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Depth resolved quantitative profiling of stratum corneum lipids and water content using short-wave infrared spectroscopy

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ABSTRACT

We show the feasibility of short wave infrared spectroscopy combined with tape stripping as a simple and noninvasive method for the analysis of lipids and the degree of hydration as a function of depth in the stratum corneum. The spectroscopic method utilizes differential detection with three wavelengths 1720, 1750, and 1770 nm, corresponding to the lipid vibrational bands that lay “in between” the prominent water absorption bands. The results are compared with other biophysical devices such as Corneometer and Sebometer.

Keywords: Water, lipids, stratum corneum, short-wave infrared spectroscopy, barrier function

1. INTRODUCTION

The intercellular lipids and the degree of hydration of the stratum corneum are the crucial determinants required for a competent skin barrier [1-2]. In the past years, quantification of the amount and composition of the extracted stratum corneum cells were performed using a range of different methods such as weighing, optical spectroscopy, microscopy [3-7]. The degree of organization of intercellular lipids and its composition has been previously studied using electron microscopic and X-ray diffraction studies [8].

Quantitative information on depth resolved lipid and water content in the SC is highly relevant for cosmetic (appearance), medical (physiological conditions) and pharmaceutical applications (penetration of drugs and creams). Previous studies report the analysis of lipids and water based on the extracts of the entire stratum corneum and quantitative information on how the lipid and water composition changes with the depth in the SC is very limited. Until now, no non-contact devices and methods have been reported for the quantitative and simultaneous measurement of skin lipids and water content, except confocal Raman spectroscopy. Recently we presented the preliminary results demonstrating the feasibility of a novel noninvasive optical method in the spectral region around 1720 nm utilizing the lipid vibrational bands that lay “in between” the prominent water absorption band for simultaneously measuring the hydration and sebum retaining ability of the skin [9]. To gain access to the deeper layers of SC, we used tape stripping, which is a well-established method for the investigation of skin permeability and barrier function, evaluation of dermatological disorders and assessment of penetration profile and efficacy of various cosmetical and dermatological formulations.

In this study, we show the feasibility of short wave infrared spectroscopy as a novel, non-invasive and easy-to-apply method for analyzing the stratum corneum components, lipids and water, as a function of depth where SC layers were removed using an adhesive tape.

2. MATERIALS AND METHODS

The infrared spectroscopic setup that we have developed utilizes differential detection with three wavelengths 1720 ± 4 nm, 1750 ± 5 nm, 1770 ± 20 nm corresponding to the lipid vibrational bands that lay “in between” the prominent water absorption bands (Fig.1). The skin surface is illuminated with these three wavelengths and the amount of water and lipids were calculated from the ratio of backscattered light to the incident intensity using an algorithm based on Beer–Lambert’s law. The wavelengths 1750 nm and 1770 nm were used for estimating the water content and 1720 nm and 1750 nm were used for the lipids content estimation. The setup (fig. 2) and algorithms has been described in more detail elsewhere [9].
Figure 1. Absorption spectrum of sebum and water measured in the spectral range between 800 to 2400 nm.

Figure 2. Schematic of the short wave infrared experimental set-up [9]: LD1 – Laser Diode (1720± 4 nm, Roithner Laser), LD2 – Laser Diode (1750± 5 nm nm, Roithner Laser), LD3 – Laser Diode (1770± 20 nm, Roithner Laser), F – Narrowband filter (1770 ± 5 nm, Spectrogon), M1, M2, M3, M4 – mirrors, M5 – Mirror with a central aperture, FM1, FM2 – Flipping mirrors , L1, L3, L5 – Aspheric lenses , L2 (f = 300 mm), L4 (f = 300 mm), L6 (f = 75 mm), L7 (f = 150 mm), L8 (f = 35 mm,)– Plano convex lenses L9 (f = 35 mm) – Biconvex lens, L10 (f = 25.4 mm, LA1951-C) P1s, P2p – polarizers , PD – photodiode (DET30B/M).
We used large D-Squame Sampling Disc with the diameter 30.16 mm for tape stripping. The applied pressure on the tape was controlled by the D-Squame pressure instrument at 225 gr/cm² for 10 seconds. Tapes were removed by fast movement with D-Squame angular tweezers to ensure minimal variations in the conditions of tape stripping and in the amount of skin removed. Measurements with all devices were repeated five times with every measurement device within small area of investigation to reduce the overlapping of the probed earlier spots.

Measurements were performed in a climate-controlled room with temperature 21±1°C and humidity 45±5 %. Prior to the experiment participant had acclimatization for 30 minutes. The measurements were performed on the forehead (T-zone) of a female volunteer (26 years) with no history of atopic dermatitis, asthma, allergy, contact dermatitis, or any other skin disorders. Forehead represents a skin area with high density of sebaceous glands in the skin along with medium level of hydration in the facial skin. We marked two areas: untreated skin – normal skin hydration levels and the second location was treated with moisturizing cream for enhanced hydration levels. The internal committee for biomedical experiments approved the study and volunteers gave written informed consent. The relative amount of water and lipids content in the stratum corneum with respect to the baseline were measured after each tape stripping with the short wave infrared spectroscopic experimental set-up, Corneometer (Courage & Khazaka) and Sebumeter (Courage & Khazaka) These reference biophysical devices were used as per the instructions given in the user manuals.

3. RESULTS AND DISCUSSION

Figure 3 shows the relative water and lipids content of the SC normalized with respect to the own maximum value (70% water content in viable epidermis [10]) obtained after tape stripping measured with SWIR as a function of tape-strip number. As expected, as the number of tape-strips increases, the level of SC hydration also increases, which indicate progressively increasing H-bonding further and further into the SC reflecting, increasing availability of water. The change in relative water content as a function of tape-strip number reveal a steeper change for the first few tapes and thereafter, there is a much less dramatic decline with tape-strip number. Similar findings were earlier reported for the water concentration profile across the SC using electron-probe analysis [11] and Confocal Raman microscopy [12].

The figure shows linear increase in capacitance in the low hydration regime corresponding to the intact skin (up to five tapes), similar to the results demonstrated previously by Boncheva et. al. [13]. Both methods, capacitance and SWIR spectroscopy proved to be sensitive to induced by moisturizing topical formulation change in stratum corneum hydration level (fig. 3). Corneometer hydration depth profile appears to flatten with increase of skin moisture content. SWIR measurement levels up, but preserves characteristic curve corresponding to SC water depth profile.

Regarding non-invasive methods reported for monitoring water content in the skin in vivo, instruments based on dielectric properties such as conductance and capacitance [14] and spectroscopic techniques, such as near-infrared spectroscopy [9], have been used. The relationship between electrical properties and water content is highly complex and not linear. The device readings using electrical measurements are affected by the presence of sebum, sweat, hairs, surface microtopography and other environmental factors such as humidity, temperature. Furthermore, sensitivity of capacitance-based method in the low hydration regime is limited. However, electrical devices and methods can be used to distinguish between dry SC, normal SC and highly hydrated SC.
The relative lipid content of the SC (normalized with respect to the maximum value measured at the baseline before tape stripping) is shown as a function of tape number measured with SWIR spectroscopic set-up (fig. 4). The SC contains non-viable corneocytes oriented parallel to the skin surface surrounded by a cornified cell envelope and intercellular regions are filled with highly organized lipids with a characteristic composition and organization. We observe that amount of intercellular lipid per layer decreases as distance into the SC from the surface increases [15-17]. The measurements of the lipids level depth profile with Sebumeter (fig 4.) show dramatic drop within two tapes striped. This number of tapes is sufficient to remove major part of superficial layer of sebum naturally occurring at the skin surface.

Several non-invasive in vivo methods such as solvent extraction, cigarette paper pads, photometric assessment, bentonite clay, and lipid-sensitive tapes have been developed to quantify skin surface lipid parameters: sebum casual level, sebum excretion rate, sebum replacement time, instant sebum delivery, follicular excretion rate, and sustainable rate of sebum excretion. Sebumetry, based on photometric measurement technique is an established photometric method in the diagnostic practice and in clinical trials, as they are time-saving and highly reproducible. Sebumeter is sensitive only to the non-bound skin surface sebaceous lipids and epidermal lipids present in the first one or two layers.

Figure 3. Relative stratum corneum water content normalized with respect to the baseline (no-strip) as a function of tape-strip number measured with SWIR and Corneometer measured on normal and moisturized skin.
One of the potential advantages of the proposed optical method is that it is insensitive to the presence and variation of other skin chromophores such as blood and melanin and can be applied independent of skin type. Moreover, the probe does not need to be in contact with the skin so that the repeated measurements can be performed on the same location without changing the skin conditions.

4. CONCLUSIONS

In this manuscript, we quantify the depth resolved changes in skin hydration and lipids using short wave infrared spectroscopic set-up combined with tape stripping and compare the results with conventional biophysical devices such as Corneometer and Sebumeter. The preliminary results demonstrate the feasibility of this novel noninvasive optical method for simultaneously measuring the hydration and sebum retaining ability of the skin as a function of depth during tape stripping. We anticipate that short wave infrared spectroscopic technique combined with tape stripping can be used as a non-invasive and easy-to-apply method for analyzing the stratum corneum components and thereby provide much more-quantitative and more reliable skin barrier function information in contrast to conventionally employed biophysical methods.

REFERENCES


