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In vivo Study of Skin Barrier Repair Kinetics with Structural and Molecular Insights Using 3D LC-OCT and Confocal Raman Spectroscopy

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1. Introduction

The skin, a complex and vital organ, comprises three primary layers: the epidermis, dermis, and subcutaneous tissue. The epidermis is further stratified into distinct layers: the stratum basale, where keratinocytes proliferate; the stratum spinosum; the stratum granulosum; and, finally, the stratum corneum (SC), the outermost protective barrier [1]. The SC is a highly specialized and essential barrier that relies not only on its structural components but also on the regulated process of desquamation (the shedding of corneocytes) and the balanced water content maintained by hygroscopic substances and lipids. It consists of flattened, anucleated cells called corneocytes, which are embedded within a complex lipid matrix of ceramides, cholesterol, and free fatty acids organized in lamellar structures. This arrangement creates a water-impermeable barrier that prevents both the entry of harmful substances and excessive water loss from the body [2]. Corneocytes, terminally differentiated keratinocytes, are filled with keratin filaments and surrounded by a cornified envelope, providing mechanical strength and resilience. Natural moisturizing factors (NMF) within corneocytes further enhance hydration by attracting and retaining water [3]. Tape stripping (TS), initially described by Wolf in 1939 for cell morphology studies and later demonstrated by Pinkus (1951) to stimulate epidermal proliferation, is commonly employed in dermatological research to sample the SC through sequential removal [4]. This method has proven valuable in studies examining skin barrier function, including experimentally induced barrier impairment, penetration studies [5], and the assessment of epidermal biomarkers in inflammatory skin diseases [6]. The invasiveness of the procedure depends on the number of strips applied; complete SC removal can be achieved after 30-40 strips [7]. TS presents methodological challenges, necessitating the use of near-infrared densitometry [8] or biochemical analysis of proteins to normalize data by quantifying the amount of SC removed during stripping [9] or to test cosmetic produtc efficacy [10]. Non-

invasive imaging methods such as confocal microscopy (reflectance, mulitphoton, etc.) are relevant for investigating the structure and thickness of the SC in vivo [11], while Raman spectroscopy offers promising alternatives for molecular analysis (proteins, lipids, NMF, water, etc.) [12]. Therefore, in the present study, Raman Confocal Microscopy (RCM) and Line-Field Confocal Optical Tomography (LC-OCT) [13] were used in combination to investigate the immediate effects of TS on the SC, followed by monitoring its reconstruction over approximately 15 days.

2. Materials and Methods

2.1 Study Population

This study was conducted in accordance with legal regulations and the principles of the Declaration of Helsinki. Approval was obtained from the SGS Proderm Institutional Review Board (IRB: 2023/030). Female subjects (n=24) between 40 and 60 years of age with healthy skin on the volar forearms were enrolled. Inclusion criteria included uniform skin color with no erythema or dark pigmentation in the test area and Fitzpatrick skin types I-III.

2.2. Experimental Design and Test Procedure

24 women were divided into two sub-panels. Sub-panel 1 (n=12) underwent sequential tape stripping (10 and 21 strips) on randomized volar forearms to disrupt the skin barrier. Sub-panel 2 (n=12) had their skin barrier disrupted via tape stripping, followed by twice-daily application of a test cream on one forearm (control on the other, sides randomized). Stripping continued (minimum 10 strips, up to 5 more) until TEWL doubled from baseline. For both, Instrumental measurements were taken at baseline, immediately post-stripping, and on days 3, 5, 8, 11, and 16 post-stripping to assess barrier repair kinetics.

2.3 Instrumental Measurements

Subjects were acclimatized in a controlled environment (22 ± 2 °C and 50 ± 7.5 % relative humidity) for at least 30 minutes prior to measurements. A template was used to ensure measurements were taken from the same area throughout the study.

a. Transepidermal Water Loss (TEWL)

TEWL was measured using a TEWAMETER® TM 300 (Courage & Khazaka, Cologne, Germany). The open chamber probe was held in place for 40 seconds per measurement to ensure a stable value. One measurement was taken per test area and assessment time.

b. Confocal Raman Spectroscopy

Raman spectra were acquired using a gen2-SCA Skin Analyzer (River Diagnostics, Rotterdam, Netherlands). High wavenumber profiles were obtained from Raman spectra (2600 to 3800 cm^{-1}) at depths of 0-40 µm +/- 1.25 µm in 3 µm steps with a 1-second integration time (25 µm pinhole). Ten profiles were recorded per test area and assessment time.

c. 3D LC-OCT Imaging

3D LC-OCT images were acquired using the DeepLive™ system (DAMAE MEDICAL, France). This system captures images measuring 1200 µm x 500 µm x 500 µm in the x, y, and z (depth) directions, with an isotropic cellular resolution of approximately 1.3 µm. Detailed characteristics of DeepLive™ and LC-OCT can be found in the literature **[14]**. Three 3D acquisitions with acceptable quality with a measurement depth of approximately 300 µm were taken per test area and per assessment time. Images were subjected to segmentation algorithms to compute the thickness of the stratum corneum **[15,16]** as illsutrated in figure 1.

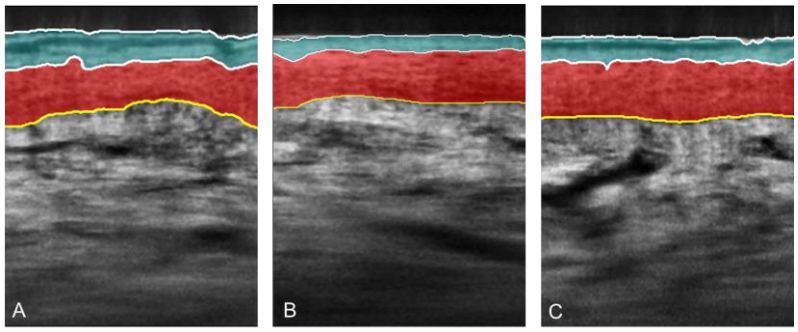

Figure 1. *Example of skin layer segmentation (stratum corneum in blue, viable epidermis in red) shown on 2D reconstructed vertical images. A : Before tape stripping, B : Immediatly after tape stripping and C : at D16 after tape stripping.*

3. Results

3.1. Measuring the barrier repair using TEWL et LC-OCT measurenents

Figure 2 presents results from TEWL measurements (Figure 2A) and SC thickness measured by LC-OCT (Figure 2B) for subset 1, subjected to 10 and 20 tape strips. The baseline TEWL measured was 8.48 ± 1.65 g/m²/h (a). Following TS, TEWL significantly increased to 14.89 ± 2.04 g/m²/h (bc) after 10 strips and further increased to 18.43 ± 3.76 g/m²/h (c) after 20 strips, both significantly different from D1. These measurements were performed immediately after TS without a stabilization period. Subsequent measurements tracked the barrier's recovery over 16 days. TEWL gradually decreased to 15.92 ± 5.56 g/m²/h (c) at day 3, 12.09 ± 2.61 g/m²/h (ab) at day 5, 11.38 ± 1.30 g/m²/h (ab) at day 8, 10.77 ± 1.73 g/m²/h (a) at day 11, and reached 8.99 ± 1.20 g/m²/h (a) by day 16. While TEWL remained significantly different from D1 at day 3, a progressive recovery toward baseline levels was observed over the subsequent two weeks, with TEWL values no longer significantly different from the baseline value from day 11 onward. In comparison, the mean stratum corneum thickness, measured before TS, was 16.46 ± 2.42 µm (b). Following the removal of 10 tape strips, the mean thickness significantly decreased to 10.13 ± 3.74 µm (a) and further reduced to 9.57 ± 3.04 µm (a) after 20 tape strips. Subsequently, a gradual increase in stratum corneum thickness was observed

over the following days. Three days post-stripping (D3), the mean thickness was 14.57 ± 2.35 µm (b), increasing to 14.83 ± 1.23 µm (b) at day 5, 16.50 ± 1.31 µm (b) at day 8, 16.60 ± 1.86 µm (b) at day 11, and reaching 17.64 ± 1.99 µm (b) by day 16. A significant decrease in SC thickness was observed after applying 10 tape strips. Adding further 10 strips resulted in no further noticeable decrease. LC-OCT measurements demonstrated a progressive recovery of SC thickness over the subsequent two weeks.

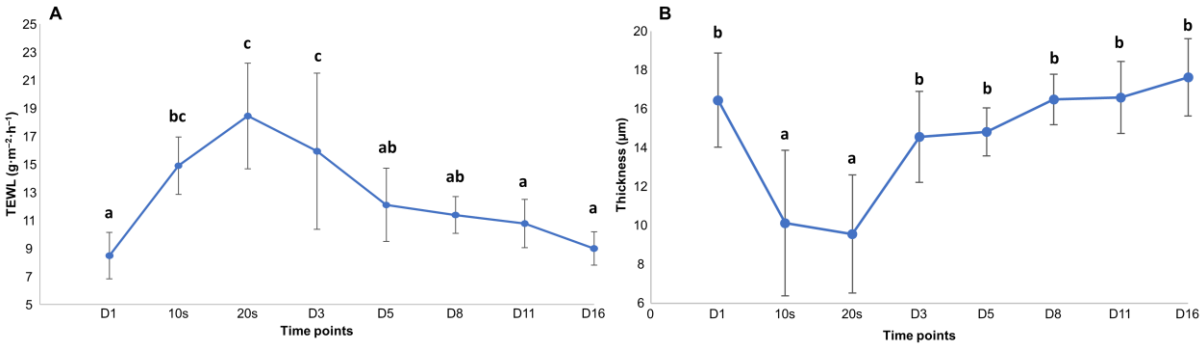

Figure 2. *(A) Transepidermal water loss (TEWL) analysis and (B) Stratum corneum thickness measured using Line-field Confocal Optical Coherence Tomography (LC-OCT). Time points are as follows: D1 (before tape stripping), 10s after 10 tape strips, 20s after 20 tape strips, D3 to D16 indicating measurement days post-tape stripping.*

Figure 3 presents results for TEWL (Figure 3A) and LC-OCT measurements of SC thickness (Figure 3B) for subset 2, subjected to 10 ± 5 tape strips. One volar forearm was used as a control, and the other was treated with a model cream. The number of tape strips was adjusted to achieve a doubling of TEWL immediately after TS. Baseline TEWL measurements at D1 were similar for both the control and treated volar forearms, at 8.51 ± 1.85 g/m²/h and 8.07 ± 2.30 g/m²/h, respectively. These values appeared consistent with subset 1. Following the application of 10 tape strips, TEWL increased in both groups, with the control forearm reaching 18.30 ± 3.71 g/m²/h and the treated forearm reaching 16.06 ± 3.13 g/m²/h. Subsequently, TEWL gradually decreased over time in both groups, indicating barrier recovery. For the control forearm, TEWL decreased to 12.79 ± 3.57 g/m²/h at Day 3 (D3), followed by 11.72 ± 3.08 g/m²/h at D5, 9.50 ± 2.51 g/m²/h at D8, 10.42 ± 1.97 g/m²/h at D11, and 9.36 ± 1.63 g/m²/h at D16. Similarly, the treated forearm saw a decrease in TEWL to 11.32 ± 3.46 g/m²/h at D3, 10.53 ± 2.84 g/m²/h at D5, 9.18 ± 2.92 g/m²/h at D8, 9.70 ± 1.88 g/m²/h at D11, and 8.39 ± 1.25 g/m²/h at D16. This demonstrates that both forearms experienced an initial increase in TEWL following tape stripping, indicative of barrier disruption, but both also exhibited a progressive recovery over the subsequent days, with TEWL trending back towards baseline levels by the end of the 16-day observation period. In figure 3B, at baseline (D1), the mean SC thickness was 17.18 ± 2.49 µm for the treated area and 18.06 ± 3.35 µm for the untreated area. Following the application of tape strips, both areas experienced a decrease in SC thickness to 13.43 ± 0.77 µm in the treated area and 13.81 ± 1.32 µm in the untreated area. Over the subsequent days, SC thickness gradually increased in both areas. In the treated area, SC thickness was 14.62 ± 1.56 µm at D3, 15.40 ± 2.01 µm at D5, 17.01 ± 3.14 µm at D8, 18.30 ±

3.15 µm at D11, and 18.62 ± 2.23 µm at D16. Similarly, in the untreated area, SC thickness was 13.89 ± 1.47 µm at D3, 14.76 ± 1.82 µm at D5, 15.91 ± 1.74 µm at D8, 17.63 ± 2.52 µm at D11, and 17.99 ± 3.63 µm at D16.

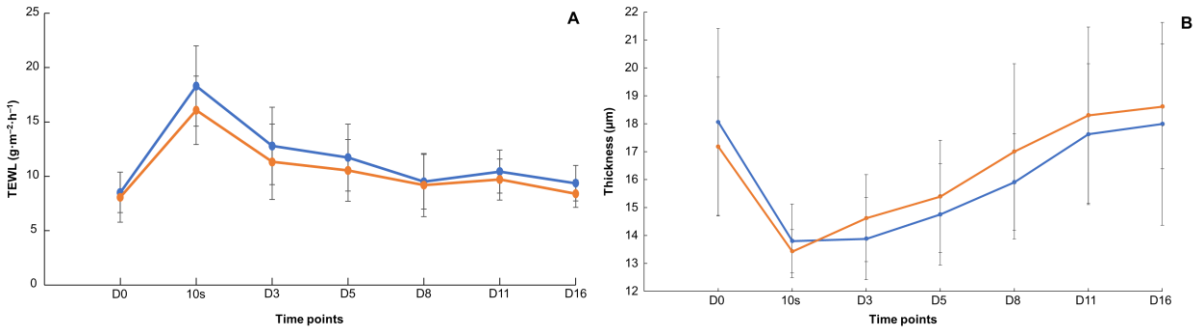

***Figure 3**. (A) Transepidermal water loss (TEWL) analysis and (B) Stratum corneum thickness measured using Line-field Confocal Optical Coherence Tomography (LC-OCT). Time points are as follows: D1 (before tape stripping), 10s after 10 tape strips, D3 to D16 indicating measurement days post-tape stripping. Blue curve: control, Orange curve: cream application.*

Figure 4 represents the percentage change in SC thickness compared to baseline before TS. At 10S post-10 tape stripping, both the treated and control areas show a significant decrease in SC thickness, with the treated area at -21.82% and the control area at -23.55%. Over time, both areas exhibit a gradual recovery in SC thickness (As seen in figure 3). However, the treated area consistently shows a higher percentage of recovery compared to the control. By Day 3, the treated area is at -14.89% versus -23.11% for the control. This trend continues through Day 5 (-10.38% treated vs -18.29% control), Day 8 (-1.01% treated vs -11.92% control), and Day 11 (6.54% treated vs -2.40% control). Notably, by Day 11, the treated area has surpassed the baseline thickness, indicating a complete recovery and a slight increase, while the control area is still below baseline. This method of representation highlights the correlation between time points rather than comparing individual data. The slope, representing the repair rate of the SC over time, is higher for the treated area (~7.1) compared to the control area (~5.3) suggest overall a faster recovery of the SC thickness for the treated area. The Day 16 time point isn't represented because the SC thickness tends to reach a plateau in both conditions, impacting the curve fitting. Additionally, it was noted that at Day 3 for the control area, the mean SC thickness is comparable to the 10S measurement, which lowered the R^2 value. Furthermore, between the 10S time point and Day 3 in Figure 3, the two curves cross each other, showing that the mean SC thickness in the treated area had a different pattern, with a quicker increase in SC thickness after stripping.

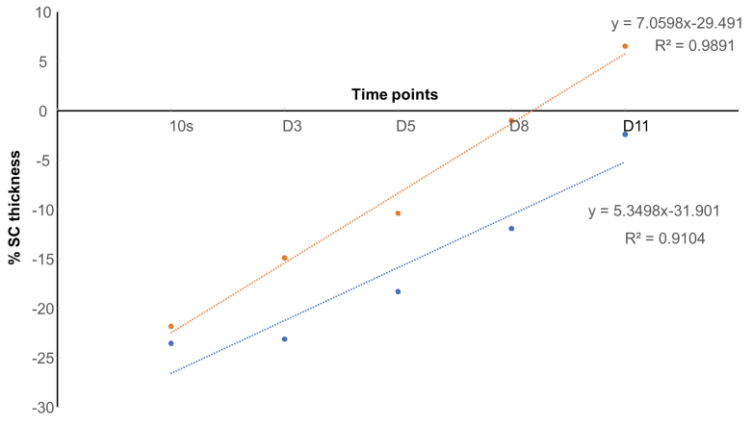

***Figure 4**. Repair kinetics of the SC from Day 1 (D1) after tape stripping to Day 11. SC thickness is expressed as a percentage compared to the baseline measurement taken before tape stripping. Time points are as follows: 10 seconds after 10 tape strips, and Days 3 to 11 (D3 to D11) indicating measurement days post-tape stripping. Blue curve: control; Orange curve: cream application.*

3.2 Molecular insights using Confocal Raman Spectroscopy

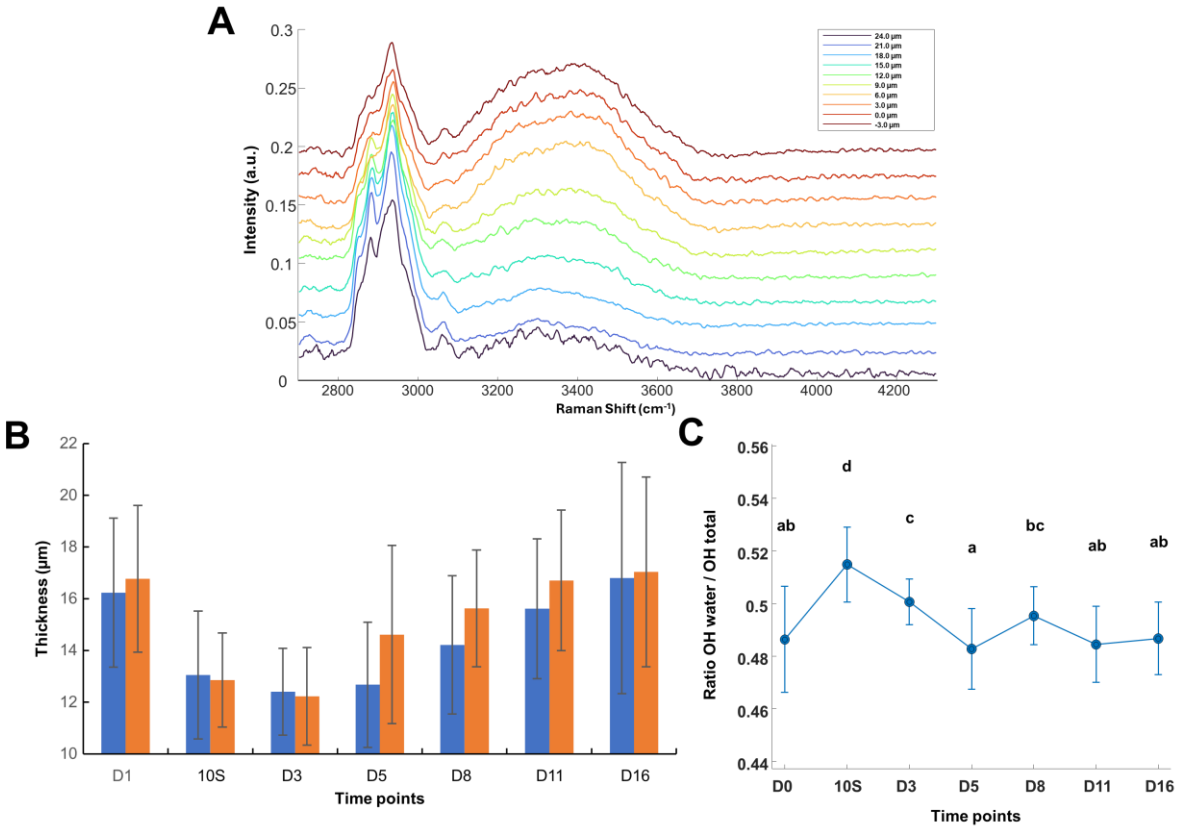

***Figure 5**. A: Example of Z profile collected by Raman spectroscopy. B: Mean SC tchikness comuted from Raman data collected from the control (blue) and treated (orange). C: Ratio asymmetric OH stretching mode / total OH band.*

Figure 5A presents Raman spectra collected from the high wavenumber region, highlighting specific bands around 2,881 cm^{-1} (antisymmetric stretching mode of CH_2 groups) and 2848 cm^{-1} (symmetric stretching mode of CH_2 groups). The Raman spectra of the human stratum corneum (SC) and its components, keratin and lipids, are observed in the 2810–3030 cm^{-1} region, with keratin specifically in the 2910–2965 cm^{-1} range. The valence vibrations of the OH group of water originate from the broad and intense Raman band at approximately 3,100–3,700 cm^{-1}. The water concentration in the SC is non-homogeneously distributed, increasing from the surface (minimal concentration) toward the bottom of the SC (maximal concentration) [12] , and is typically calculated by the intensity ratio of the 3,350–3,550 cm^{-1} water-related bands to the 2,910–2,965 cm^{-1} keratin-related Raman bands [17,18] .

Figure 5B illustrates SC thickness for both control and treated areas. At baseline, both areas exhibit similar SC thickness, with means of 16.23 ± 2.88 µm for the control area and 16.77 ± 2.84 µm for the treated area. Following tape stripping, both areas show a marked decrease in SC thickness, with the control area at 13.05 ± 2.48 µm and the treated area at 12.85 ± 1.82 µm. During the recovery phase, the treated area demonstrates a slightly faster recovery rate. By Day 3, the SC thickness means are 12.40 ± 1.67 µm for the control and 12.22 ± 1.88 µm for the treated area. By Day 5, the treated area shows a more notable increase in thickness at 14.61 ± 3.44 µm compared to 12.67 ± 2.42 µm for the control. This trend continues through Days 8, 11, and 16, with the treated area maintaining a slightly higher SC thickness, reaching 17.03 ± 3.66 µm by Day 16, compared to 16.79 ± 4.47 µm for the control. The standard deviations indicate some variability within each group, suggesting individual differences in response to the stripping and recovery process. Nevertheless, the trends depicted through Raman spectra are comparable to those observed for LC-OCT measurements (Figure 3). Any differences could be attributed to the 3µm step size used for Raman acquisition, compared to the intrinsic approximately 1 µm isotropic resolution in LC-OCT images.

In Figure 5C, the data represent the mean values and standard deviations of the ratio of the asymmetric OH stretching mode to the total OH band. It has also been described that the decomposition of this band can provide information about the different water mobility states in the SC (strongly/weakly bound, unbound water) [12]. Immediately after 10 tape strippings, the ratio increased from 0.486 ± 0.020 (ab) to 0.515+/-0.014 (d). By Day 3, it had decreased to 0.501 ± 0.009 (c). By Day 5, it had returned to a value comparable to baseline with 0.483 ± 0.015 (a). From Day 11 onwards values oscillated between 0.495 ± 0.011 and 0.487 ± 0.014. Raman spectra provide information about the molecular composition of the SC, and water is an important constituent that can be used as a marker of barrier repair, complementing TEWL measurements. The variations in the ratio derived from Raman spectra and the TEWL indicate different kinetics in the barrier function recovery compared to the actual physical thickness of the SC, which takes up to 16 days post-stripping to reconstruct. Combining modalities in assessing the SC in vivo is relevant to better understand the mechanistic aspects of barrier repair at different levels, including water gradient, water evaporation, and water organization (bounding).

5. Conclusion

Combining imaging and molecular spectroscopy appears relevant to studying the recovery of the SC after impairment induced by tape stripping. LC-OCT provides micrometric measurements of SC thickness variations over time, enabling quantification of the effect of stripping and monitoring its reconstruction. In this study, this process was observed to take approximately 16 days after the application of 10 and 20 tape strips. Alternative means of representation, such as linear fitting, can be used to enhance the differences between tested conditions, for instance, control versus treated areas, by expressing recovery as a rate to overcome inter-volunteer variability. Raman spectroscopy has proven a valuable companion technology, corroborating the observations made by LC-OCT and confirming the kinetics of barrier recovery. While tape stripping disrupts water gradient and organization at a molecular level, Raman spectroscopy is a powerful tool to study this level of information to better understand the mechanisms involved. The results collected from two small subsets of volunteers are encouraging and open perspectives to refine and optimize the protocols to access valuable information and, in the near future, provide powerful methods to test product efficacy in the context of barrier strengthening or repair.

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