In vivo analysis of wound healing by optical methods

In-vivo-Analyse der Wundheilung mit optischen Methoden

Abstract
The analysis of wound healing is important for the therapy control and for the development of drugs stimulating the healing process. Wounds cause damage to the skin barrier. A damaged stratum corneum leads to an increased water loss through the skin barrier. The standard measuring procedure for characterization of wound healing is the measurement of transepidermal water loss (TEWL). The disadvantage of this method is that it can be easily disturbed by the perspiration of the volunteers and by topically applied substances, for instance wound healing creams.

In the study presented, in vivo laser scanning microscopy and optical coherent tomography were compared concerning the application for their analysis of wound healing processes. The laser scanning microscopy allows the analysis of the healing process on a cellular level. The course of wound healing determined by laser scanning microscopy was correlated with numerical values, allowing the numerical characterization of the wound healing process.

Keywords: in vivo laser scanning microscopy, optical coherent tomography, transepidermal water loss, suction blister technique, wound healing analysis

Zusammenfassung
Die Analyse der Wundheilung ist wichtig zur Therapiekontrolle und für die Entwicklung von Arzneimitteln zur Wundheilungsförderung. Wunden verursachen eine Schädigung der Hautbarriere. Ein geschädigtes Stratum corneum verursacht einen erhöhten Wasserverlust durch die Hautbarriere. Daher ist das Standardverfahren zur Charakterisierung von Wundheilungsprozessen die Messung des transepidermalen Wasserverlusts (TEWL). Der Nachteil dieser Methode ist die Störanfälligkeit durch die Perspiratio der Probanden sowie durch topisch applizierte Substanzen, z.B. Wundheilungskremes.

In der vorliegenden Studie werden die In-vivo-Laser-Scan-Mikroskopie und die optische Koheranztomographie in ihrer Aussagekraft, Wundheilungsprozesse zu charakterisieren, verglichen. Die In-vivo-Laser-Scan-Mikroskopie ermöglicht die Darstellung des Heilungsprozesses auf zellulärer Ebene. Mit Bewertungskriterien, die die Stadien des Wundheilungsprozesses beschreiben, lässt sich der Heilungsprozess numerisch beschreiben.

Schlüsselwörter: In-vivo-Laser-Scan-Mikroskopie, transepidermaler Wasserverlust, Wundheilungsanalyse, Saugblasentechnik

Introduction
The skin is the largest organ of our body and represents a barrier to the environment. It protects the organism from water loss and penetration of harmful substances. The barrier can be damaged by mechanical and thermal action or by UV light and different diseases, such as psoriasis and atopic dermatitis [1], [2]. If the skin barrier is disturbed, the skin starts repair mechanisms [3]. The kinetic of the wound healing processes is influenced by endogenous and exogenous factors [4]. The analysis of the kinetic is important for therapy control and for the development of efficient drugs and cosmetic products, stimulating the wound healing [5].
In all cases of damaged skin, the transepidermal water loss (TEWL) through the barrier is increased. Therefore, TEWL measurements are used for the characterization of the barrier properties [6]. The disadvantage of TEWL measurement is that it can be easily influenced by the perspiration of the volunteers and by topically applied drugs and cosmetic products [7].

In the present study, laser scanning microscopy (LSM) [8], [9] and optical coherent tomography (OCT) [10], [11] were compared concerning the application for the characterization of wound healing processes.

Materials and methods

Volunteers

The investigations were carried out on 6 volunteers aged between 22 and 38 years. Approval for the investigations had been obtained from the Ethics Committee of the Charité.

Suction blister formation

Suction blisters were induced on the untreated forearms of the volunteers. The blisters were produced using the tube of a syringe, pressed upside down onto the skin. The needle nipple was connected to a vacuum pump. A vacuum of 0.3 bar was applied for approximately 1 h, until the suction blister was generated. Subsequently, the vacuum pump was disconnected [12].

TEWL-measurements

The measurements of the transepidermal water loss (TEWL) were performed using the TEWA-meter TM 210 (Courage + Khazaka Electronics, GmbH, Cologne, Germany) [6].

Optical methods

In principle, two optical methods are suitable for the characterization of wound healing – the optical coherent tomography (OCT) and the laser scanning microscopy (LSM). Both techniques are non-invasive online methods. The OCT produces images of vertical sections of the skin, analogous to biopsies, whereas, the LSM produces images of horizontal sections.

Laser scanning microscopy (LSM)

The investigations were carried out using the in vivo laser scanning microscope “Stratum” (OptiScan, Ltd., Melbourne, Australia) [8]. The hand piece of the laser scanning microscope contains the optical scanning system and is connected by optical fibers to the base station, containing the Argon laser (λ=488 nm) and the detector system. By the application of the hand piece on the skin surface, images of 200x200 µm are obtained online. Furthermore, the focus can be moved to a depth of approx. 250 µm. In the meantime, the skin can be analyzed layer by layer.

Optical coherent tomography (OCT)

OCT measurements were performed utilizing the SkinDex 300 ( Isis Optronics, GmbH, Mannheim, Germany) [10]. With this system a depth and lateral resolution in tissue can be achieved of approx. 5 µm and 3 µm, respectively.

Results and discussion

In Figure 1, the vertical section of an OCT image obtained in the non-invasive manner of intact human skin is compared to a histological section obtained from a biopsy likewise of intact human skin. The stratum corneum (SC), the epidermis (EP) and the dermis (DE) can be clearly recognized in both cases. Unfortunately, the resolution of the OCT is not sufficient to determine the real thickness of the barrier (SC). The determination of the real thickness of the stratum corneum on the histological section is also not possible, because the structure and thickness of the stratum corneum changed during the preparation of the histological section, on account of mechanical stress and hydration.

In Figure 2, a histological section of intact human skin (Figure 2a) is compared to an image obtained by non-invasive in vivo LSM measurements of likewise intact human skin. The LSM measurement produced horizontal sections in contrast to histology and OCT. In order to determine the thickness of the barrier, the laser focus has to be moved from the skin surface (Figure 2b) down to the boundary between the stratum corneum (SC) and the stratum granulosum (SG) (Figure 2c). This boundary can be clearly recognized, because the size and the structure of the cells in the SC and the SG are significantly different. In Figure 2c, the smaller cells of the SG can be seen in the upper part of the image, whilst the relatively larger cells of the SC are located in the lower part. The distance which the focus has to be moved from the skin surface down to the boundary layer, corresponds to the thickness of the SC.

The SC has a thickness between 15 and 25 µm. Only on the palms and feet, it is much thicker up to 150 µm. Therefore, the OCT measurements are unsuitable for the determination of the thickness of the skin barrier, because of the low depth resolution. In contrast to OCT, the application of LSM can achieve a depth resolution of about 250 µm. Therefore, this method is well suited for the determination of the barrier properties.

In the second part of this study, laser scanning microscopy was compared with TEWL measurements, analyzing the wound healing process after application of the suction blister technique. The disadvantage of the TEWL measurement was that it could not be applied during the first stage of the wound healing process, because the skin surface of the lesion was covered by wound secretion.
Figure 1: Comparison between a histological section (left picture) and an image obtained by OCT (right picture); the stratum corneum (SC), the epidermis (EP) and the dermis (DE) can be clearly recognized.

Figure 2: Comparison between a histological section (a) and an image obtained by LSM; in the LSM image the skin surface (b) and the boundary of the SC to the SG (c) can be clearly recognized.

The corresponding high water evaporation disturbs the TEWL measurement. Furthermore, the application of a wound healing cream is able to disturb the TEWL measurement. On the contrary, the application of in vivo laser scanning microscopy allows the determination of the formation of single cell layers during the healing process. The formation of the first cell layer of the SC is demonstrated in Figure 3. It can be seen that the formation of the new cells starts from the outer edge of the lesion and around the hair follicles. In some volunteers, a colonization of the wounds with fungi was observed; mainly in the case of a wound healing cream being applied to stimulate the wound healing. The fungi spores could be clearly recognized by LSM. One reason for the colonization of fungi in some lesions could be that the applied wound healing cream disturbs the natural formation of a protection film by the wound secretion on the surface of the lesion, which has antibacterial and antymycotic properties [13].

Figure 3: LSM image showing the formation of a new cell layer of corneocytes during the wound healing process
There are hints that the infection of wounds can be reduced or even prevented by a combined application of a wound healing cream and plasma techniques, because electrical signals stimulating the wound healing process, non-invasively and online on a cellular level. This method is less sensitive to endogenous or exogenous interferences and, additionally, it can be used for the analysis of wound colonization by fungi.

Taking into consideration the new developments in the field of laser electroscopic techniques, it can be expected that in the near future efficient and low-cost optical systems will be available, which can be used as a standard tool for therapy control of wound healing and infection processes.

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