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FULL ARTICLE

Comparison between TEWL and laser scanning microscopy measurements for the *in vivo* characterization of the human epidermal barrier

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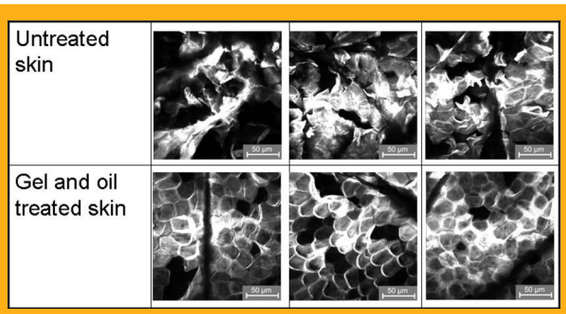
The analysis of the skin barrier properties is important in various fields of medical treatment and cosmetology. The development and improvement of topically applied substances require an objective analysis of the skin barrier characteristics. Transepidermal water loss (TEWL) measurement is the standard method to characterize epidermal barrier function. The most important disadvantage of this method though, is that it can be affected by different exogenous and endogenous factors, e.g. water content of the applied formulation and room temperature. In the present study, TEWL measurements are compared to laser scanning microscopic (LSM) measurements, concerning the use of these two methods for the non-invasive *in vivo* characterization of the epidermal barrier function. The investigations were performed prior and subsequent to treatment of dry skin with a gel mixture, developed for skin treatment after radiotherapy for cancer. The present results indicate that *in vivo* laser scanning microscopy is an appropriate method for the characterization of the skin barrier structure without interference by external factors.



LSM measurements of three volunteers.

1. Introduction

Environmental factors, such as dry and cold climate, dry air and strong winds can affect in different ways the epidermal barrier function [1, 2], which is mainly functioning on the stratum corneum (SC) and this could lead subsequently to dryness of the human skin [3, 4]. Moreover, an intact SC is crucial to main-

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tain a barrier that prevents the loss of fluids, electrolytes and other molecules from within the body and also prevents penetration of xenobiotes. Also, medical treatments, like radiotherapy for cancer, can lead to dysfunction of the epidermal barrier and subsequently to dry skin [5–7]. Different skin care products are commercially available, which intend to improve the skin parameters after radiotherapy for cancer [8–11].

The main function of the dermal barrier is to protect us against the penetration of contaminants and micro-organisms. Also, the dermal barrier prevents our body from drying out. Yet small amounts of water vapour are constantly released through the skin to the outside. The amount of water evaporated through the skin can be measured and used for characterizing the epidermal barrier function. If the dermal barrier is damaged, more water can evaporate on the skin than in the case of an intact barrier [12–15]. TEWL measurements are performed using a small, highly sensitive electrode system, through which the water vapour separated through the skin is led. These measurements have the disadvantage that they are influenced by topically applied substances containing water as a component of the mixture [16]. Additionally, the TEWL measurements must always be performed under standardized climatic conditions in terms of room temperature, air convection and ambient humidity. Last but not least, the measurements can also be affected by anxiety and instability of the volunteers during the measurements [17].

In the present study, TEWL measurements were compared to *in vivo* laser scanning microscopic measurements (LSM), in order to study the skin barrier function and the structure of the SC [18, 19]. Additionally, the skin elasticity and the SC hydration were analyzed.

A gel mixture, initially developed for skin rehydration after radiotherapy, was investigated. The aim of the study was to compare established methods assessing barrier function and related physiological parameters [20–22] with new optical methods, in the present study LSM.

2. Experimental

2.1 Substances under investigation

Two product samples, (brand names "Valeo Holundergel" and "Valeo Dehnungspflegeoel") were provided by the L'estétic GmbH. The products had originally been developed for the treatment of damaged skin following radiotherapy for cancer. According to the application instructions, the gel and the oil should be applied together on the same skin area; first the gel and afterwards the oil. The idea behind the combined application was that the gel should penetrate into the dry skin increasing SC hydration. The subsequently applied oil was intended to form a protective film on the skin surface thus reducing the transepidermal water loss forming a physical barrier.

2.2 Volunteers

The experiments were performed on 10 healthy, female volunteers, with dry skin, aged between 25 and 49 years on their volar forearms. The dry skin was characterized by corneometry. In addition, the *in vivo* laser scanning microscopy was used in order to analyze the volunteers' dermal surface structure.

The volunteers had been selected as a result of a screening of volunteers presenting dry skin without skin diseases, allergies or other systemic diseases. The study had been approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin and the volunteers had given their written informed consent.

2.3 Study design

The volunteers were advised to topically apply the gel and subsequently the oil, twice daily on the volar forearm over a period of 4 weeks. The other forearm was left untreated. The decision on which forearm the products should be applied was selected according to a randomization scheme.

After an application period of 2 and 4 weeks, respectively, the following parameters were measured on treated and untreated skin: transepidermal water loss (TEWL), skin barrier structure with laser scanning microscopy (LSM), skin elasticity using cutometry and skin hydration utilizing corneometry.

2.4 Determination of transepidermal water loss values (TEWL)

The TEWL values were measured by a TEWA-Meter 210 (Courage & Khazaka GmbH, Germany). The measurements had always been taken on the same skin site for a period of 20 s and stopped when the standard deviation was less than 0.15 g m^{-2} h^{-1}. The measurements were carried out in accordance with the published guidelines [13, 15].

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2.5 Analysis of the skin structure with laser scanning microscopy (LSM)

The LSM investigations were performed using the *in vivo* laser scanning microscope Stratum (Optilas, Ltd., Melbourne, Australia) in the fluorescent mode [23]. The system consists of a basic station containing the argon laser (488 nm), the spectrometer and the detector system. The basic station is connected by optical fibers to the hand-piece, where the optical imaging system and focus control unit are located. The size of the skin area under investigation was 250×250 µm. Before starting the LSM measurements, a fluorescent dye (0.2% fluorescein in water) was applied to the skin in order to make the cellular structure visible.

2.6 Determination of the elasticity of the skin

The elasticity of the skin before and after the application of the products was determined with the Cutometer® SEM 575 (Courage & Khazaka GmbH, Germany). The elasticity was classified by the parameters R0 (total extensibility of the skin) and R2 (overall elasticity of the skin). The lower the obtained value of R0 the firmer the skin. The closer the obtained values of R2 to "1" the higher the elasticity of the skin. These measurements were carried out on treated and untreated skin areas, on the same skin site. Values obtained before treatment were standardized to 100%.

2.7 Determination of stratum corneum hydration

The SC hydration was determined using the Corneometer® CM 820 (Courage & Khazaka GmbH, Germany). The measuring principle is based on changes in the capacity of a measuring capacitor. If the sensor of the corneometer is pressed onto the skin, the horny layer is measured within the range of variation of the capacitor field. For this purpose, the very high dielectric constant of water is used which, contrary to most other matters, can be clearly distinguished. The dielectric constant of the skin is the higher the more water is contained in the tissue. The capacity of the measuring capacitor is converted into a digital value, which is proportional to the dermal moisture level. The values were expressed in arbitrary units. The measurements were carried out in accordance with the published guidelines [24].

3. Results and discussion

The initial TEWL values of the volunteers showed a broad range between 5.1 and 10.1 g m^{-2} h^{-1}. Therefore, for better comparison of the efficacy of the different treatment modalities, the initial TEWL values of the volunteers were standardized to 100%. Whilst the average TEWL values of the untreated skin determined for all volunteers always remained constant during the four weeks (99 ± 17%), the average TEWL values of the treated skin increased by 21 ± 27% (Table 1). In principle, such a high increase should correlate with a strong damage of the epidermal barrier.

Table 1 Determination of the TEWL values before and after treatment.

	untreated skin					gel and oil treated skin				
	TEWL [g/m²/h]			Difference [%]	Difference [%]	TEWL [g/m²/h]			Difference [%]	Difference [%]
Volunteer	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 3*	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 3*
1	4.3	5.0	5.9	116	137	2.6	4.9	4.7	188	181
2	5.8	7.5	6.4	129	110	7.3	11.5	11.5	158	158
3	5.1	5.3	5.1	104	100	5.6	6.2	5.5	111	98
4	5.5	5.9	4.8	107	87	5.8	8.4	6.8	145	117
5	7.8	7.4	6.4	95	82	7.0	8.5	6.1	121	87
6	9.1	9.7	7.5	107	82	10.5	13.8	13.0	131	124
7	4.1	5.9	4.2	144	102	3.7	5.4	4.9	146	132
10	10.1	13.8	8.6	137	85	6.5	9.2	6.6	142	102
11	10.1	9.3	8.1	92	80	9.6	9.0	8.4	94	88
12	6.0	7.5	7.2	125	120	7.5	9.9	8.4	132	112
Mean value	**6.8**	**7.7**	**6.4**	**116**	**99**	**6.6**	**8.7**	**7.6**	**137**	**120**

Table 2 Determination of the elasticity [a.u.] (parameter R0) before and after treatment.

	untreated skin					gel and oil treated skin				
	Elasticity (parameter R0) [a.u.]			Difference [%]	Difference [%]	Elasticity (parameter R0) [a.u.]			Difference [%]	Difference [%]
Volunteer	*Visit 1*	*Visit 2*	*Visit 1*	*Visit 2*	*Visit 1*	*Visit 2*	*Visit 1*	*Visit 2*	*Visit 1*	*Visit 2*
1	1.05	1.03	1.37	98	130	1.11	1.12	1.03	101	93
2	1.08	1.23	1.31	114	121	1.07	1.11	1.18	104	110
3	0.91	1.15	1.02	126	112	1.18	1.01	1.03	86	87
4	1.02	1.01	0.97	100	96	1.44	0.99	0.91	69	63
5	1.61	0.99	1.07	62	67	1.03	1.08	1.05	105	102
6	1.02	1.15	1.28	113	126	1.12	1.14	1.02	102	91
7	1.10	1.01	1.00	92	91	1.07	1.06	1.39	99	130
10	0.95	1.06	1.05	112	110	1.42	1.10	1.11	78	78
11	0.98	1.00	1.07	103	110	1.02	1.22	1.02	120	100
12	0.92	0.88	0.92	95	99	1.04	1.02	1.04	98	100
Mean value	**1.06**	**1.05**	**1.10**	**101**	**106**	**1.15**	**1.08**	**1.08**	**96**	**96**

However, this result was surprising, because other skin parameters, such as skin elasticity (Tables 2 and 3) and SC hydration (Table 4) were significantly improved. Following a 4-week treatment, the SC hydration increased by 20%, and the elasticity of the skin by approx. 10%, on average. These results present evidence for an improvement in the skin barrier, with no indication of destruction during the combined gel treatment.

Comparing the cellular structure of the SC by laser scanning microscopy before and after the combined treatment, a distinct improvement in the skin barrier could be observed. This situation is demonstrated in Figure 1 for 3 volunteers.

Before treatment, the typical characteristics of dry skin could be recognized on the LSM images. The corneocytes of the SC show an irregular mountainous structure. Four weeks after gel treatment, the structure of the skin barrier had improved. The corneocytes had formed a "honeycomb-like" flat structure surrounded by intact lipid layers. Such images are characteristic for intact healthy stratum corneum.

The differences between the TEWL and *in vivo* LSM measurements can be explained by the action of the topically applied formulation.

After regular topical administration, the applied products formed a thin homogeneous protection film on the skin surface. This film reduced the continuous water flux through the skin barrier and led to an accumulation of the moisture in the stratum corneum layers and underneath. Improvements in moisture

Table 3 Determination of the elasticity [a.u.] (parameter R2) before and after treatment.

	untreated skin					gel and oil treated skin				
	Elasticity (parameter R2) [a.u.]			Difference [%]	Difference [%]	Elasticity (parameter R2) [a.u.]			Difference [%]	Difference [%]
Volunteer	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 3*
1	0.67	0.70	0.65	104	97	0.77	0.78	0.81	101	105
2	0.77	0.73	0.70	95	91	0.71	0.77	0.77	108	109
3	0.74	0.65	0.70	89	65	0.74	0.72	0.78	98	106
4	0.70	0.73	0.68	105	96	0.64	0.75	0.71	117	112
5	0.54	0.62	0.56	113	104	0.65	0.67	0.61	104	94
6	0.74	0.74	0.67	101	92	0.71	0.78	0.78	109	109
7	0.71	0.74	0.74	105	105	0.77	0.82	0.83	106	108
10	0.82	0.83	0.78	101	95	0.74	0.80	0.84	107	113
11	0.80	0.82	0.82	103	102	0.86	0.89	0.86	103	100
12	0.86	0.83	0.84	105	105	0.85	0.90	0.89	105	105
Mean value	**0.73**	**0.74**	**0.71**	**102**	**95**	**0.74**	**0.79**	**0.79**	**106**	**106**

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Table 4 Stratum corneum hydration values assessed with a Corneometer before and after treatment.

	untreated skin					gel and oil treated skin				
	skin moisture [r.u.]			Difference [%]	Difference [%]	skin moisture [r.u.]			Difference [%]	Difference [%]
Volunteer	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 3*	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 2*	*Visit 3*
1	50	54	48	108	96	47	62	65	132	138
2	57	54	60	95	105	52	59	68	113	131
3	53	50	53	94	100	46	53	56	115	122
4	54	50	54	93	100	52	54	60	104	115
5	64	55	61	86	95	59	63	65	107	110
6	55	56	57	102	104	51	62	56	122	110
7	49	48	51	98	104	43	61	58	142	135
10	59	48	50	81	85	49	55	53	112	108
11	52	57	53	110	102	52	59	59	113	113
12	50	49	55	98	110	47	49	55	104	117
Mean value	**54**	**52**	**54**	**96**	**100**	**50**	**58**	**60**	**116**	**120**

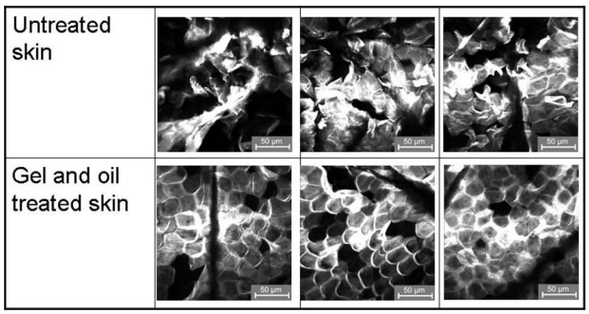

Figure 1 LSM measurements of three volunteers.

content and the elasticity of the skin were the consequences. As the TEWL sensor had been applied directly on the skin surface during the TEWL measurements, the protection film formed by the gel mixture was physically abrogated or the direct water release measured. As a result, the water accumulated in the skin layers underneath was released. This, in turn, resulted in a strong temporary increase in TEWL values. Consequently, the TEWL measurements were disturbed and could not be applied directly in this experimental setting for the characterization of the epidermal barrier function. This effect is enhanced with the increasing efficacy of the protective film which was formed on the skin surface.

4. Conclusion

The results presented in this paper demonstrate that the *in vivo* LSM measurements give a more detailed and in this setting a more accurate evaluation of the state of the skin barrier than TEWL measurements. Furthermore, LSM measurements are suited to analyze the efficacy of topically applied medical drugs and cosmetic products on the cellular level. Additionally, LSM measurements are not influenced by the disturbances caused by the topically applied products, by room temperature, by high ambient air humidity, or by the movements of volunteers.

LSM data reflect the state of the skin barrier, which is understandable also to consumers not specialized in skin measuring technology.

The *in vivo* LSM systems are still very expensive, and no quantitative assessment has been validated so far. Due to the rapid progress in the field of optoelectronics in spectroscopy, it can be expected that such LSM systems will become more affordable and widely used in the near future.

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Theognosia Vergou obtained her medical school diploma at the University of Crete, Faculty of Health Sciences, School of Medicine, Heraklion, Greece in 2001. She was a resident of Dermatology-Venereology at 'A. Sygros' Hospital, University of Athens, Greece and received her granting of title of medical specialty in 2008. Since 2008 she has been a clinical fellow in the Photobiology, Psoriasis and Clinical Trials Unit, University Clinic, 'A. Sygros' Hospital. She received a scholarship from the 'J. D. Stratigos' foundation and attended the International Training Program in Dermatology in Harvard Medical School, Boston, MA, USA in 2007–2008. She is a Ph.D. candidate at the University of Athens. She is currently a research fellow at the Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology and Allergology, Charite University Hospital, Berlin, Germany.

Sabine Schanzer studied at the University of Applied Sciences in Berlin from which she graduated as a Chemical Engineer, specialized in Organic and Inorganic Chemistry. From 1980 to 1991 she worked at the Academy of Science in Berlin, initially at Institute of Organic Chemistry, moving to the Institute of Optics and Spectroscopy in 1988. In 1992, she joined the Center of Experimental and Applied Cutaneous Physiology at the Department of Dermatology at the Charité – Universitätsmedizin Berlin. Here she is in charge of skin physiological measurements as well as cosmetic and clinical studies.

Heike Richter was trained as a communications engineer from 1976–1979. From 1990–1991 she received her training as a technical assistant (physics). From 1986–1991 she was affiliated with the Academy of Sciences, Central Institute of Optics and Spectroscopy. Since 1992 she has been working with the Center of Experimental and Applied Skin Physiology of the Department of Dermatology at the Charité – Universitätsmedizin Berlin. Her main focus is on optical and spectroscopic investigations.

Ragna Pels studied at the Medical School of the University of Saarland and of the University of Berlin. Afterwards she worked as resident at the Department of Dermatology, Charité Universitätsmedizin Berlin. In 2001 she received her doctor degree at the Humboldt University of Berlin. Since 2006 she has been working as a dermatologist in an office for medical supply. Her main interests are skin care and protection, barrier function and skin changes due to surgery, chemotherapy and radiation.

Gisela Thiede studied at the Martin-Luther-University Halle, Department of Organic Chemistry, where she completed her master's degree in chemistry. After obtaining her doctorate as Dr. rer. Nat she joined a biotechnological institute where she worked as a project leader doing research in the field of liposomal drug delivery systems. Since 2006, she has been affiliated with the Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology, Venerology and Allergology, of the Charité – Universitätsmedizin Berlin, with drug delivery systems, skin penetration and follicular penetration being in the focus of her research activities.

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Martina C. Meinke studied chemistry at the Free University of Berlin and did her Ph.D. in 1994. After graduation she built up and headed a laboratory for environmental analysis in Brandenburg until 1999. Then she switched to the medical diagnostics field and was employed by the Charité University Hospital. After a postgraduate study she was awarded the title medical physicist in 2006. Since 2007 she is assistant professor for medical physics. Her main focus is on optical spectroscopy and diagnostics of blood and skin as well as electron paramagnetic resonance measurements in skin.

Wolfram Sterry studied Medicine at the University of Cologne. From 1984 to 1991 he was the Vice chairman of the Department of Dermatology at the University of Kiel and from 1991 to 1994 Professor and chairman of the Department of Dermatology at the University of Ulm. In 1994 he became a Professor and the chairman of the Department of Dermatology at the University Hospital Charité in Berlin. Prof. Sterry was Dean of the Medical Faculty at the University of Ulm (1993–1994) and at the Charité Berlin (1995–1997). He is Editor and Associated Editor of different scientific Journals.

Joachim W. Fluhr received his medical training in Mainz, Germany and Strasbourg, France. He performed his internship for dermatology in Karlsruhe. From 2000–2002 he worked as a post-doc in San Francisco, USA. From 2002–2007 he served as an attendant and the head of the skin physiology lab in Jena, Germany. Between 2007 and 2010 he worked as medical director in a CRO. Since January Dr. Fluhr has been heading the division of dermato-surgery at the department of dermatology, Charité – Universitätsmedizin Berlin, Germany. His main interest in research is the non-invasive biophysical assessment of skin physiology and skin inflammation.

Juergen Lademann studied at the Moscow State University, the Physical Faculty, Quantum Electronics Department, where he completed his master's degree. In the year 2000, Prof. Lademann was appointed Professor of Dermatology at the Charité University Hospital Berlin. He is the Editor of the international journal "Skin Pharmacology and Applied Skin Physiology" and the President of the„International Society of Skin Pharmacology and Physiology".

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