Skin barrier restoration upon topical use of two 5% dexpanthenol water-in-oil formulations on freshly tattooed skin: results from a single-blind prospective study

Topical dexpanthenol has been used in the aftercare of new tattoos for years, but formal studies in this setting are lacking. The current article outlines the results of an investigator-blinded, prospective study assessing the effects of Bepanthen® Ointment and Bepanthen® Emulsion (both Bayer Consumer Care; Basel, Switzerland), two 5% dexpanthenol water-in-oil formulations, on skin barrier restoration when applied on freshly tattooed skin for 14 days. The temporal pattern of transepidermal water loss after tattooing was assessed in 54 subjects. The application of both products was associated with a virtually complete skin barrier restoration; cosmetic performances were well perceived by study participants leading to high product acceptability.

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In Western countries, the prevalence of tattooed people currently ranges from 10% to 30%, and continues to rise (Kluger, 2015; Kluger and De Cuyper, 2018). Following a tattooing session, an acute aseptic inflammatory reaction develops and the skin is warm and sensitive to touch; this occurs irrespective of the size of the tattoo or the length of the session (Sperry, 1992; Kluger, 2012). Since the epidermis is repeatedly punctured by the tattoo needle to introduce pigments and dyes into the dermis, the stratum corneum skin barrier is also violated and has to undergo a repairing process (Sperry, 1991).

If the skin barrier is damaged, there will be an increased loss of water, electrolytes and proteins, and an increased risk for infections (Simunovic and Shinohara, 2014; Antonov et al, 2016). A fresh tattoo that becomes infected or undergoes physical trauma (e.g. scratching because of dry skin) may not heal (Liszewski et al, 2016). Therefore, an adequate aftercare is critical for achieving successful skin barrier restoration and wound healing (Kluger and De Cuyper, 2018). In the absence of complications, it normally takes 2–4 weeks until the skin is healed after tattooing (Kluger, 2012; Tucker, 2012).

Despite the fact that tattoos are becoming increasingly popular, no official guidelines exist that provide tattoo aftercare instructions (Liszewski et al, 2016). A 5% dexpanthenol-containing ointment (Bepanthen® Ointment; Bayer Consumer Care; Basel, Switzerland) has been used by tattooists for years in the aftercare of new tattoos, although formal studies in this setting are lacking. Its use and recommendation are rather based on anecdotes and experience (Tucker, 2012). In different models, topical dexpanthenol has been shown to act as moisturiser and skin barrier restorer; it also prevents skin irritation, stimulates skin regeneration and promotes wound healing (Proksch et al, 2017).

The study presented here was conducted to investigate the effects of two 5% dexpanthenol water-in-oil formulations — Bepanthen Ointment and Bepanthen® Emulsion (Bayer Consumer Care; Basel, Switzerland) — on transepidermal water loss (TEWL) when applied on freshly tattooed skin 4–8 times daily for 14 days. Measurement of TEWL is a non-invasive and sensitive method to quantify stratum corneum barrier function (Pinnagoda et al, 1990; Antonov et al, 2016). Furthermore, the cutaneous tolerability, as well as the subjective performance and acceptability of the two formulations, were assessed.

METHODS

The trial was conducted in freshly tattooed healthy adult subjects under supervision of a dermatologist at Eurofins Evic Product Testing Romania SRL, in Bucharest, Romania, between December 2017 and May 2018. The study was performed according to the requirements of the Declaration of Helsinki with all its amendments

and in accordance with the guidelines for the evaluation of the efficacy of cosmetic products. Subjects gave written informed consent to participate after they had been informed about the study. The two study preparations were provided by Bayer Consumer Care AG, Basel, Switzerland, and comprised Bepanthen Ointment (Product A) and Bepanthen Emulsion (Product B). Both cosmetic products are water-in-oil formulations containing 5% dexpanthenol.

Study design

This was an investigator-blinded, prospective, intra-individual comparison study in healthy adult subjects having received two new tattoos of comparable size. The size of a tattoo was to be no larger than what the tattoo artist was able to accomplish within 2 hours (dorsal body parts were not permitted). Study visits took place at screening, baseline (day 1), as well as on study days 2, 7 and 14. There was no overnight confinement of study subjects.

At the screening visit, subjects selected the desired tattoos from a catalogue. In addition, the skin areas planned to be tattooed were dermatologically assessed. On day 1, the subjects went to the predefined professional tattoo parlour (2nd Face Tattoo & Body Piercing, Bucharest, Romania) where the tattoos were made in a standardised fashion on the selected skin areas after an ink allergy had been excluded. Black ink was used for all tattoos.

At the time of completion, a thin layer of petroleum jelly was applied over the new tattoos and then the tattooed areas covered with a plastic wrap. After 4 hours, the plastic wrap was removed at the study site and the tattoos washed with an antibacterial soap. Subsequently, for each subject, the study products were assigned to the two new tattoos. Each product was assigned to one tattoo only, thereby allowing an intra-individual comparison of product performances. The allocation of tattoos to treatment (Product A or Product B) was conducted according to a balanced randomisation list.

Following baseline assessments (day 1, approximately 4 hours after the tattooing session), the respective study product was applied to the assigned tattoo by the subjects under supervised conditions. During the following 14 days, the products were administered by the subjects at home. Each product was to be applied with the fingers 4–8 times daily by gentle massage and in enough quantity to cover the involved area. Compliance was assured by weighing each tube with study product before and after the application period.

Subjects and assessments

Healthy male and female subjects between 18 and 60 years of age with white or light brown skin [type II-IV on Fitzpatrick scale (Fitzpatrick, 1988)] and wishing to receive two new tattoos (according to the scheduled procedures) were to be enrolled. For inclusion, females had to be non-pregnant. Female subjects of childbearing age were required to use reliable methods of contraception during the study.

Study participants were required to have no history of allergic reaction to cosmetic products. Subjects were excluded if they had allergies to tattoo inks or to any ingredient of the test products, an active skin disease that would interfere with clinical assessments, or sunburn at the sites of planned tattoos. Subjects were further excluded if they had taken drugs interfering with the immune system within 30 days prior to the study, had diseases able to compromise immune system response (e.g. autoimmune disease, diabetes, or human immunodeficiency virus infection), or participated in another trial within 30 days before study start.

During the study, the subjects were instructed to avoid hot showers, hot bathing, saunas, Turkish baths, swimming in a pool, or anything which could irritate the freshly tattooed areas (e.g. scratching, picking the crusts, wearing bracelets, or exposure to ultraviolet rays). They were also not allowed to use other topical preparations on the newly tattooed areas during the study course. Those subjects who presented with tattooed skin requiring medical treatment after the tattooing session (e.g. due to moderate/severe inflammation or infection) had to discontinue study participation.

Before the instrumental measurements of TEWL (MPA Tewameter® TM300, Courage & Khazaka, Cologne, Germany) during study visits, subjects stayed in an air-conditioned room (20 ± 2 °C, 45 ± 15% relative humidity) for at least 15 minutes. Consumption of hot drinks (e.g. coffee or tea) was not permitted during and also within 2 hours before a measurement.

Over the course of the study, the measurements were made on the same tattooed areas; if necessary they were cleaned at first. TEWL was assessed at screening on areas planned to be tattooed. TEWL was further determined on each of the two test areas on day 1 (approximately 4 hours after tattooing and immediately before first application of study products [= baseline], and 1 hour after treatment initiation) as well as on study days 2, 7 and 14.

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Table 1. Mean change from baseline in TEWL following topical application of Product A or Product B on freshly tattooed skin 4-8 times daily over 14 days.

	Product A	p-value* for Product A	Product B	p-value* for Product B
Day 1 (BL)	79.14 ± 15.98	-	76.84 ± 16.95	-
Day 1 (1 h after BL)	-12.99 ± 19.25	0.001	-14.85 ± 18.20	0.001
Day 2	-26.16 ± 25.30	<0.001	-21.34 ± 21.09	<0.001
Day 7	-60.23 ± 17.35	<0.001	-54.04 ± 19.61	<0.001
Day 14	-62.62 ± 18.39	<0.001	-58.96 ± 23.49	<0.001

N = 54. Data are given in g/m²/h. All values are presented as mean ± standard deviation.
BL = Baseline assessment = Mean TEWL value at approximately 4 hours after the tattooing session and immediately before first topical application of study products.
Product A = Ointment containing 5% dexpanthenol;
Product B = Emulsion containing 5% dexpanthenol.
**For mean change from baseline, Dunnett's two-tailed t-test.*
Note: A reduction of TEWL reflects improvement in skin barrier function.

At each study visit, dermatological examinations were performed by the investigator, a dermatologist, who was unaware of treatment allocation. To determine the cosmetic performance and acceptability of study products, the subjects had to complete a validated self-assessment questionnaire when they visited the study centre on days 2, 7 and 14. The questionnaire design was based on the guidance provided by the American Society for Testing and Materials (ASTM, 2016).

For the assessment of cosmetic performance, the subjects had to rate various statements on a numerical scale ranging from 1 to 5 (1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree). The questionnaire specified the following performance parameters: overall suitability for aftercare of tattooed skin, convenience of application, presence of unpleasant residues, protection and comfortability of tattooed skin, characteristics related to soothing, smoothing, softening and moisturisation, and appearance of the tattoo. Acceptability was determined by asking the question: "What is your overall opinion of each of the products you tested?" Predefined rating options were: "Liked very much", "Liked somewhat", "Neither liked nor disliked", "Disliked somewhat" and "Disliked very much". Frequency and severity of local/systemic adverse events (AEs) were assessed by the investigator at every visit at the study centre, which included the evaluation of diaries that the subjects had to complete at home.

Statistical methods

The temporal pattern of TEWL after tattooing was described for both the test area treated with Product A and the test area treated with Product B. Repeated measures analysis of variance (ANOVA) was conducted to test if there was any difference between the mean TEWL values determined at baseline (i.e. after tattooing but before first product application), day 1 (1 hour after baseline assessment), day 2, day 7 and day 14. The Dunnett's two-tailed t-test was used to identify any significant mean change in TEWL between baseline and post-baseline measurements. For all tests, the level of significance was set at 0.05. Data collected by means of the questionnaire were assessed descriptively for Products A and B. All statistical analyses were performed using SPSS (version 22, IBM, Armonk, NY).

No formal sample size calculation was performed. It was planned to have at least 50 subjects evaluable for TEWL changes. Based on historical data from TEWL studies in healthy subjects outside the tattoo setting, it was expected that scientifically meaningful results can be obtained with the selected sample size (Gehring and Gloor, 2000; Proksch and Nissen, 2002; Stettler et al, 2017).

RESULTS

In total, 67 healthy Caucasian subjects were enrolled and 56 (32 females, 24 males) completed the study. The mean age of study completers was 37 years (range: 18-57 years). Eleven study participants prematurely discontinued the study for personal reasons unrelated to study procedures. Moreover, two subjects were excluded from TEWL analysis due to non-compliance with study product application on the tattooed areas: One subject applied ≤6% of the dispensed study products (Products A and B) and one subject applied a topical preparation different from the study products. The most frequently selected locations for tattooing were the lower arm and leg.

Transepidermal water loss

Tattooing caused a pronounced skin barrier dysfunction as reflected by an approximately 7-fold increase in mean TEWL for the tattooed test areas at baseline when compared with the non-tattooed test areas at screening (Product A: 79.14 vs. 11.55 g/m²/h; Product B: 76.84 vs. 11.37 g/m²/h). Upon topical use of both Product A and Product B on the freshly tattooed skin areas, a substantial reduction in TEWL was observed over the 14-day measurement period without discernible differences between study products *[Figures 1* and *2]*. For both products, mean TEWL values determined over time were significantly different in the ANOVA analysis ($P<0.001$ each). By day 14, mean TEWL decreased to approximately 17 g/m²/h (Product A: 16.51 g/m²/h; Product B: 17.88 g/m²/h), which was close to the TEWL values assessed on non-tattooed areas at the screening visit. The decreases in TEWL reflected improvements in skin barrier function.

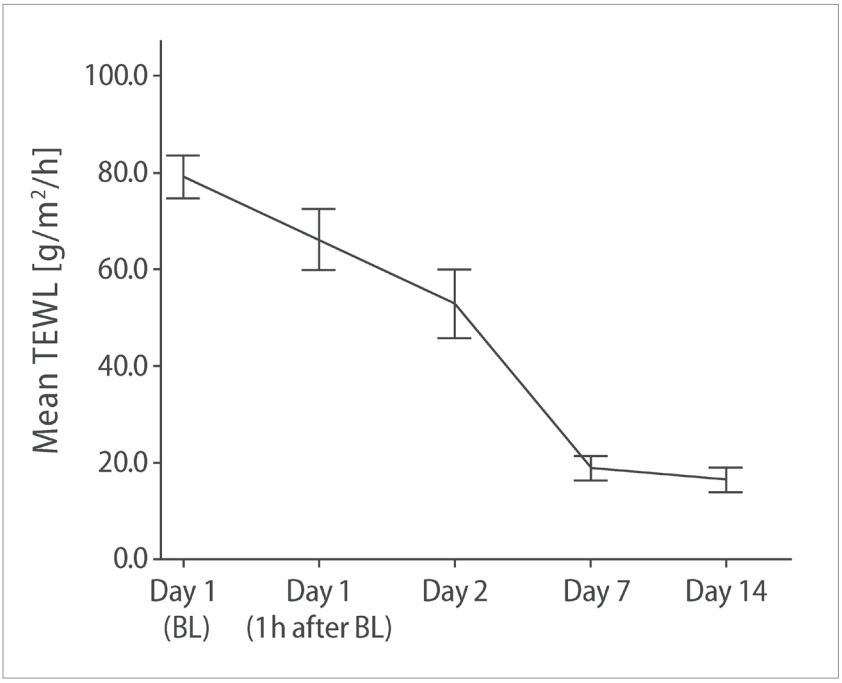

Figure 1. *Mean (±95% confidence interval) TEWL following topical application of Product A (ointment containing 5% dexpanthenol) on freshly tattooed skin 4-8 times daily for 14 days. BL = Baseline assessment at approximately 4 hours after the tattooing session and immediately before treatment initiation.*

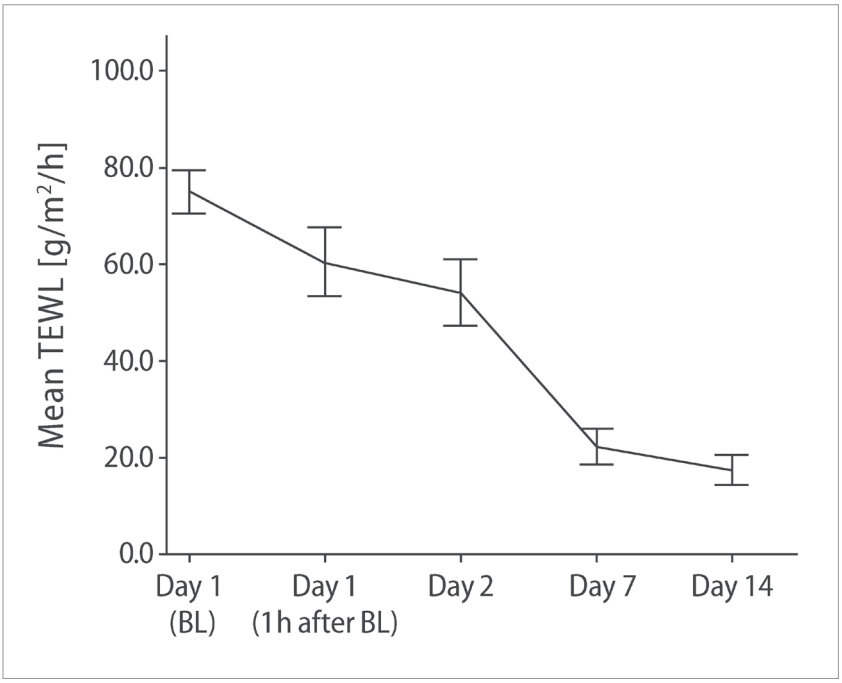

Figure 2. *Mean (±95% confidence interval) TEWL following topical application of Product B (emulsion containing 5% dexpanthenol) on freshly tattooed skin 4-8 times daily for 14 days. BL = Baseline assessment at approximately 4 hours after the tattooing session and immediately before treatment initiation.*

Table 1 shows TEWL results assessed over the study course in terms of changes from baseline. Following the tattooing session, application of both Product A and Product B was associated with a substantial recovery of the skin barrier as reflected by the negative values for TEWL change from baseline. For both products, the mean change from baseline was statistically significant at all post-baseline assessments. In addition, the temporal pattern of TEWL changes from baseline was comparable between Products A and B.

Self-assessment questionnaire

The performance of both products was appreciated by the overwhelming majority of study participants. The statements related to cosmetic performance were consistently rated as 5 (strongly agree) or 4 (agree) by at least 88%–90% of subjects at all assessments, thereby suggesting that both products perform favourably with regard to convenience of application, presence of unpleasant residues, protection and comfortability of tattooed skin, as well as soothing, smoothing, softening and moisturisation features, and appearance of the tattoo. Over the entire course of the study, 95%–98% of subjects strongly agreed or agreed with the overall statement that Product A is suitable for aftercare of tattooed skin; for Product B, 93%–96% strongly agreed or agreed with this statement. Similar results were obtained for the acceptability of study products. Specifically, on days 2, 7 and 14, 93%, 98% and 96%, respectively, of subjects liked very much or liked somewhat Product A; for Product B, the respective proportions were 93%, 95% and 95%.

Tolerability

Over the study course, the local reaction caused by the tattooing session improved as a reflection of skin healing. All subjects showed an uncomplicated healing process of the skin areas affected by the new tattoos. In particular, there were no bacterial infections. For both study products, no AE considered unrelated to the tattooing execution was recorded. Following application of Product A, 7 subjects just reported discomfort sensations (mostly stinging) for up to 15 minutes during study days 1–3. Similarly, after administration of Product B, 6 subjects reported discomfort sensations (stinging, heat and/or tightness) for up to 20 minutes duration during study days 1–3, except one subject who reported a slight and transient sensation of tightness on day 7.

DISCUSSION

The quantification of TEWL is one of the most

reliable methods to assess the functionality of the skin barrier, which is primarily located in the stratum corneum (Bouwstra and Ponec, 2006; Antonov et al, 2016). Following a tattooing session, the stratum corneum skin barrier is damaged and needs restoration. This single-blind prospective 2-week study in healthy subjects investigated the temporal course of skin barrier restoration (based on TEWL data) in freshly tattooed skin areas upon topical use of 5% dexpanthenol water-in-oil products. Tolerability, performance and acceptability of the two products were also studied. To the best of the authors' knowledge, this is the first study that has assessed TEWL on newly tattooed human skin.

The findings of this study can be summarised as follows: (1) tattooing caused a pronounced skin barrier dysfunction as reflected by an approximately 7-fold increase in mean TEWL; (2) the use of both 5% dexpanthenol water-in-oil formulations (ointment and emulsion) was associated with a virtually complete barrier restoration when applied 4-8 times daily over 14 days; (3) the performance and acceptability of both products were favourably rated by the overwhelming majority of subjects; and (4) both products were well tolerated when administered on freshly tattooed skin for 14 days.

The average TEWL value quantified on non-tattooed areas at the screening visit of our study (~11.5 g/m^2/h) is in the same ballpark as previously reported TEWL data, which were assessed by the same method and under virtually identical experimental conditions on unchallenged skin of healthy subjects (~6-7 g/m^2/h) (Stettler et al, 2017). However, the observed increase in TEWL following tattooing is markedly greater than the one measured by Stettler et al (2017) after experimentally inducing skin barrier dysfunction (SDS challenge). Specifically, sodium dodecyl sulfate (SDS) challenge caused an approximately 3-fold increase in mean TEWL compared with unchallenged skin of healthy subjects, while in this study the execution of a tattoo was associated with a 7-fold increase. This may be explained by the more traumatic process of tattooing than the topical application of a skin irritant (SDS).

In the aforementioned study by Stettler et al (2017), it took approximately 3 weeks until TEWL had normalised (i.e. the skin barrier was fully restored) in the skin areas challenged with SDS and left untreated with a topical care product. After 7 days, there was just a 26% reduction in mean TEWL from baseline (i.e. time point immediately after SDS challenge).

On the contrary, the authors observed in the current study that the reduction in mean TEWL from baseline to day 7 was as high as 76% and 70% for the tattooed areas treated with Product A and Product B, respectively. Irrespective of the product applied, an almost complete barrier restoration was achieved for the tattooed areas by day 14. Our observations are particularly noteworthy as skin barrier damage at baseline was more pronounced in the present study than the one reported by Stettler et al (2017). Our results indicate that the topical use of both Product A and Product B accelerated skin barrier restoration following tattooing. It has already been previously shown that the topical application of 5% dexpanthenol-containing formulations enhances skin barrier repair in experimentally damaged human skin (Proksch and Nissen, 2002; Stettler et al, 2017; Proksch et al, 2017). The mechanism of action by which dexpanthenol restores skin barrier function has not fully been elucidated, but there is evidence that dexpanthenol generates a hydrated environment which facilitates enzyme functioning necessary for skin barrier repair (Proksch et al, 2017). In addition, dexpanthenol promotes epidermal regeneration of wounded skin by enhancing epidermal differentiation and lipid synthesis (Giménez-Arnau, 2016).

The performance and acceptability of both study products were favourably rated over the course of the study by at least 88%–90% of study participants. Apart from the physical properties of the two water-in-oil formulations, providing protection and comfortability of the tattooed skin, the moisturisation potential of dexpanthenol (Proksch et al, 2017), that keeps fresh tattoos hydrated and thus prevents itching, may have led to the observed high ratings. A dexpanthenol-related expedited healing process may have further contributed to the high acceptability of study products. Not only that moistened wounds heal generally faster (Tucker, 2012), dexpanthenol also facilitates wound healing as a result of enhanced fibroblast activity (Oguz et al, 2015). In fact, in different models of superficial injury, topical dexpanthenol has been shown to improve the wound-healing process (Proksch et al, 2017).

With regard to safety, both study products applied on the new tattoos were well tolerated, thereby confirming anecdotal reports that adverse reactions due to topical dexpanthenol are rarely observed during aftercare of tattoos (Bregnbak et al, 2016).

A limitation of the study is the absence of a placebo or 'no treatment' arm. However, to withhold tattoo aftercare was considered unethical.

Conflict of interest statement
Anna Macura-Biegun, Peter Kurka and Sonja Trapp are employees of Bayer Consumer Care AG, Basel, Switzerland. The other authors report no conflicts of interest.

CONCLUSION

Both 5% dexpanthenol water-in-oil formulations investigated in this study are considered suitable for aftercare of tattooed skin. Their use was associated with a virtually complete skin barrier restoration — as assessed by TEWL measurements — when applied 4–8 times daily over 14 days, and was well tolerated. The cosmetic performances were well perceived by the overwhelming majority of subjects leading to a high acceptability of both products. All subjects showed an uncomplicated healing process of the skin affected by the new tattoos. The results of this study provide scientific evidence for the integration of a 5% dexpanthenol water-in-oil formulation into the aftercare of tattooed skin. The tested products represent adequate options in this setting and may contribute to a low incidence of tattoo complications. WINT

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