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Comparison of Transepidermal Water Loss and Laser Scanning Microscopy Measurements to Assess Their Value in the Characterization of Cutaneous Barrier Defects

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Key Words
In vivo laser scanning microscopy · Transepidermal water loss · Wound healing · Suction blister technique

Abstract
The exact qualitative and quantitative analysis of wound healing processes is a decisive prerequisite for optimizing wound care and for therapy control. Transepidermal water loss (TEWL) measurements are considered to be the standard procedure for assessing the progress of epidermal wound healing. The damage to the stratum corneum correlates with an increased loss of water through the skin barrier. This method is highly susceptible to failure by environmental factors, in particular by temperature and moisture. This study was aimed at comparing TEWL measurements and in vivo laser scanning microscopy (LSM) for the characterization of the epidermal wound healing process. LSM is a high-resolution in vivo method permitting to analyze the kinetics and dynamics of wound healing at a cellular level. While the TEWL values for the individual volunteers showed a wide scattering, LSM permitted the wound healing process to be clearly characterized at the cellular level. However, a comparison between the two methods was very difficult, because the results provided by LSM were images and not numerical. Therefore, a scoring system was set up which evaluates the stages of wound healing. Thus, the healing process could be numerically described. This method is independent of any environmental factors. Providing morphologically qualitative and numerically quantitative analyses of the wound healing process and being far less vulnerable to failure, LSM is advantageous over TEWL.

Introduction

Wound Healing

An efficient wound healing to close dermal lesions in keeping with both functional and aesthetic requirements continues to be a serious medical problem. Scientific knowledge about professional caring and treatment of

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chronic wounds is still scarce. While the range of modern wound dressings and other therapeutic components has become almost vast, studies are still missing which would demonstrate the efficiency of these often empirically applied wound therapeutics. Wound healing is a highly intricate biological and chemical process which takes place in several overlapping stages [1]. An essential prerequisite for understanding this process and using the new insight for scientific wound studies is the representation of the wounds at a cellular level in vivo. The existing methods and surrogate criteria suitable for wound healing analysis, for therapy control and, last but not least, for the development of drugs to support the wound healing process are of limited relevance [2]. So far, transepidermal water loss (TEWL) measurements have been the standard method to analyze epidermal barrier defects in vivo [3]. Laser scanning microscopy (LSM) is the expression and result of fast-moving developments in high-precision optical technologies. In this study, the value of TEWL and LSM measurements for the characterization of wound healing processes shall be directly compared.

TEWL Measurements

TEWL measurements are established in clinical routine. They are used particularly in allergology and neonatology and in the treatment of burn wounds [4].

The TEWL method is based on the physical principle that the damaged skin is more permeable to water than an intact cutaneous barrier [5, 6]. The TEWL is defined as the quantity of water that passes through the skin to the surrounding atmosphere per hour and 1 cm^2 of area. The pressure of the body water generated by the diffusion of the body water, which is a highly sensitive integral signal, can be quantitatively acquired and measured as TEWL. The more water passes through the skin to the outside, the more pronounced is the barrier defect [7]. The acquired fluid volume is a surrogate criterion describing the barrier function although indirectly, but precisely by a numerical value [8]. This means that the wound healing process becomes quantitatively measurable. Using the TEWL method, defects of the protective function of the skin can be detected already at a very early stage. Other important benefits are that the measurements can be carried out in situ and repeated as often as required. Thus, measuring profiles can be prepared in a spatial and temporal dimension without having to damage the examined skin areas any further [9–11].

A very important disadvantage of the TEWL method is its strong dependence on defined and constant conditions on both the environment and the wound itself. In an aqueous environment, the TEWL method cannot be applied, as it is unable to accurately detect minimum fluid differences. Consequently, this method is unsuitable for evaluating the important initial phase of wound healing, i.e., the exudative phase. In addition, especially moist wound treatment is a basic and generally accepted postulate of modern wound management. In all three phases of wound healing, i.e., in the exudative, the granulation and with some restrictions also in the epithelialization phase, modern moist wound dressings prevent the wound from drying out. Consequently, the TEWL method can be applied to a limited extent for evaluating the epidermis. Moreover, this method is highly susceptible to failure in the presence of topically applied water-containing substances or other protective films on the skin. Finally, TEWL measurements strongly depend on environmental factors. Even minor fluctuations, for example, in the air humidity or the room temperature, lead to significant measuring errors. In addition, the patient must not sweat during the TEWL measurements [12, 13].

LSM Measurements

The confocal LSM permits the representation of biological structures in samples featuring a layer thickness between 150 and 200 μm. Its lateral and depth resolution extends to approximately 5 μm. In most cases, the visual field is 250 × 250 μm in size. Various modes are available for laser scanning microscopes. The reflection mode uses the differences in the refractive index of various biological structures to image these structures. Since these differences are minor in the human skin, high-contrast images can be obtained only by considerable technical effort. Fluorescence microscopy provides a simpler solution. It uses the fluorescence properties of biomolecules or fluorescent markers to represent cellular structures. The images obtained in this mode are mostly sharp and of high contrast.

With the recent development of in vivo LSM systems, which can be directly applied on any human skin area, new promising potentials have opened up for LSM in medicine. Here, either miniaturized systems are used or laser scanning microscopes consisting of a base and a handpiece, which accommodates the optical imaging and scanning system and the control unit for the focus position. Connected to the base by optical fibers, the handpiece can be flexibly moved over the skin surface.

The LSM is advantageous over TEWL measurements, because it is not disturbed by external factors, such as air humidity, room temperature and water content of topically applied substances.

Table 1. TEWL measuring values for healthy skin and wound in 1 volunteer from day 1 to day 10

	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10
Healthy skin	9.4	11.8	8.6	13	10.5	10	10	11	10	12
Wound region	62	71	107	31	51	20	17	12.3	24	12

Objectives

The objective of this study was to compare the value of the standard TEWL method to the LSM as a recent methodological approach for the analysis of wound healing processes in vivo. For better comparison of the TEWL and LSM results, a numerical scoring system had to be developed for the LSM assessment of the wounds. The subject of this study was the investigation of the epithelialization phase of the epidermis.

Materials and Methods

Volunteers

Standardized investigation and wound conditions were needed to methodologically compare the two methods TEWL and LSM. The investigations were carried out on 12 healthy Caucasian volunteers with skin type II according to the classification by Fitzpatrick [14], aged between 18 and 40 years. Ten of the volunteers were male, the remaining 2 female. Before the investigations started, the study had been assessed and approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin according to the Good Clinical Practice guidelines.

Creation of Defined Wound Conditions

Defined and reproducible skin defects were generated using the suction blister technique according to Kiistala and Mustakallio [15], separating the epidermis from the dermis at the level of the basal membrane. The blisters were generated in an air-conditioned room of constant air humidity at a room temperature of 21°C. Before the experiments started, the volunteers had rested in the test room for approximately 15 min. In a vacuum of 200 mm Hg that persisted for a period of 2 h, 1 suction blister was produced on the volar side of the left forearm of each volunteer [16]. Thereafter, the top of the blister was removed by forceps and scalpel. The evoked surface wound healed within a few days without scarring.

TEWL Measurement

The TEWL measurements were carried out with the tewameter TM 210® (Courage and Khazaka Electronic GmbH, Cologne, Germany) under constant temperature and humidity conditions. During the measurements, the volunteers did not move and showed no emotional reactions.

In vivo LSM

The LSM is a novel non-invasive real-time online method providing horizontal two-dimensional images using optical fibers [17–19]. For the in vivo investigations on the kinetic assessment of wound healing, a dermatological laser microscope 'Stratum' (Opti Scan Ltd., Melbourne, Australia) was applied [20–22]. The mobile handpiece of this microscope accommodates the optical imaging system and the focusing system, which are both connected to the laser and detection system via optical fibers [23, 24]. This permits the signals to be derived from any region of the integument. The system used was an argon laser system of $\lambda = 488$ nm. Its measuring area is 250 × 250 μm in size, and the detection depth extends to 250 μm. The focus position can be manually adjusted on the handpiece, thus permitting different skin layers to be analyzed and also the acquirement of depth dimensions. A fluorescent dye (0.1% fluorescein in water) was applied onto the base of the wound to differentiate the cellular tissue structures. The images of all measurements were saved stating the number of the volunteer and the measuring time and subsequently used for evaluating the wound healing. A scoring system was used to evaluate the progress of wound healing and to quantitatively compare TEWL and LSM.

Results

TEWL Measurements

Following the generation of the suction blisters, the TEWL was measured in all volunteers on the entire wound area on 7 consecutive days. The measuring values for the wound area in 1 volunteer are exemplarily shown in table 1.

The reduced TEWL clearly demonstrates the clinically relevant healing process.

Figure 1 shows the daily TEWL values for the measurements on all artificially generated wounds and on the untreated skin of all 12 volunteers during the experiments as mean values. To improve the comparability of the results, the basic concentrations of the TEWL values, which differed strongly for the 12 volunteers, were standardized to 100%. The TEWL value of the intact skin was proportionally related to the mean value of the initial TEWL values of the wound areas. The resulting value of the intact skin corresponded to 11%. The standard deviation of the measurements varied between 15 and 25%.

A quick decline of the TEWL values of all wounds can be observed during the first 5 days. In the case of the untreated wound, the TEWL values increased again on

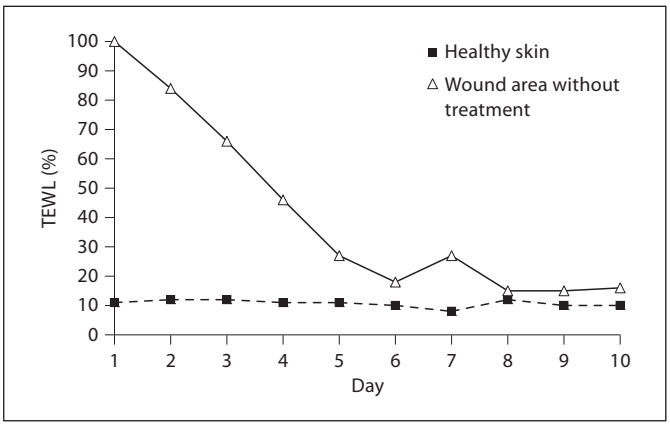

Fig. 1. TEWL mean values of the 12 volunteers from day 1 to day 10 of the experiment.

Table 2. Wound healing progress; change in wound areas with means ± SD

Day	Wound area, %
1	100 ± 30.4
2	91 ± 26.0
3	79 ± 20.8
4	73 ± 19.9
5	66 ± 19.1
6	61 ± 17.4
7	49 ± 21.0
8	38 ± 16.4
9	27 ± 15.6
10	15 ± 12.3

day 7. However, not before day 8 does the TEWL value measured in the wound area start assimilating the value detected in the intact skin area. Contrary to the uniform decline of the TEWL values of the wound, the TEWL values of the untreated skin show an almost constant course, independent of the day the data were acquired.

Laser Scanning Microscopy

Using LSM, the individual skin layers can be visualized at cellular resolution for analysis (fig. 2). The stratum corneum showed flattened, keratinized, coreless corneocytes surrounded by lipid layers. The cells of the deeper lying stratum spinosum are much smaller, with cell cores being recognized. At a depth of 200 μm, the papillary structure became visible.

In order to accurately represent the different skin layers, the systems had to be focused on the relevant epidermal layer. The analysis of the thickness of the stratum corneum is an important parameter for assessing the progress of wound healing and, in particular, for comparing various wound healing processes. The thickness of the stratum corneum is defined as the distance between the skin surface and the boundary layer from the stratum corneum to the stratum spinosum. This vertical measurement was technically realized by displacing the plane of the focus into the respective skin layers [25].

After the removal of the top of the blister and, consequently, the separation of the epidermis from the dermis at the level of the basal membrane, a wound surface became visible which was covered with wound secretions that quickly dried out and formed a crusty structure. In the images provided by LSM (fig. 3), the regeneration of the stratum corneum could be detected after 5 days. It was clearly visible that the corneocytes regenerated not only from the borders of the lesion, but also from 'isolated islets' out of the wound area. These 'islets' quickly grew so that a first row of corneocytes covered the cell surface after 7 days. With the formation of more and more new cell layers, which could be separately observed by adjusting the focus, the stratum corneum finally completely regenerated, although its structure seemed to be rather irregular at that early stage. After 10 days, the skin surface had recovered its original homogeneous structure.

Considerable differences were observed regarding the spontaneous healing of the individual volunteers during a period of 10 days after removal of the suction blister. Table 2 shows the progress of wound healing as temporal change of the mean values of the wound areas of all volunteers. For the sake of better comparability of the results, the basic values of the wound areas were standardized to 100%. While volunteer 10 exhibited a wound area with a maximum residual defect of 31% when the volunteers of the group were compared, the wound areas of volunteers 2 and 4 were found to have completely healed showing 0% residual defect each. For the group of patients who participated in these investigations, the average wound healing progress amounted to 7.6% reduction in wound area per day. Related to the total group this means that after 10 days a residual wound defect of 15% of the original wound area remained on average.

The morphological analysis by LSM also revealed isolated cases of fungal particles. Bacteria could not be imaged (fig. 4).

Numerical scoring criteria were stipulated to permit a dynamic analysis of the wound healing process beyond the description of the individual findings, as well as a comparison of different lesions (table 3).

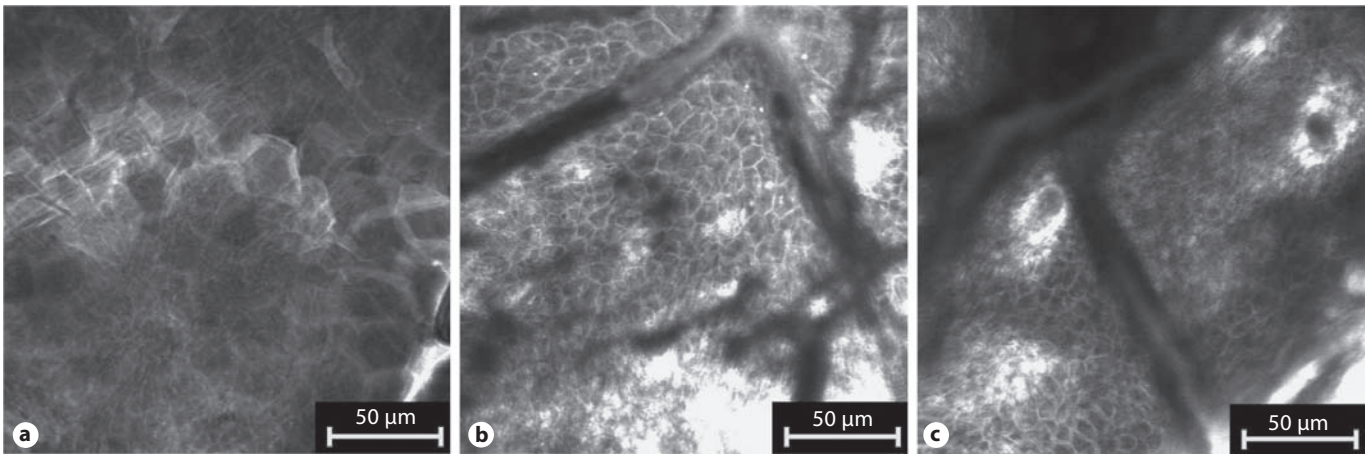

Fig. 2. Characterization of the individual epidermal layers with their typical cellular structures by LSM. **a** Stratum corneum. **b** Stratum basale. **c** Stratum papillare.

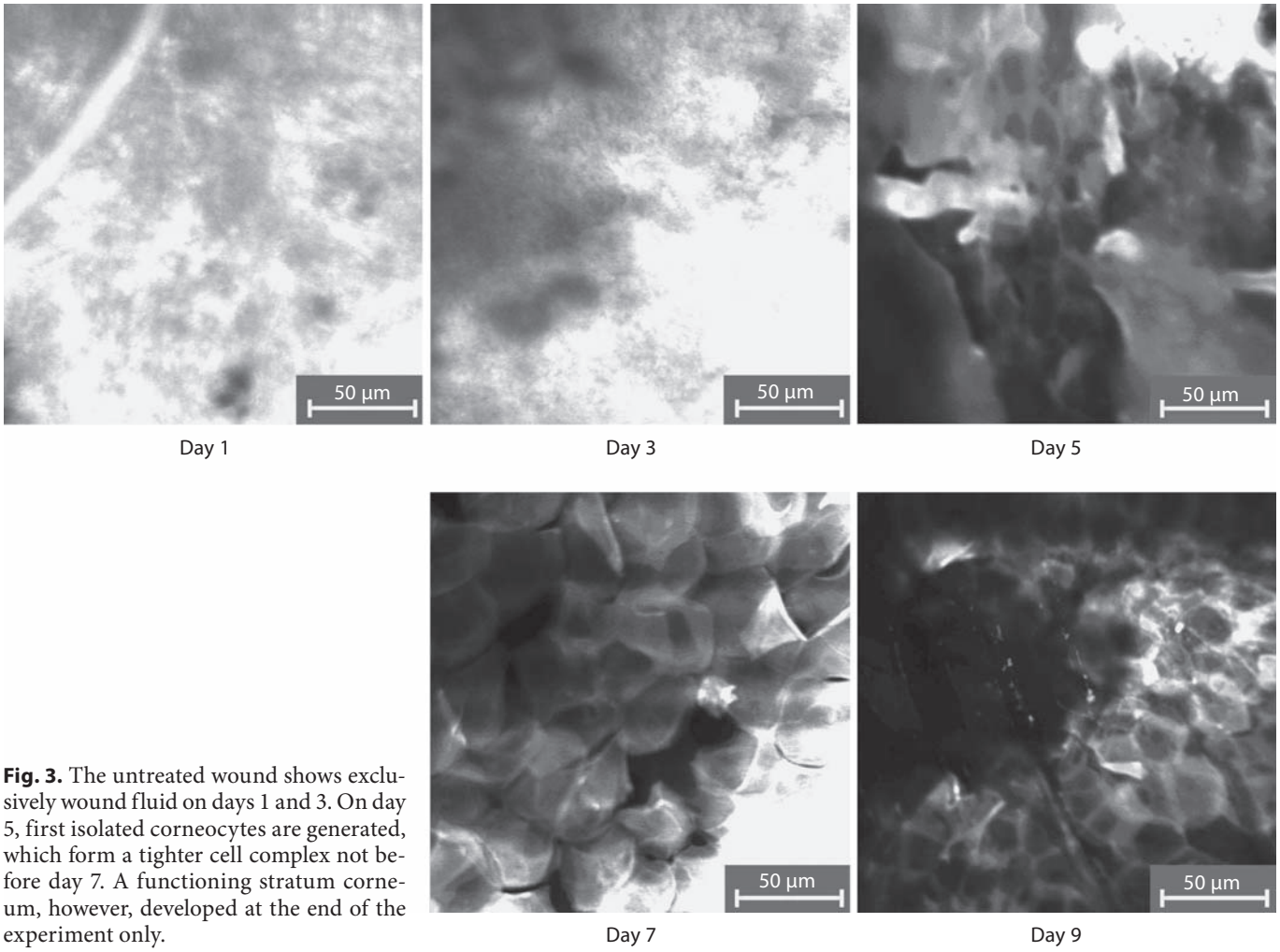

Fig. 3. The untreated wound shows exclusively wound fluid on days 1 and 3. On day 5, first isolated corneocytes are generated, which form a tighter cell complex not before day 7. A functioning stratum corneum, however, developed at the end of the experiment only.

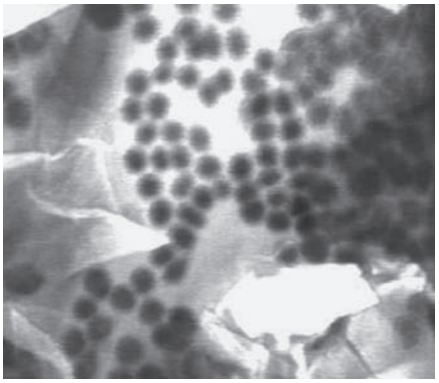

Fig. 4. Fungi were visualized by LSM.

Table 3. Phases of wound healing and their codes for a comparative assessment by LSM in vivo

Phase	Definition	Code
I	The lesion is covered by wound secretions which form a crust	1
II	The first layer of the stratum corneum is forming	2
III	The stratum corneum has completely reepithelialized	3

Table 4. Wound scoring: mean value for each measuring point from the data of the respective phases of the 12 volunteers

Day	Score mean value
1	1.0
3	1.1
5	1.2
7	1.9
9	2.5

This score was developed specifically considering the comparability of the wound healing speed in the different volunteers. At the same time, it is a prerequisite for evaluating various treatment principles for the specific lesion.

Every volunteer underwent wound scoring according to the above-mentioned criteria at any day of the investigations. Subsequently, the mean value was calculated for each measuring point from the data of the respective phases of all the 12 volunteers (table 4). A mean value of 1.5 at one measuring point meant, for example, that one half of the volunteers was in phase I and the other half was in phase II on the given conditions.

As a result of the measurements, it could be shown that based on phase I on day 1, phase II was reached on day 5, on average, while phase III was reached on day 10, on average.

Discussion

In this study, TEWL and LSM measurements were directly compared for their suitability to assess wound healing processes on defined evoked epidermal lesions.

Both these methods are in vivo diagnostic procedures without any influence upon the investigated skin area. Thus, both measuring methods can be repeated as often as required. Moreover, both methods are suitable to assess the changes in the horizontal plane of the wound.

Considering all the measuring results, both methods proved suitable for describing dynamics correlating with the clinically visible healing progress. Applying the TEWL method, the wound area had reached a TEWL value corresponding to that of the healthy skin after 7 days. Using the LSM method, a first closed layer of corneocytes could be detected after 7 days (fig. 2).

An essential advantage of the TEWL method is the possibility to acquire measuring values, which permit the wound healing process to be quantified. An exact quantification, in turn, is indispensable for comparing various wound healing processes. The TEWL method provides a relatively high measuring accuracy, with even short-term changes being detected. In all wounds investigated, for example, a short-term increase in the TEWL was noticed on day 7 (fig. 1).

Referring to the LSM method, its most important advantage is that it can describe the skin surface structures both superficially and in depth at an accuracy that is almost equal to conventional histology. This makes a qualitative three-dimensional assessment of the wound and healing conditions possible. Here, the main focus is on the qualitative statement on the wound healing kinetics. By means of a morphological representation, it can be demonstrated, for example, that the healing process does not exclusively start from the border of the wound, as has been widely postulated so far, but also from small epithelial islets inside the wound area. Thus, LSM decisively contributes to new insights in the mechanisms of wound healing.

The hair follicles, in turn, contain pluripotent stem cells, which enable the epidermis to regenerate out of the center of the wound. Consequently, the human skin can regenerate its epidermis in two ways: by epithelialization

from the wound edges and by epithelialization from the hair follicles. This could explain why larger superficial wounds can also heal spontaneously.

The high resolution of LSM at the cellular level made it possible to represent contaminating fungal particles. According to the conventional microbiological examination, these particles were apathogenic germs. Bacteria, however, could not be detected by LSM. The measurability or 'quantitative significance' of the LSM, compared to the TEWL standard method, was tested by comparing different wounds.

A disadvantage of the LSM compared to the TEWL method is that the immediately available pictorial summary does not provide a numerical value for assessing the wound in the horizontal plane. Yet, the healing process in the wound area represented by LSM can be quantified indirectly by adding an evaluation system, in which comparable healing phases are assigned a numerical point value. The quantitative comparison is then possible by determining the time span required to reach the relevant phase. Both the comparison of results obtained from different volunteers with equivalent wounds and the comparison with the TEWL data on the same wound areas indicated the plausibility, validity and reproducibility of the LSM. However, using the calibrated focus of the LSM to determine the thickness of the stratum corneum illustrates the decisive quantitative measurement potential of the method. The laser beam is focused on the skin surface and the position is marked. Next, the beam is moved deeper into the skin until the boundary between the stratum corneum and the stratum granulosum becomes visible. As the cells in the stratum corneum and in the stratum granulosum have a very different structure, this boundary layer is easy to recognize.

The increasing thickness can be considered to be a direct measure of the healing progress. This is a potential for characterizing differently growing thicknesses on various skin areas or for comparing the healing process using different therapeutic strategies (e.g. topical application).

The numerical scoring system semiquantitatively records the qualitative and morphological observation of the wound healing. One cell layer of the stratum corneum is approximately 1 μm thick, whereas the fully reepithelialized stratum corneum is 10–15 μm in thickness. The scoring system used here consists of 3 phases from the absence of the stratum corneum via the formation of its first cell layer to its full reepithelialization. The time relation to all the volunteers permits a statement on the average progress of wound healing and on the expected increase in thickness of the stratum corneum for primary epidermal wounds. The results of the present study show, for example, that the thickness of the stratum corneum increases not before day 5 on average.

The TEWL method provides a high measuring accuracy as long as the epithelial cover has not yet been completely formed. In this study, this was the case approximately until day 7 (fig. 1). Thereafter, the measured transepidermal fluid loss remained constant. Using LSM, however, it could be demonstrated that although a closed epithelial layer existed from approximately day 7, the wound healing process was not yet concluded. Not before a multilayer of stratum corneum had stabilized from day 10 was the wound healing process finished. This means that LSM is capable of imaging the complete wound healing process.

All in all, the LSM proves to be of higher qualitative significance than the TEWL method. Being able to measure the thickness of the stratum corneum, the LSM provides the advantage of an exact quantitative wound assessment in the vertical plane. Applying a point score, an adequate quantitative horizontal plane measurement is also possible. In addition, LSM is not affected by external factors like air humidity, water, components of topically applied substances, etc. An essential disadvantage of LSM is that it is still rather expensive and available in specialized laboratories only. Considering the development of prices for other computerized optical systems, it can be assumed that the price of laser scanning microscopes will considerably drop in the future.

Conclusion

Taking into account the benefits and drawbacks of the two methods, the LSM seems to be advantageous over TEWL measurements, the hitherto standard procedure for epidermal wound analysis.

This high-resolution in vivo method permits the kinetics and dynamics of wound healing to be evaluated. By means of this method, it is possible not only to exactly measure the layer thickness, but also to visualize the formation of epithelial islets. The method is feasible and independent of any environmental factors.

Acknowledgements

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