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ORIGINAL ARTICLE

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Confocal Raman spectroscopy is suitable to assess hair cleansing-derived skin dryness on human scalp

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Abstract

Background: The purpose of this pilot study was to provide information about the washout-dependent depletion of important skin components in the horny layer of the scalp. They were taken as markers for scalp drying effects of cosmetic cleansing products and were measured directly in vivo.

Method: In vivo confocal Raman spectroscopy was used to measure the depletion of the total natural moisturizing factor (total NMF) and some of its components (urea and lactic acid) as well as a fraction of stratum corneum lipids, after repeated washing with a standard shampoo on the human scalp.

Results: The measurements showed a reduction in the amount of NMF and lipids of the stratum corneum caused by repeated shampooing.

Conclusion: Confocal Raman spectroscopy is an innovative technology that can be used successfully in vivo on the hairy scalp. The loss of the most important skin components caused by hair washing can be quantified directly with this technology. The method is valuable to support the development cosmetic cleansing products, as it is suitable to directly compare the effects of different product candidates on the human scalp in a most realistic way.

KEYWORDS
confocal Raman spectroscopy, dry scalp, hair cleanser, in vivo, NMF, scalp, stratum corneum lipids

1 | INTRODUCTION

The horny layer (stratum corneum, SC), the outermost layer of the skin, is the most important skin layer that keeps the skin in a good condition. The SC cells (bricks) that are embedded in a matrix of intercellular lipophilic substances (mortar) represent the physical barrier. This mortar is produced during the process of differentiation of keratinocytes and represents the chemical barrier against the entrance of foreign substances and excess of loss of water.[1,2] Ceramides, cholesterol and free fatty acids are lipids found in the SC which play a very important role in supporting skin barrier function.[3,4]

NMF is a mixture of water-soluble amino acids found only in the corneocytes as a product of the final stage of the differentiation process. NMF such as glycine, histidine, lactic acid, and urea are very important for the water binding capacity of the SC and thus for the maintenance of the water balance in this layer.[3,4] Robinson et al. found that exposing the skin to water (10 min soaking) is significantly reducing the NMF level.[5]

Scalp skin in comparison with other body areas has distinctive features. It contains a high number of hair follicles accompanied with large sebaceous glands and a dense net of nourishing blood vessels.[6] Furthermore, the scalp SC with an average of 12 cell layers is slightly

thicker than that of the face but thinner than that of the trunk and forearm.[7] Therefore, the thickness of scalp SC can be assumed to approximately 15 μm.

There are several factors that could impair the barrier function including external factors such as ultraviolet radiations, low relative humidity, and cold temperatures, but also personal habits for instance frequent washing or bathing along with a frequent use of surfactants and water.[8] Warner et al. documented SC damage after long-term exposure to water including disturbance of the barrier lipid bilayer und boosting the SC permeability, which in turn leads to skin irritation.[9] Regular hair washing with water and surfactants is challenging the barrier integrity, especially due to the repeated loss of NMF and lipids.

Confocal Raman spectroscopy (CRS) is a noninvasive technology that allows examining and exploring properties of the skin without invasively compromising the skin structure.[10] The Raman effect is described elsewhere.[11] The characteristics of the bands in the RAMAN spectra, their magnitude, location, and shape provide specific qualitative and quantitative information as concentration, type, and structure of chemical bonds, and the structure of molecules in a sample.[12] Thus, CRS can be used to deliver important information about various parameters such as lipids, water, and NMF.

Caspers et al. were the first to determine the concentration of defined depth profiles of NMF components noninvasively in the SC using CRS in vivo.[10,13,14]

CRS was also used in vivo to observe the effects of antiage cosmetic products on the level of hydration and NMF.[15] The effects of the investigated products increased the level of hydration and enhanced NMF.

Surfactants are the major component in cleansing products facilitating the removal of dirt, sebum, and sweat from the skin, scalp and hair. They also support the normal process of desquamation. On the other hand, the use of cleansing products may have damaging effects on the skin, including barrier damage and skin irritation, characterized by itching and dryness.[16,17] The cause is that surfactants increase the removal of moisturizing skin molecules as NMF and solubilize the SC lipids that form the main skin barrier. Although there are different types of surfactants, anionic surfactants are the most commonly primary surfactants used in hair washing products due to their excellent foam forming capability.

In this study, we used CRS in vivo to measure the depletion of the total natural moisturizing factor (total NMF) and some of its components (urea, and lactic acid) as well as the major compounds of SC lipids (free fatty acids and ceramides) after repeated washing with a standard shampoo on the human scalp.

2 | MATERIALS AND METHODS

2.1 | Participants

This nonmedical study on healthy human subjects was executed according to the principle requirements of the declaration of Helsinki and according to the main principles of Good Clinical Practice (GCP). Volunteers were informed orally and written on the study details including potential risks and inconveniences. They provided their written consent before they were included in the study. Three female healthy subjects, 18–30 years old, with healthy, dandruff-free scalp were enrolled in the study. Before the study start, all participants were interviewed to assure that all inclusion and exclusion criteria were met.

2.1.1 | Inclusion criteria

The included subjects were nonsmoking females with an average hair length and a body mass index (BMI) of 30 or below.

2.1.2 | Main exclusion criteria

Subjects were excluded in case of pregnancy or lactation, scaly scalp, or freshly dyed hair; moles, tattoos, scars, irritated skin, topical medication on the scalp within the last 7 days prior to the start of the study; systemic therapy with immunosuppressive drugs (e.g. corticosteroids) and/or antihistamines (e.g., antiallergics) within the last 7 days prior to the start of the study.

2.2 | Raman spectroscopy

The instrument used in this study was a "gene2-SCA Ultimate" manufactured by RiverD International B.V., Rotterdam, Netherlands. This instrument is a confocal RAMAN system of high sensitivity designed for in vivo skin analysis. In this study, a pinhole of 50 μm was used to record the Raman signal in a depth of up to 28 μm in the scalp skin. The gen2-SCA Ultimate has two built-in lasers (wave class 3B lasers, 671 nm and 785 nm). In this study we used the laser with a wavelength of 785 nm which is near-infrared (NIR). It records the "Raman fingerprint region" with wavenumbers from 400 to 1800 cm^{-1}. All components measured display a distinct and intense Raman spectrum in this range.

2.3 | Test product

A mild shampoo was used, consisting of 12.5% of wash active (sodium lauryl ether sulfate with 2 mol ethylene oxide).

2.4 | Procedure

The scalp covered with terminal hair makes it difficult to bring the surface in close contact to the window of the Raman device. To facilitate the measurement, the subject's head was bedded on a specific headrest. The subject's hair was fixed with hair ties to form a parting. A 3M™ Transpore™ Medical Tape (3M Deutschland GmbH, Neuss, Germany) was used to avoid the contact between the remaining nonfixed hair with the measurement window, as shown in Figure 1. Since the laser

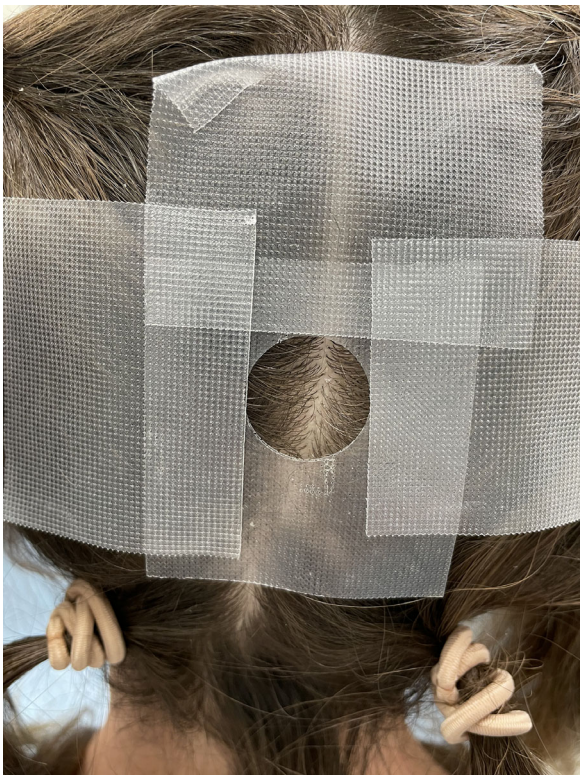

FIGURE 1 Test area: A parting was formed with hair ties followed by covering the surrounding hair with a 3M™ Transpore™ Medical Tape

FIGURE 2 Placement of the subject's head on a specific head rest above the measurement window of the Raman device

beam in CRS is very thin (only 1 μm wide), it can be used to measure directly on the hairy scalp skin between hair and hair follicles. Visual control with the integrated light microscope was used to control the correctness of the measurement area (Figure 2).

Raman measurements were taken at baseline. Afterward, 0.5 ml of the test product was applied to the test area followed by a washing procedure. Washing with gentle rotating movements was done over the entire selected area for 2 min, inducing foam. The test area was rinsed with water for 30 s. The washing process was repeated twice; to a total of 3 washings. After that, the test area was dried with a hairdryer. After 30 min, Raman measurements were repeated.

Spectra were measured in steps of 4 μm from the skin surface until a depth of 28 $\pm$ 4 μm to ensure that the complete SC of the scalp was assessed despite of technical variations in detecting the skin surface.

Approximately 8–10 profiles per test area of 500 $\times$ 500 μm were taken using an integration time of 5 s. The total assessment time was approximately 15 min per subject.

3 | RESULTS

3.1 | Subjects

Three female subjects 24.3 $\pm$ 4.04 years old who fulfilled all inclusion and exclusion criteria successfully completed the study according to the protocol.

3.2 | Washout effects

Figure 3 shows the total amount of total NMF, lactate (pH4), urea, and the sum of free fatty acids and ceramides calculated as the area under the curve from the detected skin surface to a depth of 15 μm.

The *y*-axis (arbitrary unit/cm^2) refers to the Raman signal in arbitrary units per cm^3 of skin multiplied with the skin depth in cm. Figure 3A shows the NMF content of the scalp. A clear drop in the amount of total NMF between baseline and 30 min after hair washing was observed. This decrease represents the washout effect of the shampoo on the amount of total NMF. NMFs constituents such as Lactate (pH4) and urea show a different loss due to the shampoo application (Figure 3B and C).

Raman measurements allow to measure two fractions of the SC lipids. The first fraction is cholesterol/ cholesterol esters and the second one is free fatty acids and ceramides, which accounts to approximately 2/3 of SC lipids. Raman measurements of free fatty acids and ceramides were chosen as they represent the majority of lipids in the SC. A further discrimination of free fatty acids and ceramides is not possible with Raman spectroscopy.[18] A clear reduction in the amount of free fatty acids and ceramides is shown (Figure 3D) 30 min after the washing procedure.

4 | DISCUSSION

The skin of the human scalp is unique. It produces the hair on the head, and therefore, its physiological functioning impacts hair quality[19] and plays an important social role and thus has a direct

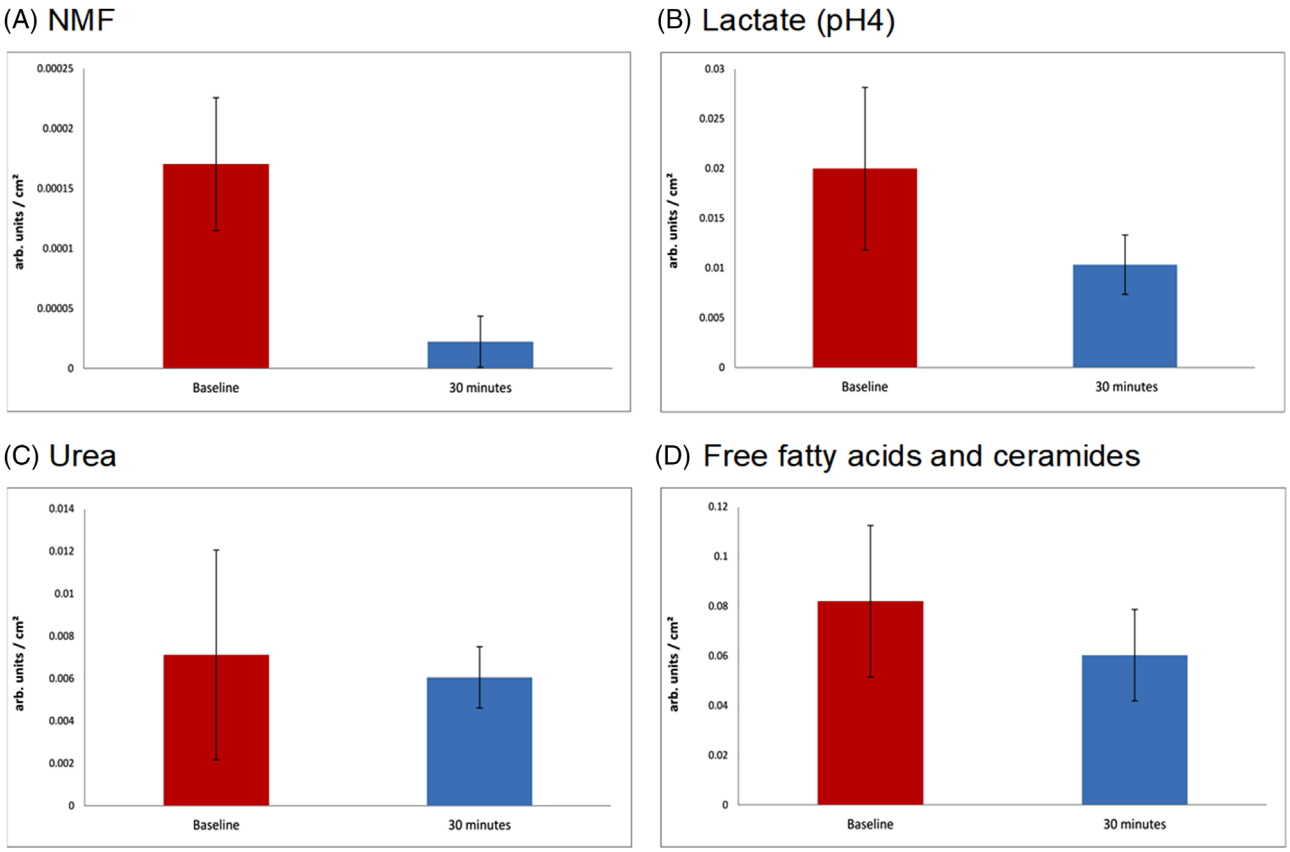

FIGURE 3 Amount of (A) total NMF, (B) lactate (pH4), (C) urea, and (D) free fatty acids and ceramides before and 30 min after hair washing (mean results and SD)

impact on human well-being. New measurement technologies like CRS enable to examine the condition of the scalp and hair in vivo and noninvasively.

Human scalp has thinner SC and a lower NMF content compared with other body regions as, for example, the skin on the extremities.[20] Some conditions like dandruff only exist on the scalp. In dandruff, the NMF content and SC hydration level is lower than on dandruff free scalp.[21] Due to such specific scalp conditions, it is very important to study the effects of hair care product where they are used, namely on the scalp.

In this study, repeated hair washing with a lauryl ether sulfate-model-shampoo that simulates a wide range of shampoos on the European market was investigated to determine the loss of natural moisturizing factor components and skin barrier lipids from the scalp caused by hair washing. In this in vivo setting, already after three shampooing cycles, the level of total NMF, lactate, and an important fraction of SC lipids clearly depleted. The sensitivity to a washout of the investigated molecules was found different for different molecules. Possibly this washout-pattern is characteristic for the investigated product.

Raman measurement in vivo on the scalp has some limitations. The hair in the test area has to be parted very carefully to enable direct contact of the Raman-measurement-window with the scalp and avoid disturbing fluorescence signals from melanin of hairs hit by the Raman-laser-beam. Also, due to the more complex device handling, the time required to perform scalp measurements is clearly longer compared with other body regions.

5 | CONCLUSION

Confocal Raman spectroscopy is an innovative technology that can be used successfully in vivo on the hairy scalp. The loss of the most important skin components by hair washing could be quantified directly with this technology. We assume that the measured molecules are excellent markers to predict drying out and barrier damage on the scalp. The method is suitable to directly compare the effects of treatments in vivo on the human scalp and therefore in a most realistic way.

The method also appears promising to be used in advanced claim support studies to demonstrate mildness and scalp friendliness of hair cleansing products.

Further research on larger test panels and with a variety of different surfactant-products would be needed to obtain more knowledge on the discriminating power and reproducibility of the method.

CONFLICT OF INTEREST

The study was financed by the proderm GmbH. We declare no conflict of interest. All authors are employees of the proderm Institute.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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