stripping procedure detected similar amounts of the fluorescent dye compared to the reference area but just up to the fourth tape strip. By means of the laser scanning microscopy and the tape-stripping procedure, at least a trend of building a protecting effect by BC1 was shown but it was not completely effective against water-soluble substances as promised by the manufacturers. One possibility for the incomplete effectiveness of BC1 could be a thesis described by Zhai et al (27) which stated that a number of cream solutions probably develop a protective effect against

some but not all irritative or allergenic substances. BC2 showed neither a protective effect against glycerol trinitrate nor against sodium fluorescein. The results of the tape-stripping procedure detected even a significantly higher amount of the fluorescing dye ($P < 0.05$) compared to the reference area. Likewise, after the application of glycerol trinitrate, the blood flow increased above the results of the reference area but insignificantly. All in all, BC2 seems to boost the penetration of at least the investigated hydrophilic topically applied substances. Marks et al (28) described, that the affinity of a substance for a BC and even for the stratum corneum could influence the results. Thus, the penetration of a substance could be aggravated by a higher solubility for BC2.

Summarizing the findings of this study, it can be concluded that all 3 tested methods can be theoretically used to evaluate the effectiveness of BC. The advantageous appeared to be the tape-stripping procedure, which enables the detection of the penetration depth, quantity, and the accurate amount of applied substances. Also, with the help of the laser scanning microscopy, it is possible to detect the penetration of fluorescent substances online exactly and without time-consuming procedures. However, the results are rather semi-quantitative. Also, laser doppler flowmetry represents a suitable method, which achieved better comparable results than the 2 other methods. However, this method is highly susceptible for various interferences. Another benefit of all 3 methods is the applicability *in vivo* and in humans without irritating effects. Regarding the effectiveness of the used BC, Vaseline showed a 100% protective effect followed by BC1 and afterwards BC2, which showed no protective effect. For all 3 methods, the same ranking of the BC was performed.

Finally, it can be assumed that BC should be used only supportively to prevent irritative damages to the skin. But it is necessary to use additional protective measures like reducing the contact time to an irritative substance or using gloves. Furthermore, the present study showed that it is important to analyse the protective effect of BC with adequate and standardized methods as described above.

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