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SYMBIO® DERMAL

New Microbiotic Care with Bacterial Lysate against Dry Skin

H-J. Müller, E. Jaspers, A. Hecht

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abstract

This application study examined *in vivo* the effect of a microbiotic care product (**SYMBIO®** DERMAL) on dry skin prone to atopic dermatitis. The microbiotic care contained a complex of lysed, non-pathogenic *Escherichia coli* and *Enterococcus faecalis* bacteria additionally to nourishing substances. It thus follows the "emollients plus"-principle as described in the current European guidelines for treatment of atopic dermatitis [5]. Before and after applying the microbiotic care for four weeks, the following tests were carried out on defined skin testing areas: **(i)** determination of the transepidermal water loss (TEWL); **(ii)** determination of hydration, **(iii)** evaluation of dryness/flaking by *in vivo* touching evaluation, **(iv)** clinical-dermatological assessment with final questionnaire and **(v)** patch safety test. As shown after four weeks of application, the microbiotic care reduced transepidermal water loss by an average of around 22%, increased skin moisture by an average of almost 50% and reduced dryness/flaking by an average of almost 50%. Consequently, the microbiotic care supported the barrier function of dry skin. Previously performed *in vitro* studies also proved immunomodulatory properties of the bacterial lysate complex. According to clinical-dermatological test criteria, the microbiotic care was very well tolerated. It received the 5-star-rating ("clinically tested" &"very good") from Dermatest.

1. Introduction

The human skin is one of the largest and most versatile organs in the human body. On the one hand, it has barrier function and prevents invasion of harmful substances like toxins and pathogenic microorganisms. Additionally, the human skin helps to maintaining body's water balance.

Epidermal lipids (horn fats) seal the intercellular spaces between the keratinocytes and form a diffusion barrier. The density of this lipid- and cell network is a basic requirement for a healthy and resilient skin that retains sufficient moisture. Impaired horn fats promote water loss and subsequent xerosis. Dry skin solely can already promote inflammation, for instance the "eczéma craquelé" in elderly people [4]. Additionally, a disturbed skin barrier sets the course for atopic dermatitis (neurodermatitis).

The physiological skin microbiota supports barrier function: By providing colonization resistance, the microbiota protects the skin from microbial infections. The skin microbiota also interacts with the immune system of the host. For instance, *Staphylococcus epidermidis* inhibits the release of proinflammatory cytokines from keratinocytes [2]. *S. epidermidis* also increases gene expression of antimicrobial peptides like human β-defensin 2 [2] and stimulates resident skin T-lymphocytes [3]. *S. epidermidis* thereby enhances the skin's immune defense and thus contributes to skin protection and health.

In most skin dysfunctions and diseases, the skin microbiota has changed. In atopic dermatitis, for example, more pathogenic *Staphylococcus aureus* colonise the skin, which can cause infections. At the same time, the skin produces fewer antimicrobial peptides, which promotes the infection process [1].

This application study examines the effect of a new microbiotic skincare on dry skin prone to neurodermatitis. In addition to different lipids, which act as emollients, the product contains inactivated (lysed) apathogenic *Escherichia coli* and *Enterococcus faecalis*. As shown by preliminary *in vitro* studies, the bacterial lysate complex can modulate immunological skin reactions.

The new microbiotic skincare follows the new principle "emollients plus" in the basic care for atopic dermatitis as described in the current European guideline for the treatment of atopic dermatitis [5]: Active components like plantlet extracts or bacterial lysates are added to the emollients [5]. The lysates of certain bacteria can improve lesions and influence the skin microbiota as well as the skin's immune system [5,9].

2. Material & Methods

2.1 Skincare products

We tested a new type of water-in-oil emulsion (cosmetic Symbio® DERMAL, SymbioPharm GmbH). Beside other skincare ingredients, it contained lysed *Escherichia coli* (*E. coli*)

and *Enterococcus faecalis* including their metabolites produced up to their lysis.

Ingredients: *E. coli*/*Enterococcus faecalis* fermented lysate, caprylic/capric triglyceride, squalane, pentylene glycol, glycerine, Simmondsia chinensis oil, Prunus Amygdalus Dulcis oil, Cetyl PEG/PPG-10/1 dimethicone, Persea Gratissima castor oil, Oenothermal oil Panthenol, Butyrospermum Parkii Butter, Tocopheryl Acetate, Cera Alba, Aqua, Betaine, Cetyl Palmitate, Sodium Gluconate, Sodium Hyaluronate, Sodium Lactate, Ceramide-NP, Cholesterol, Glyceryl Dibehenate, Phytosphingosin Acid, Tocopherol, Ceramide 6 II.

For comparison and control, the identical skincare without bacterial lysate and untreated control areas on the left forearm were used, respectively.

2.2 Application study

Institutions involved

- **RSC Pharma GmbH & Co. KG,** Gleibergring 23, 35396 Gießen, Germany (contract researcher),
- **Dermatest® GmbH**, Engelstrasse 37, 48143 Münster, Germany (assessor),
- **SymbioPharm GmbH**, Auf den Lüppen 10, 35745 Herborn, Germany (product development)

Study participants

20 male and female adults between the ages of 23 and 66 years meeting the inclusion criterion were enrolled **(Table 1** (see Annexe page 1)**)**. The inclusion criterion was very dry skin or skin prone to neurodermatitis, but not in need of medical treatment. In the test and control areas, all test subjects initially showed dry or very dry, sometimes atopic skin.

Exclusion criteria were severe or chronic skin inflammation; severe internal or chronic diseases; medication that might affect skin reaction such as glucocorticoids/antiallergics/topical immunomodulators; application of skincare products or substances containing active ingredients 7-10 days before start of studies, severe allergies or any previous severe side effects from cosmetics; sunbathing or solarium visits during the study, cancer.

Study design

Preliminary *in vitro*-studies (cytotoxicity test prior to marketing authorization according to ISO 10993 and "Skin Irritation Test" using a reconstituted epidermis (Epi Derm™)) had confirmed the safety of the bacterial lysate (unpublished data).

The Ph. Eur. 5.1.-test for adequate antimicrobial preservation passed the microbiotic care with "Criterion A". The microbiotic care showed *in vitro* inhibitory effects on several pathogens, including *Staphylococcus aureus* (unpublished data).

The bacterial lysate modulated *in vitro* the release of cytokines Interleukin (IL)-6 and IL-8 in a TNF-α provoked inflammation of epidermal skin cells (HaCaT cell line).

The subsequent application study included:
(i) determination of the transepidermal water loss (TEWL),
(ii) determination of hydration
(iii) assessment of dryness/flaking,
(iv) clinical-dermatological evaluation with final questionnaire and
(v) patch safety test.

Each subject had a dry, partially atopic test area on the extremities of each body half **(Table 1** (see Annexe page 1)**)**. The subjects applied the microbiotic care to the test areas on the right body half. The product without bacterial lysate was applied to the test areas on the left body half. Both occurred daily in the morning and evening for four weeks. The application was double-blinded. The control areas remained untreated. The use of any other products in the test areas was prohibited.

2.2.1 Determination of the transepidermal water loss (TEWL)

Transepidermal water loss (TEWL) is a measure of skin barrier function. The evaporimeter probe with two sensors was used to measure the vapor pressure gradient arising within the chamber and between the skin and the surrounding air. TEWL in the test areas was measured using the Tewameter® evaporimeter (Courage + Khazaka electronic GmbH). Decreases in TEWL indicate an improvement in skin barrier function such that less water is lost through the skin barrier.

Both test areas as well as the control areas had a diameter of approximately 3 cm each. 20 TEW measurements were taken at each test area, out of which an average value was calculated.

Measurements were performed before and after the four-week application period.

2.2.2 Determination of skin hydration

Changes in skin capacitance were used to study epidermal hydration *in vivo*. The Corneometer CM 825 (Courage and Khazaka electronic GmbH) was used to measure the electrical capacitance of the skin. Prior to the measurement, the test subjects were standardized for 45 min in a 22°C room

with a relative humidity of 60%. Measurements were performed before and after the four-week application period, ten to twelve hours after product application. Three replicate measurements were taken on three different points within each test and control area, which each had a diameter of approximately 3 cm. Out of each of the three measurements an average value was calculated.

2.2.3 Evaluation of dryness and flaking

Specially trained dermatologists assessed the parameters dryness and flaking visually and by touching the skin (*in vivo*-touching evaluation). For this, they used an analog scale that ranged from "no intensity" (0.00) to "maximum intensity" (100.00). Intensity 0 corresponds to excellent skin conditions without any dryness or flaking. Very dry, flaky skin has an intensity of 100 **(Figure 1).** The evaluations were performed before and after the four-week application period. The diameter of the test areas was extended to approximately 5 cm. Control areas were not considered as this evaluation method by default only assesses treated skin.

Fig.1 Analog scale for the evaluation of dryness and flaking

2.2.4. Clinical-dermatological assessment with final questionnaire

Before and after the four-week application period, the subjects were dermatologically examined. Additionally, each subject could daily refer to the dermatologists if necessary.

Dermatological assessment criteria were as follows:
(i) redness,
(ii) flaking and
(iii) dryness.

At the end of the study, each subject completed two questionnaires, each for the microbiotic skincare and the lysate-free care. Here, they answered general question about the skincare products and about how the skin subjectively felt after applying the products **(Tables 5a, 5b** (see Annexe page 10) **and Figure 5a)**.

2.2.5 Patch safety test

After four weeks of application; the skincare products were applied in concentrations of 5 mg/15µL to patch test strips (Curatest® F Adhesive Strip, Lohmann & Rauscher GmbH & Co. KG, REF 30062). Two discs remained uncoated (control), one disc was coated with the microbiotic care, another disc was coated with the skincare without bacterial lysate. The strips were applied to healthy skin of the upper back.

Under standardized lighting, evaluation of possible skin reactions was performed after 24, 48 and 72 hours of exposition 30 min after the tapes were detached.

3. Results

3.1 Transepidermal water loss (TEWL)

After four weeks of application, the microbiotic care had reduced TEWL by 26,7% on average, compared to the start. The skincare without bacterial lysate reduced TEWL by 17,75% on average. In control areas, TEWL was reduced by 4,85% on average **(Tables 2a, 2b and 2c** (see Annexe page 2) **and Figure 2)**.

3.2. Hydration

After four weeks of application, the microbiotic care increased skin moisture by 51,37% on average, compared to the start. The skincare without bacterial lysate increased skin moisture by 53,67%. In control areas, skin moisture increased by 5,23% on average. **(Tables 3a, 3b and 3c** (see Annexe page 5) **and Figure 3)**.

3.3. Dryness and flaking

After four weeks of application, the microbiotic care improved dryness and flaking by 47,94% on average, compared to the start. The skincare without bacterial lysate improved dryness and flaking by 46,46% on average **(Tables 4a and 4b** (see Annexe page 8) **and Figure 4)**.

3.4. Clinical-dermatological assessment with final questionnaire

No irritation or sensitivity effects were reported in any subject during and after the four-week application period. Correspondingly, none of the subjects consulted the dermatologist. No test interruptions or medical treatments were needed. The microbiotic skincare as well as the skincare without bacterial lysate were very well tolerated.

In the finale questionnaire, the subjects adressed several criteria related to the microbiotic skincare and the skincare without bacterial lysate. The criteria were as follows: consistency, skin feeling, relaxation of skin tension, spreadability,

tolerance, suitability for sensitive skin, skin soothing and suitability for very dry skin prone to neurodermatitis. The majority of the subjects rated both skincare products in the criteria-order listed as "exactly right", "(very) pleasant", "I (fully) agree", "(very) quickly absorbed", "very good and good", "I totally/tend to agree" and "I totally/tend to agree". (details see **Tables 5a, 5b** (see Annexe page 10) **and Figure 5a**)

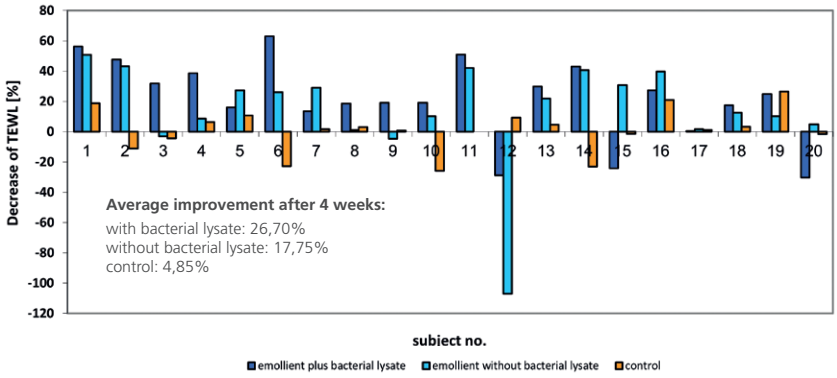

Fig. 2 Decrease in transepithelial water loss (TEWL) after four weeks of applying the microbiotic care and the skincare without bacterial lysate.

3.5. Patch safety test

The patch test results did not reveal any sensitivity or irritation from the test products – neither in the test areas nor in the control areas **(Tables 6a, 6b, 6c** (see Annexe page 18)**)**.

It received the 5-star rating ("clinically tested" & "very good") from Dermatest.

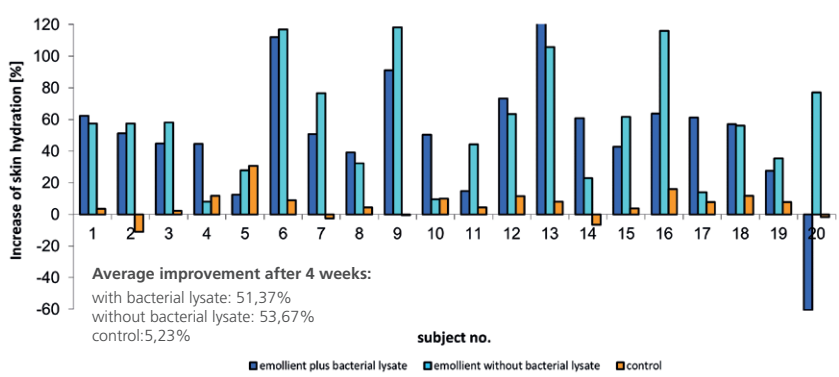

Fig. 3 Increase in skin hydration after four weeks of applying the microbiotic care and the skincare without bacterial lysate.

4. Discussion

Altered skin conditions are often associated with an impaired quality of the patient's daily life. Beside skin diseases like atopic dermatitis or acne also dry skin is problematic. Patients need to apply special skincare to prevent exacerbation of dryness and flaking and to maintain skin's barrier function.

Filaggrin gene mutations are the highest risk factor for developing atopic dermatitis [7]. The protein filaggrin is part of the *stratum corneum* and helps to connect the keratinocytes with each other. Then the skin barrier is intact and the *stratum corneum* is sufficiently hydrated. However, mutated filaggrin genes do not produce enough filaggrin molecules. In consequence, the skin's barrier function is disturbed which increases transepidermal water loss. This promotes dry skin and eczema [7]. Dry skin is also likely to promote penetration of allergens that lead to allergenic sensitization, asthma and hay fever [7]. Reducing skin dryness is the key to reduce the frequency, duration and severity of inflammation in atopic dermatitis [7].

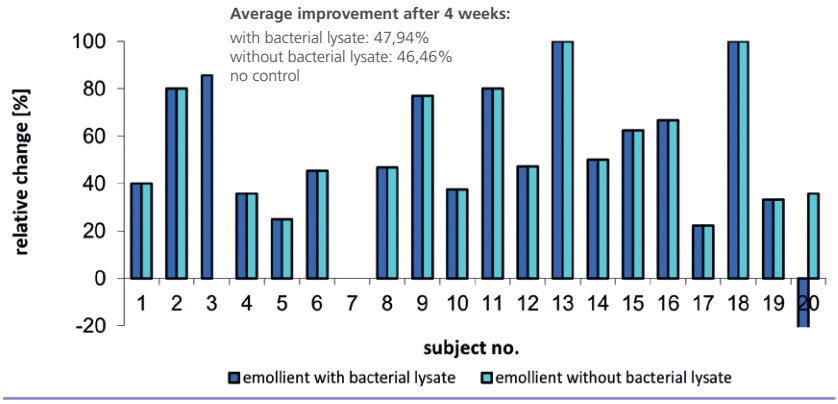

Fig. 4 Decrease in skin dryness and flaking after four weeks of applying the microbiotic care and the skincare without bacterial lysate. An assessment of untreated skin areas as a control was not performed.

The current European guideline for the treatment of atopic dermatitis describes emollients as "extremely helpful for AE (atopic eczema = atopic dermatitis) patients". It recommends using emollients twice a day to reduce skin dryness [5,7].In recent years, so called "emollients plus" products for topical treatment have been developed. Beside emollients they also contain active components. The current European guideline recommends emollients plus for the basic therapy of atopic dermatitis. Often these products are neither fulfilling the definition of nor needing a license as a topical drug [5].

They are cosmetics such as Aderma Exomega Control and Avene XeraCalm A.D. They contain plant saponins, flavonoids and riboflavins or bacterial lysates from *Aquaphilus dolomiae* or *Vitreoscilla filiformis* [5] as active components. In general, live bacteria as especially the lactid acid bacteria *Lactobacillus paracasei*, *L. brevis* and *L. fermentum* influence the skin positively. They support growth of the health promoting skin-bacterium *Staphylococcus epidermidis* and inhibit growth of undesired skin-bacteria like *Staphylococcus aureus*. In cosmetics, however, the use of inactivated bacteria as lysates or extracts is more common [1]. *Aquaphilus dolomiae* and *Vitreoscilla filiformis* extracts have been scientifically investigated for beneficial properties for skin health. *In vitro* studies and clinical research have shown their immunomodulatory properties. For example, the modulate the release of interleukin-8 or induce the expression of human beta-defensin (HBD)-2 [8,9]. Many human epithelial tissues produce beta-defensins. They fight pathogenic bacteria without eliciting inflammatory immune responses. The skin epithelia express HBD-1 constitutively; HBD-2 is additionally induced when inflammation occurs [1]. Keratinocytes increase their HBD-2-expression when exposed to *Staphylococcus aureus* [1]. In atopic dermatitis, high levels of HBD-2 in the *stratum corneum* are associated with impaired skin barrier and the severity of the disease [12]. They are "fight indicators", demonstrating how the skin epithelial cells fight potentially pathogenic bacteria of an aberrant skin microbiota. Therefore, substances which promote HBD-2-formation support the epithelial immune defense.

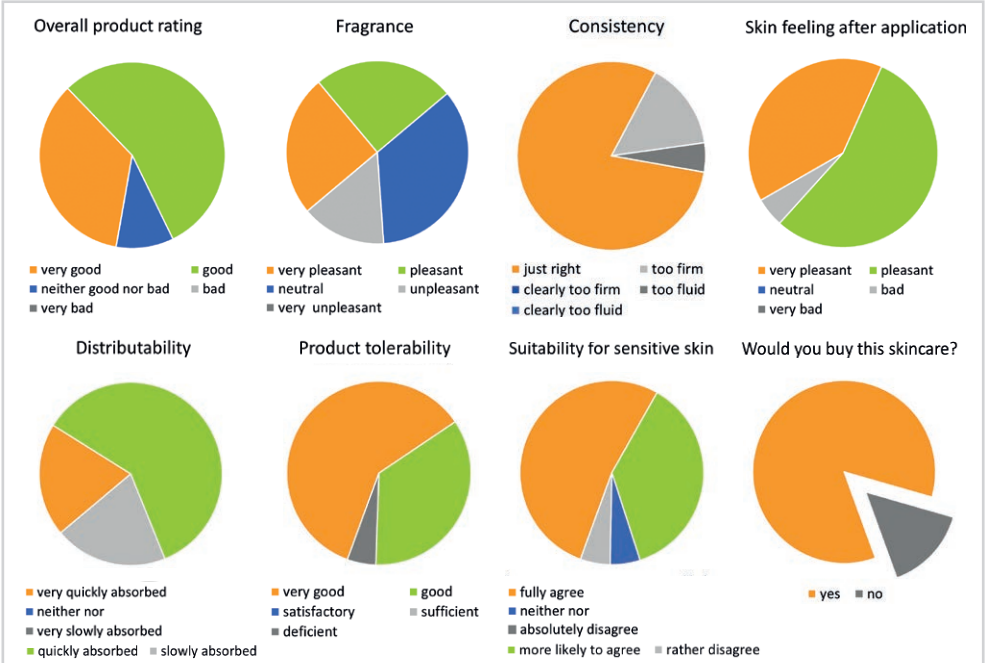

Fig. 5a Results (graphics) of the questionnaire on the application of the microbiotic care (right half of the body) - corresponding to **Table 5a** (download at https://www.sofw.com/images/a-symbio-2106-e.pdf).

This application study shows that a complex of lysed, non-pathogenic *E. coli* and *Enterococcus faecalis*-bacteria helps to improve dry skin or skin prone to neurodermatitis: 40% of the TEWL-reduction was achieved by the bacterial lysate complex. The nourishing ingredients brought about the further 60% of the TEWL-reduction. TEWL is one of the most important parameters to evaluate the epidermal permeability barrier function of the skin. Low values of this parameter are the main characteristic of the healthy skin which is capable of retaining moisture. Consequently, substances which reduce TEWL improve epidermal barrier function of the skin.

The nourishing ingredients exhibited further positive effects: Within for weeks, they decreased skin's dryness and flaking, while in parallel skin moisture was increased. Consequently, the new microbiotic skincare supports the epidermal skin barrier of dry skin and skin prone to neurodermitis, following the new principle of "emollients plus". The risk factor "impaired epidermal skin barrier" for developing atopic dermatitis is therefore reduced.

Beside an impaired skin barrier, an immunological imbalance is believed to contribute to the inflammatory lesions of atopic dermatitis [7]. Especially in the case of acute excema inflammation is strong [7]. The close interaction between microbes and human immune system is known for the intestinal microbiota and also occurs between the skin microbiota and the skin's immune system [1]. Inactivated bacteria (lysates or extracts) can possibly contribute to the immunomodulatory effects of the skin microbiota. The complex of lysed, non-pathogenic *E. coli* and *Enterococcus faecalis* bacteria used in Symbio® DERMAL exhibited *in vitro* immunomodulatory capacities like modulating cytokine-responses. This might support improvement of skin conditions. As one example, the lysed *E. coli* and *Enterococcus faecalis*-bacteria reduced the interleukin-8 release, much like lysed *Aquaphilus dolomiae* [8] does. Interleukin- 8 is considered to be a mediator of inflammation in psoriasis [11]. Interleukin-8 recruits neutrophils and promotes their degranulation [10]. Therefore, the lysed *E. coli* and *Enterococcus faecalis* used in this study can probably have a positive effect on the risk factor "immunological imbalance", which increases susceptibility to inflammation.

Beside health effects, tolerance is another important aspect for daily use of a skincare product for dry skin. According to clinical-dermatological test criteria, the microbiotic care was very well tolerated. No irritation or sensitivity effects were reported. The subjects were very content: As shown in the results of the questionnaires, 85% of the subjects would buy the microbiotic care.

5. Conclusion

According to dermatological assessments, the microbiotic care Symbio® DERMAL supports dry skin prone to neurodermatitis in multiple ways: It increases skin moisture, reduces dryness and flaking and – with special contribution of the lysed *E. coli* - and *Enterococcus faecalis* - bacterial strains - reduced TEWL. As shown in preliminary *in vitro* studies, the complex of lysed bacterial strains can modulate the immune system to promote antiinflammatory reactions. Consequently, the newly developed microbiotic skincare has positive impacts on a disturbed skin barrier and probably also to an inflammatory immunological imbalance - two risk factors for the development of atopic dermatitis.

Conflict of interest:

- Dr. Hans-Jörg Müller is employed as Director Business Development by the SymbioPharm GmbH.
- Dr. Elke Jaspers (mikroLogos GmbH) is working as scientic consultant for the SymbioPharm GmbH.
- Angelika Hecht is employed in the Public Relation Department of the SymbioPharm GmbH.

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Annex

1. Test group

Table 1: Gender, age, skin type of the 20 subjects

Subject No.	Gender	Age	Skin	Location of the test area (one each per body half)	Location of the control area (one area)*
1	In	65	dry/sensitive	shin	left forearm
2	In	60	dry/sensitive	shin	left forearm
3	In	25	combination skin/sensitive	shin/wrest	left forearm
4	m	62	dry/sensitive/atopic	arm, leg	left forearm
5	In	41	dry/sensitive	leg	left forearm
6	In	56	dry/sensitive	elbow	left forearm
7	In	64	dry/sensitive	arm	left forearm
8	In	27	dry/sensitive	shin	left forearm
9	In	45	dry/sensitive/atopic	leg	left forearm
10	In	48	dry/sensitive	arm, abdomen, leg	left forearm
11	m	50	dry/sensitive/atopic	shin	left forearm
12	In	46	dry/sensitive	shin	left forearm
13	In	63	dry	shin	left forearm
14	In	66	dry/sensitive	shin	left forearm
15	In	24	dry/sensitive/atopic	shin	left forearm
16	In	53	dry/sensitive	shin	left forearm
17	In	46	very dry	arm	left forearm
18	In	23	very dry/sensitive	elbow	left forearm
19	In	34	dry/atopic	elbow	left forearm
20	In	37	dry	hand	left forearm

*** Exception:** When determining dryness/flaking with *in vivo* touching-evaluation, controls were not performed (details see Material & Methods).

2. Transepidermal Water Loss (TEWL)

Tables 2a, **2b** and **2c** show the individual measurement results of the transepidermal water loss in g/hm^2 in the control or test areas of the 20 subjects. The differences between the TEWL values at the start of the application and the end of the application are also reported. Negative values result from decreasing transepidermal water loss during the test period.

Table 2a: Test area of the **right** half of the body under the application of the **microbiotic care**: TEWL results of its twice-daily use before and after four weeks of application

Proband No.	Test area under special microbiotic care before	after 4 weeks	Difference	% change
1	19,5	8,5	-11	-56,41
2	13	6,8	-6,2	-47,69
3	21,3	14,5	-6,8	-31,92
4	27,7	17	-10,7	-38,63
5	18,6	15,6	-3	-16,13
6	17,1	6,3	-10,8	-63,16
7	16,2	14	-2,2	-13,58
8	16	13	-3	-18,75
9	27,3	22,1	-5,2	-19,05
10	19,8	16	-3,8	-19,19
11	14,1	6,9	-7,2	-51,06
12	5,9	7,6	1,7	28,81
13	19,7	13,8	-5,9	-29,95
14	18,3	10,4	-7,9	-43,17
15	15	11,4	-3,6	-24
16	17,6	12,8	-4,8	-27,27
17	16,2	16,1	-0,1	-0,62
18	18,3	15,1	-3,2	-17,49
19	13,2	9,9	-3,3	-25
20	16,2	21,1	4,9	30,25
Average	17,6	12,9	-4,7	-26,7
Minimum	5,9	6,3	-11	-63,16
Maximum	27,7	22,1	4,9	30,25
Standard deviation	4,8	4,5	4	24,21
variance	22,6	20,4	16,3	586,03

Table 2b: Test area of the **left** half of the body using the **skin care without bacterial lysate**: TEWL results of its twice-daily use before and after four weeks of application

No.	Test area under comparative product before	after 4 weeks	Difference	% change
1	17,9	8,8	-9,1	-50,84
2	11,1	6,3	-4,8	-43,24
3	23.0	23,7	0,7	3,04
4	22,8	20,8	-2,0	-8,77
5	20,8	15,1	-5,7	-27,40
6	14,1	10,4	-3,7	-26,24
7	18,8	13,3	-5,5	-29,26
8	16,2	16,0	-0,2	-1,23
9	17,4	18,2	0,8	4,60
10	18,5	16,6	-1,9	-10,27
11	15,4	8,9	-6,5	-42,21
12	5,8	12,0	6,2	106,90
13	19,1	14,9	-4,2	-21,99
14	17,9	10,6	-7,3	-40,78
15	14,3	9,9	-4,4	-30,77
16	16,3	9,8	-6,5	-39,88
17	16,9	16,6	-0,3	-1,78
18	18,3	16,0	-2,3	-12,57
19	17,5	15,7	-1,8	-10,29
20	16,0	15,2	-0,8	-5,00
Average	**16,9**	**13,9**	**-3,0**	**-17,75**
Minimum	5,8	6,3	-9,1	-50,84
Maximum	23,0	23,7	6,2	106,90
Standard deviation	3,8	4,4	3,5	33,25
Variance	14,6	19,0	12,5	1105,40

Table 2c: Control area: TEWL results before and after four weeks

Proband No.	Control area before	after 4 weeks	Difference	% change
1	13,8	11,2	-2,6	-18,84
2	7,2	8	0,8	11,11
3	9,4	9,8	0,4	4,26
4	15,5	14,5	-1	-6,45
5	14,7	13,1	-1,6	-10,88
6	7,5	9,2	1,7	22,67
7	11,2	11	-0,2	-1,79
8	9,5	9,2	-0,3	-3,16
9	13,5	13,4	-0,1	-0,74
10	7,8	9,8	2	25,64
11	7,2	7,2	0	0
12	7,5	6,8	-0,7	-9,33
13	6,2	5,9	-0,3	-4,84
14	9,6	11,8	2,2	22,92
15	7,8	7,9	0,1	1,28
16	9	7,1	-1,9	-21,11
17	7,4	7,3	-0,1	-1,35
18	6,2	6	-0,2	-3,23
19	28,6	21	-7,6	-26,57
20	6,5	6,6	0,1	1,54
Average	10,3	9,8	-0,5	-4,85
Minimum	6,2	5,9	-7,6	-26,57
Maximum	28,6	21	2,2	25,64
Standard deviation	5,2	3,7	2,1	13,82
Variance	27	13,4	4,2	190,92

3. Determination of skin hydration with the corneometer

Skin moisture-values of the 20 test subjects averaged over three measurements and calculation of the differences between before and after are shown. The values are given in g/hm^2.

Difference = differences in skin hydration values

delta (%) = average percentage change in humidity due to the application, referenced to the initial value

Table 3a: **Skin hydration** before and after four weeks of applying the **microbiotic care**

Proband No.	Test area under special microbiotic care before	Test area after 4 weeks	Difference	Delta (%)
1	21,2	34,4	13,2	62,26
2	21,3	32,2	10,9	51,17
3	19	27,5	8,5	44,74
4	16,6	24	7,4	44,58
5	22,6	25,4	2,8	12,39
6	14,9	31,6	16,7	112,08
7	18,1	27,3	9,2	50,83
8	16,3	22,7	6,4	39,26
9	18,9	36,1	17,2	91,01
10	19,1	28,7	9,6	50,26
11	28,1	32,2	4,1	14,59
12	14,2	24,6	10,4	73,24
13	18,6	44,9	26,3	141,4
14	16,3	26,2	9,9	60,74
15	20,6	29,4	8,8	42,72
16	17,6	28,8	11,2	63,64
17	11,6	18,7	7,1	61,21
18	16,3	25,6	9,3	57,06
19	23,1	29,5	6,4	27,71
20	11,4	4,5	-6,9	-60,53
Average	**18,3**	**27,7**	**9,4**	**51,37**
Minimum	11,4	4,5	-6,9	-60,53
Maximum	28,1	44,9	26,3	141,4
Standard deviation	4	7,8	6,4	40,12
Variance	15,7	60,8	41,4	1609,75

Table 3b: **Skin hydration** before and after four weeks of applying the **skincare without bacterial lysate**

No.	Test area under comparative product before	after 4 weeks	Difference	Delta (%)
1,0	17,9	28,2	10,3	57,5
2,0	18,1	28,5	10,4	57,5
3,0	15,8	25,0	9,2	58,2
4,0	21,1	22,8	1,7	8,1
5,0	19,1	24,4	5,3	27,8
6,0	16,5	35,8	19,3	117,0
7,0	15,9	28,1	12,2	76,7
8,0	22,3	29,5	7,2	32,3
9,0	14,8	32,3	17,5	118,2
10,0	27,1	29,7	2,6	9,6
11,0	21,9	31,6	9,7	44,3
12,0	13,4	21,9	8,5	63,4
13,0	20,9	43,0	22,1	105,7
14,0	17,5	21,5	4,0	22,9
15,0	18,8	30,4	11,6	61,7
16,0	13,1	28,3	15,2	116,0
17,0	16,5	18,8	2,3	13,9
18,0	17,3	27,0	9,7	56,1
19,0	18,4	24,9	6,5	35,3
20,0	7,4	13,1	5,7	77,0
Average	**17,7**	**27,2**	**9,5**	**53,7**
Minimum	7,4	13,1	1,7	8,1
Maximum	27,1	43,0	22,1	118.24
Standard deviation	4,1	6,3	5,6	35,4
Variance	16,7	40,1	31,7	1252,9

Table 3c: **Skin hydration** before and after four weeks in the **control area**

Proband No.	Control area before	Control area after 4 weeks	Difference	Delta (%)
1	33,2	34,4	1,2	3,61
2	32,2	28,7	-3,5	-10,87
3	33,1	33,8	0,7	2,11
4	25,3	28,3	3	11,86
5	24,8	32,4	7,6	30,65
6	27,8	30,3	2,5	8,99
7	35,8	34,9	-0,9	-2,51
8	26,6	27,8	1,2	4,51
9	38,5	38,3	-0,2	-0,52
10	35,2	38,7	3,5	9,94
11	34,6	36,1	1,5	4,34
12	16,3	18,2	1,9	11,66
13	33,6	36,3	2,7	8,04
14	32,4	30,3	-2,1	-6,48
15	34,8	36,1	1,3	3,74
16	28,7	33,3	4,6	16,03
17	26,8	28,9	2,1	7,84
18	29,6	33,1	3,5	11,82
19	31,2	33,6	2,4	7,69
20	31,1	30,6	-0,5	-1,61
Average	**30,6**	**32,2**	**1,6**	**5,23**
Minimum	16,3	18,2	-3,5	-10,87
Maximum	38,5	38,7	7,6	30,65
Standard deviation	5	4,6	2,4	8,85
Variance	25,4	21,5	5,9	78,29

4. Evaluation of dryness/flaking by *in vivo*-touching evaluation

Table 4a: Intensities of **dryness/flaking** before and after application of the **microbiotic care** and calculation of the differences. Negative values indicate a decrease in dryness and flaking.

Proband No.	Microbiotic special care before	after 4 weeks	Difference	relative change (%)
1	50,00	30,00	-20	-40
2	75,00	15,00	-60	-80
3	70,00	10,00	-60	-85,7
4	70,00	45,00	-25	-35,7
5	60,00	45,00	-15	-25
6	55,00	30,00	-25	-45,5
7	70,00	70,00	0	0
8	75,00	40,00	-35	-46,7
9	65,00	15,00	-50	-76,9
10	80,00	50,00	-30	-37,5
11	50,00	10,00	-40	-80
12	85,00	45,00	-40	-47,1
13	35,00	0,00	-35	-100
14	60,00	30,00	-30	-50
15	80,00	30,00	-50	-62,5
16	75,00	25,00	-50	-66,7
17	90,00	70,00	-20	-22,2
18	50,00	0,00	-50	-100
19	90,00	60,00	-30	-33,3
20	70,00	85,00	15	21,4
Average	**67,8**	**35,3**	**-32,5**	**-47,94**
Minimum	35	0	-60	-100
Maximum	90	85	15	21,4
Standard deviation	14,6	23,9	19,1	31,6
Variance	214,4	569,7	364,5	1000

Table 4b: Intensities of **dryness/flaking** before and after application of the **skincare without bacterial lysate** and calculation of the differences. Negative values indicate a decrease in dryness and flaking.

No.	Comparative product before	after 4 weeks	Difference	relative change (%)
1	50,0	30,0	-20,0	-40,00
2	75,0	15,0	-60,0	-80,00
3	70,0	70,0	0,0	0,00
4	70,0	45,0	-25,0	-35,70
5	60,0	45,0	-15,0	-25,00
6	55,0	30,0	-25,0	-45,50
7	70,0	70,0	0,0	0,00
8	75,0	40,0	-35,0	-46,70
9	65,0	15,0	-50,0	-76,90
10	80,0	50,0	-30,0	-37,50
11	50,0	10,0	-40,0	-80,00
12	85,0	45,0	-40,0	-47,10
13	35,0	0,0	-35,0	-100,00
14	60,0	30,0	-30,0	-50,00
15	80,0	30,0	-50,0	-62,50
16	75,0	25,0	-50,0	-66,70
17	90,0	70,0	-20,0	-22,20
18	50,0	0,0	-50,0	-100,00
19	90,0	60,0	-30,0	-33,30
20	70,0	45,0	-25,0	-35,70
Average	**67,8**	**36,3**	**-31,5**	**-46,46**
Minimum	35,0	0,0	-60,0	-100,00
Maximum	90,0	70,0	0,0	0,00
Standard deviation	14,6	21,8	16,2	28,40
Variance	214,4	473,4	263,4	806,80

5. Results of the final questionnaires

Table 5a: Results (text) of the questionnaire on the application of the **microbiotic care** (right half of the body)

Own skin type:

[12 x] dry skin
[2 x] sensitive skin
[1 x] normal skin
[5 x] atopic

1. Which areas have been treated?
[9 x] right shin
[1 x] right shin, right wrist
[1 x] right arm, right leg
[3 x] right elbow
[2 x] right leg
[2 x] right Arm
[1 x] right arm, abdomen, right leg
[1 x] right hand

2. What did you like most about the product?
[1 x] is easy to distribute
[1 x] the neutral fragrance and good distributability, reduces the itching
[1 x] helps very well against itching on the skin after showering
[3 x] is quickly absorbed
[1 x] suppleness
[1 x] neutral fragrance, sustainable feeling of care
[1 x] moisturizing effect, non-perfumed/no artificial unpleasant smell
[1 x] the product hydrates the skin
[1 x] is quickly absorbed, odorless, makes the skin supple
[1 x] very well tolerated
[1 x] everything
[1 x] milky creamy lotion, free of fragrances
[1 x] yield, relief of itching
[1 x] smell, hydrates the skin a lot
[1 x] the packaging
[1 x] is quickly absorbend, smells pleasant
[1 x] it smells quite pleasant
[1 x] no data

3. What did you not like about the product?
[1 x] smell, difficult to distribute and absorption takes long time
[1 x] takes some time before for the product is absorbed
[1 x] is not quickly absorbed
[1 x] the smell is not so tolerable
[1 x] is absorbed slowly
[1 x] smell
[1 x] my skin was still very dry after application
[13 x] no data

4. How do you rate the product as a whole?
[7 x] very good
[11 x] good
[2 x] neither good nor bad
[0 x] bad
[0 x] very bad

5. How do you assess the fragrance of the product?
[5 x] very pleasant
[5 x] pleasant
[7 x] neutral
[3 x] unpleasant
[0 x] very unpleasant

6. How do you assess the consistency of the product?
[0 x] is clearly too firm
[3 x] is too firm
[16 x] just right
[1 x] is too fluid
[0 x] is clearly too fluid

7. How do you assess the skin feeling after the application of the product?
[8 x] very pleasant
[11 x] pleasant
[0 x] neither nor
[1 x] bad
[0 x] very bad

8. How do you assess the following product attributes:

	fully agree	**agree**	**neither nor**	**do not agree**	**don't agree at all**
a) leaves a soft skin feeling	[9 x]	[9 x]	[1 x]	[1 x]	[0 x]
b) provides intense moisture	[8 x]	[8 x]	[1 x]	[3 x]	[0 x]
c) leaves a smooth and supple skin feeling	[10 x]	[8 x]	[1 x]	[1 x]	[0 x]
d) reduces skin redness	[6 x]	[3 x]	[10 x]	[1 x]	[0 x]

e) makes the skin look healthier	[4 x]	[9 x]	[6 x]	[1 x]	[0 x]
f) reduces the tension of the skin	[9 x]	[7 x]	[3 x]	[0 x]	[1 x]
g) leaves no unpleasant residues on the skin	[8 x]	[8 x]	[3 x]	[1 x]	[0 x]
h) the product soothes irritated skin	[5 x]	[7 x]	[7 x]	[0 x]	[1 x]
i) relieves the itching	[5 x]	[3 x]	[11 x]	[1 x]	[0 x]
j) has a regreasing effect	[6 x]	[10 x]	[4 x]	[0 x]	[0 x]
k) helps with severely dry skin	[7 x]	[11 x]	[1 x]	[0 x]	[1 x]
l) helps with skin prone to neuroderma-titis 1 x no data	[2 x]	[8 x]	[8 x]	[1 x]	[0 x]
m) leaves a protective effect on the skin	[5 x]	[10 x]	[4 x]	[1 x]	[0 x]

9. How do you assess the distributability of the product?
[4 x] is absorbed very quickly
[12 x] is absorbed quickly
[0 x] neither nor
[4 x] is slowly absorbed
[0 x] is very slowly absorbed

10. How do you assess the tolerability of the product?
[12 x] very good
[7 x] good
[0 x] satisfactory
[0 x] sufficient
[1 x] deficient because: [1 x] my skin was still tense and rough

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11. Assess the following statement: "The product is suitable for sensitive skin."
[10 x] fully agree
[7 x] more likely to agree
[1 x] neither nor
[1 x] rather disagree
[0 x] absolutely disagree

12. Assess the following statement: "The product has a soothing effect on the skin and is particularly suitable for very dry skin prone to neurodermatitis."
[6 x] fully agrees
[10 x] are more likely to agree
[2 x] neither nor
[1 x] rather disagrees
[0 x] I absolutely disagree
[1 x] I can't judge because I don't have sensitive skin

13. Would you buy the product after this application test?
[17 x] yes
[3 x] no, because [1 x] the skin is still dry
[1 x] my skin needs oilier cream
[1 x] does not help my skin

Table 5b: Results of the questionnaire on the application of the **skincare without bacterial lysate** (left half of the body)

Own skin type:
[12 x] dry skin
[2 x] sensitive skin
[1 x] normal skin
[5 x] atopic

1. Which areas have been treated?
[9 x] left shin
[1 x] left shin, left wrist
[1 x] left arm, left real leg
[3 x] left elbow
[2 x] left leg
[2 x] linker Arm
[1 x] left arm, abdomen, left leg
[1 x] left hand

2. What did you like most about the product?
[1 x] easy to apply
[1 x] the neutral fragrance, the good distributability and relieves the itching
[1 x] absorbs quickly, easy to distribute, pleasant smell
[3 x] moves in quickly
[1 x] suppleness
[1 x] neutral fragrance, relaxed skin feeling
[1 x] moisturizing effect, neutral smell
[1 x] the product makes the skin moisturised
[1 x] absorbs well, smell, creaminess - in short: everything!
[1 x] odourless, makes supple skin, can be easily distributed
[1 x] light, creamy consistency without fragrance
[1 x] yield, relief of itching
[1 x] the consistency, feel
[1 x] the packaging
[1 x] moves in quickly, smells pleasant
[1 x] it was very pleasant to the skin, pleasant fragrance
[2 x] no data

3. What did you not like about the product?
[1 x] does not have the same effect compared to the other product
[1 x] no essential effect
[1 x] is not absorbed very quickly
[1 x] the smell is not tolerable
[1 x] is absorbed slowly
[1 x] smell
[14 x] no data

4. How do you rate the product as a whole?
[8 x] very good
[11 x] good
[1 x] neither good nor bad
[0 x] bad
[0 x] very bad

5. How do you assess the fragrance of the product?
[6 x] very pleasant
[5 x] pleasant
[7 x] neutral
[2 x] unpleasant
[0 x] very unpleasant

6. How do you assess the consistency of the product?
[0 x] is clearly too firm
[1 x] is too firm
[17 x] just right
[2 x] is too fluid
[0 x] is clearly too fluid

7. How do you assess the skin feeling after the application of the product?
[8 x] very pleasant
[11 x] pleasant
[1 x] neither nor
[0 x] bad
[0 x] very bad

8. How do you assess the following product attributes:

	fully agree	**agree**	**neither nor**	**do not agree**	**don't agree at all**
a) leaves a soft skin feeling	[9 x]	[10 x]	[1 x]	[0 x]	[0 x]
b) provides intense moisture	[8 x]	[8 x]	[3 x]	[1 x]	[0 x]
c) leaves a smooth and supple skin feeling	[9 x]	[9 x]	[2 x]	[0 x]	[0 x]
d) reduces skin redness	[3 x]	[5 x]	[12 x]	[0 x]	[0 x]

e) makes the skin look healthier	[5 x]	[9 x]	[4 x]	[2 x]	[0 x]
f) reduces the feeling of tension of the skin	[7 x]	[10 x]	[3 x]	[0 x]	[0 x]
g) leaves no unpleasant residues on the skin	[9 x]	[10 x]	[1 x]	[0 x]	[0 x]
h) the product soothes irritated skin	[5 x]	[8 x]	[7 x]	[0 x]	[0 x]
i) relieves the itching	[4 x]	[2 x]	[13 x]	[0 x]	[1 x]
j) has a regreasing effect	[6 x]	[10 x]	[3 x]	[1 x]	[0 x]
k) helps with severely dry skin	[7 x]	[7 x]	[4 x]	[2 x]	[0 x]
l) helps with skin prone to neurodermatitis	[3 x]	[5 x]	[8 x]	[2 x]	[0 x]
[1 x] I don't know [1 x] no data					
m) leaves a protective effect on the skin	[6 x]	[11 x]	[3 x]	[0 x]	[0 x]

9. How do you assess the distributability of the product?
[2 x] is absorbed very quickly
[16 x] is absorbed quickly
[0 x] neither nor
[2 x] is absorbed slowly
[0 x] is absorbed slowly

10. How do you assess the tolerability of the product?
[12 x] very good
[8 x] good
[0 x] satisfactory
[0 x] sufficient
[0 x] deficient because:

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11. Assess the following statement: "The product is suitable for sensitive skin."
[10 x] fully agrees
[8 x] more likely to agree
[1 x] neither nor
[0 x] rather disagrees
[0 x] absolutely disagrees
[1 x] I can't judge because I don't have sensitive skin

12. Assess the following statement: "The product has a soothing effect on the skin and is particularly suitable for very dry skin prone to neurodermatitis."
[8 x] fully agrees
[8 x] more likely to agree
[2 x] neither nor
[1 x] rather disagrees
[0 x] I absolutely disagree
[1 x] I can't judge because I don't have sensitive skin

13. Would you buy the product after this application test?
[17 x] and
[3 x] no because [1 x] it didn't have the desired effect
[1 x] I have not noticed any improvement
[1 x] my skin needs oilier cream

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6. Patch safety test

Table 6a: Results of the **patch test** with the **microbiotic care** on clinically healthy upper back skin

Proband No.	Diagnosis	24 hours	48 hours	72 hours
1	healty skin	–	–	–
2	healty skin	–	–	–
3	healty skin	–	–	–
4	healty skin	–	–	–
5	healty skin	–	–	–
6	healty skin	–	–	–
7	healty skin	–	–	–
8	healty skin	–	–	–
9	healty skin	–	–	–
10	healty skin	–	–	–
11	healty skin	–	–	–
12	healty skin	–	–	–
13	healty skin	–	–	–
14	healty skin	–	–	–
15	healty skin	–	–	–
16	healty skin	–	–	–
17	healty skin	–	–	–
18	healty skin	–	–	–
19	healty skin	–	–	–
20	healty skin	–	–	–

Table 6b: Results of the **patch test** with the **skincare without bacterial lysate** complex on clinically healthy upper back skin

Proband No.	Diagnosis	24 hours	48 hours	72 hours
1	healty skin	–	–	–
2	healty skin	–	–	–
3	healty skin	–	–	–
4	healty skin	–	–	–
5	healty skin	–	–	–
6	healty skin	–	–	–
7	healty skin	–	–	–
8	healty skin	–	–	–
9	healty skin	–	–	–
10	healty skin	–	–	–
11	healty skin	–	–	–
12	healty skin	–	–	–
13	healty skin	–	–	–
14	healty skin	–	–	–
15	healty skin	–	–	–
16	healty skin	–	–	–
17	healty skin	–	–	–
18	healty skin	–	–	–
19	healty skin	–	–	–
20	healty skin	–	–	–

Table 6c: Control: Results of the **patch test without product application** on clinically healthy upper back skin

Proband No.	Diagnosis	24 hours	48 hours	72 hours
1	healty skin	–	–	–
2	healty skin	–	–	–
3	healty skin	–	–	–
4	healty skin	–	–	–
5	healty skin	–	–	–
6	healty skin	–	–	–
7	healty skin	–	–	–
8	healty skin	–	–	–
9	healty skin	–	–	–
10	healty skin	–	–	–
11	healty skin	–	–	–
12	healty skin	–	–	–
13	healty skin	–	–	–
14	healty skin	–	–	–
15	healty skin	–	–	–
16	healty skin	–	–	–
17	healty skin	–	–	–
18	healty skin	–	–	–
19	healty skin	–	–	–
20	healty skin	–	–	–