Int J Clin Exp Med 2018;11(5):4490-4499
www.ijcem.com /ISSN:1940-5901/IJCEM0068103

Original Article

Non-invasive assessment of changes and repair dynamics post irritant intervention in skin barrier

Ou Qin*, Yimei Tan*, Wencai Jiang, Qin Fu, Yafei Xu, Changqing Jiang

*Department of Skin and Cosmetic Research, Shanghai Skin Disease Hospital, Shanghai, China. *Equal contributors.*

Received October 28, 2017; Accepted March 19, 2018; Epub May 15, 2018; Published May 30, 2018

Abstract: This study aimed to investigate the changes of skin conditions after interventions of sodium lauryl sulfate (SLS) and tape stripping (TAPE), and explore the correlation of parameters between different non-invasive tools. Twenty-three healthy volunteers were enrolled in this randomized, controlled study, and 4 evaluating skin surfaces on their left forearms were randomly divided into SLS, TAPE, filter, and control groups. Skin surfaces in SLS and TAPE groups were intervened by SLS and tape stripping respectively. Changes of skin conditions were recorded by non-invasive devices. SLS and TAPE both worsened the skin conditions according to the elevated ICD scores. Compared with control, the TAPE group showed increased transepidermal water loss (TEWL) values. Thicker epidermal thickness was observed in the TAPE group, while thinner cuticle thickness by RCM finally recovered to normal level. Roughness by OCT in TAPE declined first and then recovered, whereas reduced roughness was observed in VC98 detection. Blood flow volume detected by OCT was unchanged in TAPE, while flux by FLPI was raised. Compared to the filter group, SLS exhibited raised TEWL and decreased thickness data, while reduced epidermal thickness by OCT ultimately elevated. Roughness declined, while roughness by OCT finally recovered. Flux by FLPI decreased, whereas blood flow volume by OCT presented an instant reduction followed by a recovery. This study displays the changes of skin conditions post irritation, and discloses a positive correlation of flux parameters between OCT and FLPI as well as a positive correlation of moisture parameters between CM825 and VC98.

Keywords: Skin, sodium lauryl sulfate, tape stripping, non-invasive technique, correlation

Introduction

Increasing evidence has demonstrated that monitoring and quantitative evaluation of the skin is imperative to prevent suspected dermatic disorders in the early diagnosis or promote the recovery process of irritated skin [1, 2]. Stratum corneum (SC), which is the outer layer of epidermis, serves as the primary skin barrier, and parameters of SC such as thickness, hydration and roughness/smoothness usually applied in diagnosis and evaluation of skin [1, 3]. In addition, transepidermal water loss (TEWL) in SC is the typical measurement for assessing barrier function of SC. Blood flow is known as an important index reflecting the biological function and pathological changes in skin microcirculation [4]. Regarding evaluation of SC, sodium lauryl sulfate (SLS), and tape stripping, which mimic chemical and acute physical irritants respectively, usually act as models of skin irritation in studies, meanwhile, appropriate detection devices are used to measure the change and recovery of stimulated skin.

Emerging devices for detecting skin conditions are non-invasive tools, which have advantages in visualizing structure and managing skin recovery compared with traditional excisional biopsy: (1) Non-invasive devices examine the whole skin lesion instead of the selected slices from the lesion; (2) Real-time assessments for skin are realized by non-invasive devices to examine the dynamic changes of SC rather than a static condition [3, 5, 6]. Non-invasive tools have gained numerous applications in dermatology, however, they vary in characteristics such as resolution, clinical applicability, and accuracy, and the correlation of similar parameters between different devices is seldom assessed. Thus, we conducted a randomized,

controlled study using several non-invasive techniques, including TEWAMETER®TM 210, optical coherence tomography (OCT), Reflectance Confocal Microscopy (RCM), Corneometer CM 825®, Visioscan® VC 98, Chromameter® CR400 and Full-field laser perfusion imager (FLPI), to investigate changes of skin conditions after irritants resulting from SLS and tape stripping, and explored the correlation of parameters between different non-invasive tools.

Methods

Participants

Healthy volunteers (23) were enrolled in this study in Shanghai Skin Disease Hospital between May 2017 to Jul 2017. The inclusion criteria were as follows: (1) Age between 18-70 years; (2) No previous stimulation was applied on flexor surface of the forearm within 24 h prior to intervention; (3) No insolation within 3 months before intervention; (4) Able to follow the protocol strictly. Participant with the following conditions were excluded: (1) Pregnancy or lactation; or plan for pregnancy; (2) Allergic or highly sensitive to cosmetics; (3) Received antihistamine agents within one week or immunosuppressive agents within one month before intervention; (4) Had scars, pigments, atrophy, nevus flammeus or other skin blemishes on the evaluating skin surface which might affect the test results; (5) Participated in other clinical studies in the same time. The Ethics Committee of Shanghai Skin Disease Hospital approved the study and each patient signed the informed consent.

Interventions

The left forearm of each volunteer was selected as the evaluating site, and the flexor side of forearm was randomly divided into 4 evaluating surfaces and marked with each area of 2*2 mm, which were categorized into SLS, TAPE, filter and control groups respectively. The detailed interventions in each group were as follows. SLS group: Finn chamber with 20 μL of SLS (99.9% purity; Merck, Darmstadt, Germany) solution was applied to the evaluating site of SLS group for 24 h. Filter group: As a control to the SLS group, a piece of filter with 20 μL distilled water was applied to the evaluating site of filter group for 24 h. Tape stripping group: Corneofix was used to perform the consecutive tape stripping 25 times on the evaluating site of the TAPE group with a constant power and the same direction. Control group: Evaluating site in control group served as the reference to the TAPE group and no intervention was performed.

Assessment

Clinical assessments were analyzed according to Japanese irritant contact dermatitis institution scoring criteria (ICD scores): 0-no response; 1-mild erythema; 2-mild to moderate erythema; 3-clear erythema; 4-erythema, papule or edema; 5-erythema or blister; 6-corrosive response (bleb or necrosis). This visual scoring about the degree of skin irritation was measured by two independent investigators and the average score was collected.

Evaluations of skin conditions were performed by various non-invasive devices in laboratories with stable conditions in which the temperature was 20±2°C and the humidity ranged from 40% to 60%. Participants were required to wash their forearms with warm water and subsequently sat for 30 minutes before testing at each visiting point. Parameters including TEWL, hydration, thickness, roughness, blood flow, homogeneity, and ITA° were recorded at pre-interventions (D0), at 1 day post intervention (D1), 2 days post intervention (D2) and 3 days post intervention (D3) respectively.

TEWAMETER®TM 210 (Courage & Khazakaelectronic GmbH (CK), Germany) was adopted to measure TEWL during observation time. Capacitance was evaluated by Corneometer® CM825 (Courage & Khazakaelectronic GmbH (CK), Germany) to quantify hydration of SC. VivoSight OCT (Michelson Diagnostics Ltd, UK) was used to detect the epidermal thickness, blood flow volume, and roughness with the *in vivo* imaging depth of 1-2 mm. SKIN-VISIOSCAN® (Courage & Khazakaelectronic GmbH (CK), Germany) was applied to analyze surface evaluation of roughness (SER) and homogeneity (HOM) values with surface evaluation of the living skin (SELS) software. RCM (VivaScope 3000; Lucid Inc, Rochester, NY, USA) images of the evaluating surface site provided the cuticle thickness. FLPI (Moor instrument, UK) was used to detect blood flow in the superficial skin with a maximum depth of 1 mm. Chromameter® CR400 (Konica Minolta, Osaka, Japan) was

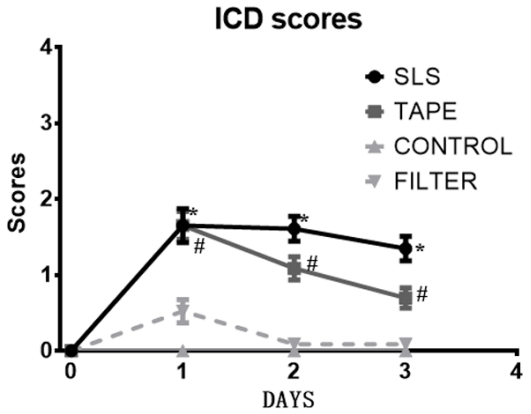

Figure 1. ICD scores of the SLS, TAPE, control, and filter group. SLS increased ICD scores at D1, D2, and D3 compared with the filter group. Meanwhile, ICD scores were also elevated in the TAPE group at D1, D1 and D3 compared with control group. Comparison between groups was determined by t test. $P<0.05$ was considered significant.

adopted to measure skin colors, and individual typological angle (ITA°) is an index determining the degree of skin colors or pigmentations.

Statistics

Statistical analysis was performed by SPSS 22.0 software (IBM, USA) and GraphPad 6.0 software (GraphPad, USA). Data is mainly presented as mean ± standard error. Comparison between two groups was determined by t test. Pearson correlation was used to determine the correlation between variables. $P<0.05$ was considered significant.

Results

Skin assessments by ICD scores

ICD sores were applied to assess the skin conditions during the observation period. As presented in **Figure 1**, SLS dramatically increased ICD scores at D1, D2, and D3 compared with filter group. Meanwhile, ICD scores were also elevated in TAPE group at D1, D1 and D3 compared with control group.

TEWL and hydration evaluation

TM 210 was adopted to evaluate barrier function of skin, which disclosed that TEWL in SLS group was remarkably raised at D1, D2 and D3 after intervention compared with filter group, and TEWL in TAPE group was also increased from D1 to D3 compared with control group (**Figure 2A**). Furthermore, hydration was assessed by CM825 which displayed that SLS group presented similar hydration at D1 and elevated hydration at D2 compared with the filter group, and subsequently recovered to the hydration level of the filter group at D3 after operation. TAPE raised hydration of skin at D1 and D2 compared with the control group, whereas the hydration returned to control level at D3 in TAPE group (**Figure 2B**).

Evaluation of epidermal thickness, roughness, and blood flow by OCT

Data of epidermal thickness, blood flow volume and roughness were provided by OCT as shown in **Figure 3**. Compared with filter group, epidermal thickness in SLS group was decreased at D1 and subsequently presented an increase, which resulted in a similar epidermal thickness at D2 and raised epidermal thickness at D3 (**Figure 3A**). In the TAPE group, no change of epidermal thickness was found after interventions. Regarding blood flow volume, it showed a dramatically increase in SLS group at D1 compared with the filter group, and then the raised blood flow volume gradually dropped and recovered to the level of the filter group until D3 after intervention, while the TAPE didn't affect blood flow volume during D1 to D3 (**Figure 3B**). Additionally, SLS interventions leaded to a burst decrease of roughness in skin at D1, which subsequently recovered to consistent level of roughness with the filter group, and the similar trend of roughness was also found in the TAPE group (**Figure 3C**).

Evaluation of roughness, smoothness and homogeneity by VC98

Evaluated by VC98, SLS reduced SER which was decreased from D1 to D3 compared with the filter group, additionally SER in the TAPE group showed no change at D1 and D2, but was lower than that in the control group at D3 after intervention (**Figure 4A**).

Homogeneity in the SLS group showed an increase but no change was observed at D2 and D3 compared with filter group (**Figure 4B**). While, homogeneity in the TAPE group was reduced during the observation period from D1 to D3 compared with the control.

Evaluation of cuticle thickness by RCM

As exhibited in **Figure 5**, cuticle thickness was abated in the SLS group during D1 to D3 com-

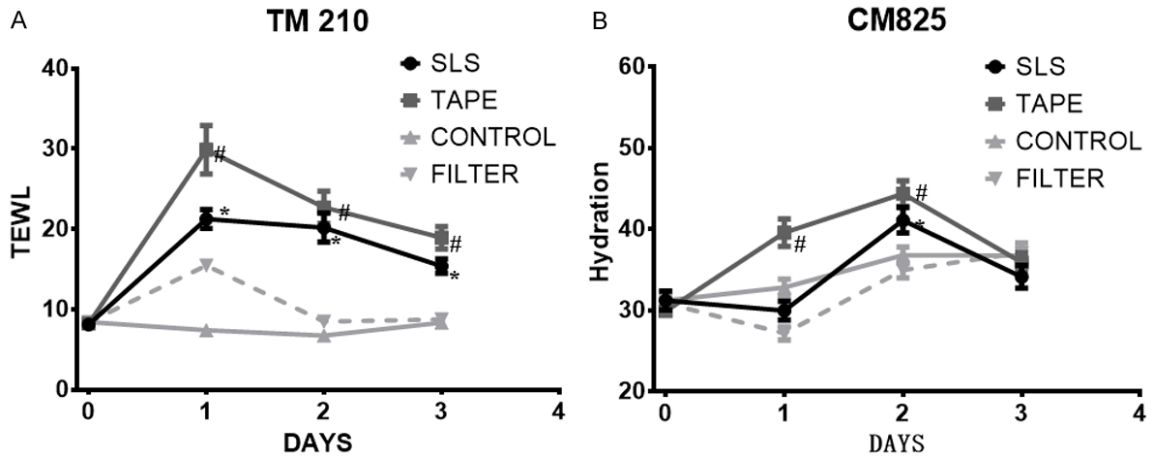

Figure 2. TEWL and hydration. A. TEWL in the SLS group was remarkably raised at D1, D2 and D3 after intervention compared with filter group, meanwhile, TEWL in TAPE group was also increased from D1 to D3 compared with control group. B. The SLS group presented similar hydration at D1 and elevated hydration at D2 compared with the filter group, and subsequently recovered to the hydration level of the filter group at D3 after operation. TAPE raised hydration of skin at D1 and D2 compared with the control group, whereas the hydration returned to control level at D3 in the TAPE group. Comparison between groups was determined by t test. $P<0.05$ was considered significant.

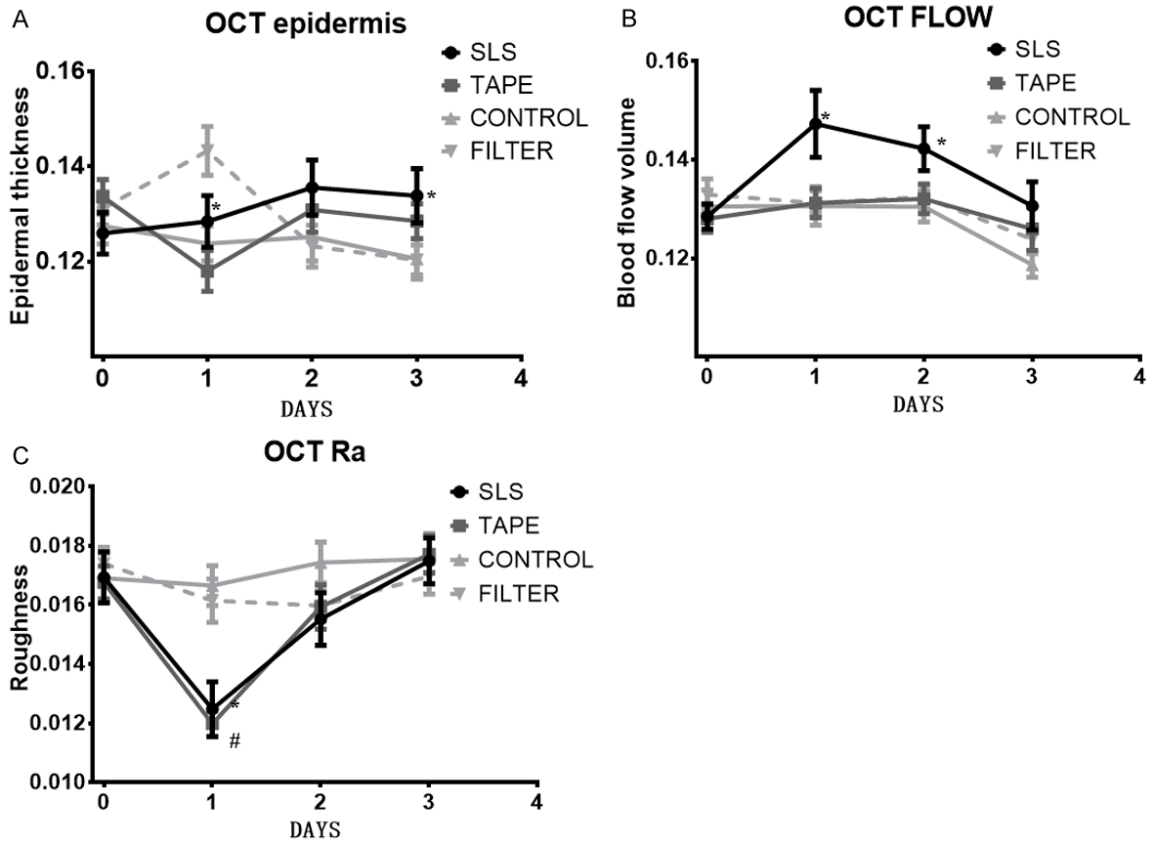

Figure 3. Epidermal thickness, blood flow volume, and roughness by OCT. A. Compared with filter group, epidermal thickness in SLS group was decreased at D1, and subsequently presented an increase which resulted in the raised epidermal thickness at D3. B. In the TAPE group, no change of epidermal thickness was found after interventions. Regarding blood flow volume showed a dramatically increase in SLS group at D1 compared with the filter group, and then the raised blood flow volume gradually dropped and recovered to the level of filter group until D3 after intervention, while the TAPE didn't affect blood flow volume during D1 to D3. C. SLS interventions leaded to a burst decrease

of roughness in skin at D1, which subsequently recovered to consistent level of roughness with the filter group, and the similar trend of roughness was also found in the TAPE group. Comparison between groups was determined by t test. P<0.05 was considered significant.

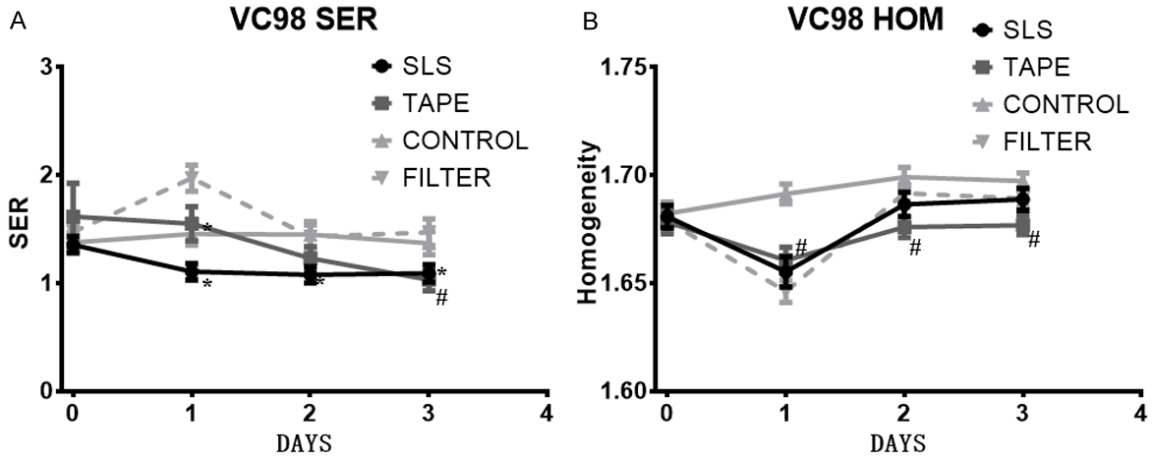

Figure 4. SER and homogeneity by VC98. A. SER in SLS was decreased from D1 to D3 compared with filter group, and SER in the TAPE group showed no change at D1 and D2 but was lower than SER in the control group at D3 after intervention. B. Homogeneity in the SLS group showed an increase but no change was observed at D2 and D3 compared with the filter group. Homogeneity in the TAPE group was reduced during the observation period from D1 to D3 compared with the control. Comparison between groups was determined by t test. P<0.05 was considered significant.

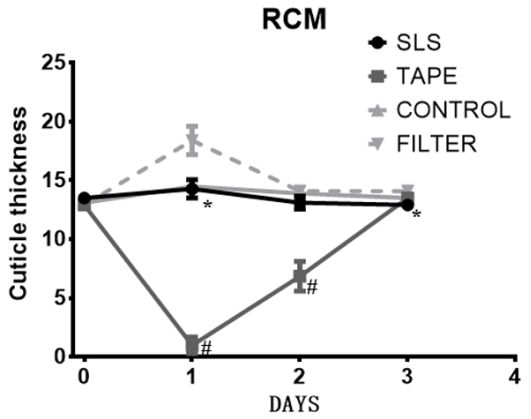

Figure 5. Cuticle thickness by RCM. Cuticle thickness was generally decreased in the SLS group during D1 to D3 compared with thenfilter group. TAPE interventions led to a rapid weakening of cuticle thickness which was observed at D1, while the cuticle thickness was continually recovered and turned to be similar with that in the control group. Comparison between groups was determined by t test. P<0.05 was considered significant.

pared with the filter group. TAPE interventions led to a rapid weakening of cuticle thickness which was observed at D1, whereas the cuticle thickness was continually recovered and turned to be similar with that in the control group.

Evaluation of flux and DC by FLPI

Through the detection of FLPI, flux in SLS group was improved after intervention with the elevated flux values at D1 and D2, and it fell to the level of filter group at D3 (**Figure 6**). In addition, flux in the TAPE group was raised compared with the control group from D1 to D3 after intervention.

Skin color assessments by CR400

ITA° data was supplied by CR400 which displayed descending ITA° compared with filter group at D2 to D4, meanwhile, no change of ITA° was found after TAPE intervention compared with the control group (**Figure 7**).

Correlations of similar parameters between different devices

Pearson test was conducted to assess the correlation between similar parameters which were detected by different non-invasive tools (**Figure 8**). As shown in **Figure 8B**, blood volume flow by OCT was positively correlated with flux by FLPI (R=0.163, P=0.002). Furthermore, hydration by CM825 was positively correlated with homogeneity by VC98 (R=0.125, P=0.017) (**Figure 8C**). No correlation between epidermal

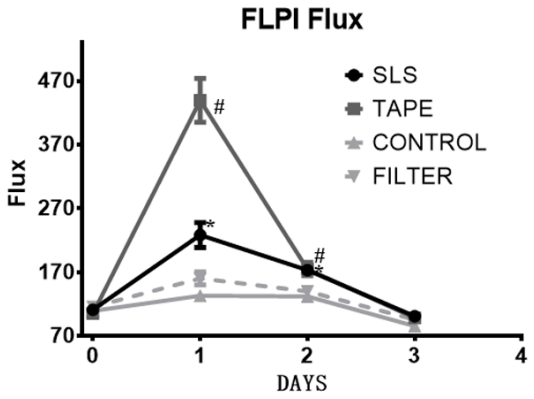

Figure 6. Flux by FLPI. Flux in SLS group was improved after intervention with the elevated flux values at D1 and D2, and it fell to the level of filter group at D3. Additionally, flux in the TAPE group was raised compared with the control group from D1 to D3 after intervention. Comparison between groups was determined by t test. P<0.05 was considered significant.

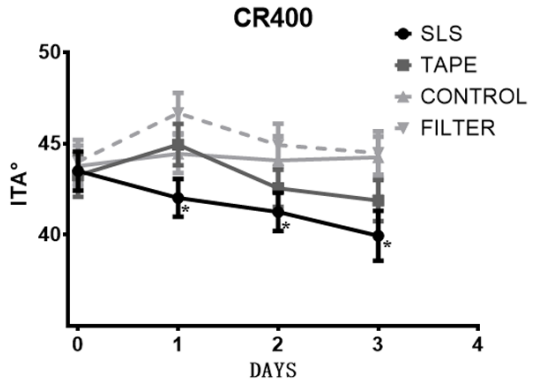

Figure 7. ITA° by CR400. SLS group displayed descending ITA° compared with the filter group at D2 to D4, meanwhile, no change of ITA° was found after TAPE intervention compared with the control group. Comparison between groups was determined by t test. P<0.05 was considered significant.

thickness by OCT and cuticle thickness by RCM was observed (R=0.055, P=0.290) (**Figure 8A**), and the correlation between roughness by OCT and SER by VC98 either (R=-0.060, P=0.247) (**Figure 8D**).

Correlation of similar parameters between devices at different time points

Correlation of parameters between different tools at each time point are displayed in **Tables 1**-**4**. At D4 after intervention in filter group, epidermal thickness by OCT was positively correlated with cuticle thickness by RCM (R=0.485, P=0.019) (**Table 1**). However, at D4 in control group, hydration by CM825 was positively correlated with homogeneity by VC98 (R=0.485, P=0.019) (**Table 3**). No other correlation was found between parameters by different devices.

Discussion

Our study applied various non-invasive devices to evaluate the response of skin in 23 healthy patients who were intervened by SLS and tape stripping. SLS and TAPE both worsened the skin conditions according to the ICD scores which were elevated after interventions. Compared with the control group, tape stripping produced the following major changes: (1) TEWL values which reflected the driage via skin barrier were increased after tape stripping; (2) Thickness related data were measured by OCT and RCM, which showed that a thicker epidermal thickness and a thinner cuticle thickness after tape stripping respectively, while the cuticle thickness finally recovered to normal level; (3) Roughness related data were generally decreased, with the roughness by OCT declined immediately but recovered to normal level, and the SER by VC98 reduced until long observation; (4) blood flow volume detected by OCT was unchanged, whereas flux by FLPI was raised. The main changes in the SLS group compared with the filter group were as followings: (1) TEWL was also elevated; (2) Both epidermal thickness by OCT and cuticle thickness by RCM in SLS group decreased while subsequently epidermal thickness elevated during long-time recovery; (3) Roughness by OCT showed an immediately reduce and a subsequent recovery, and SER by VC98 was declined; (4) Blood flow volume by OCT presented an immediately reduce followed by recovery to normal level, and flux by FLPI decreased.

Tape stripping is a traditional method in dermatological studies, which removes the layers of SC progressively. Basing on this consequence of tape stripping, it is applied as a model of acute irritant to skin or used to promote penetration rates of drugs [7]. SLS as a surfactant, its harmful effects on skin has been confirmed by several studies, thus it is widely applied to mimic the chemical irritants which penetrate into SC [8, 9]. Both tape and SLS led to damage of skin, furthermore, a repair response in the

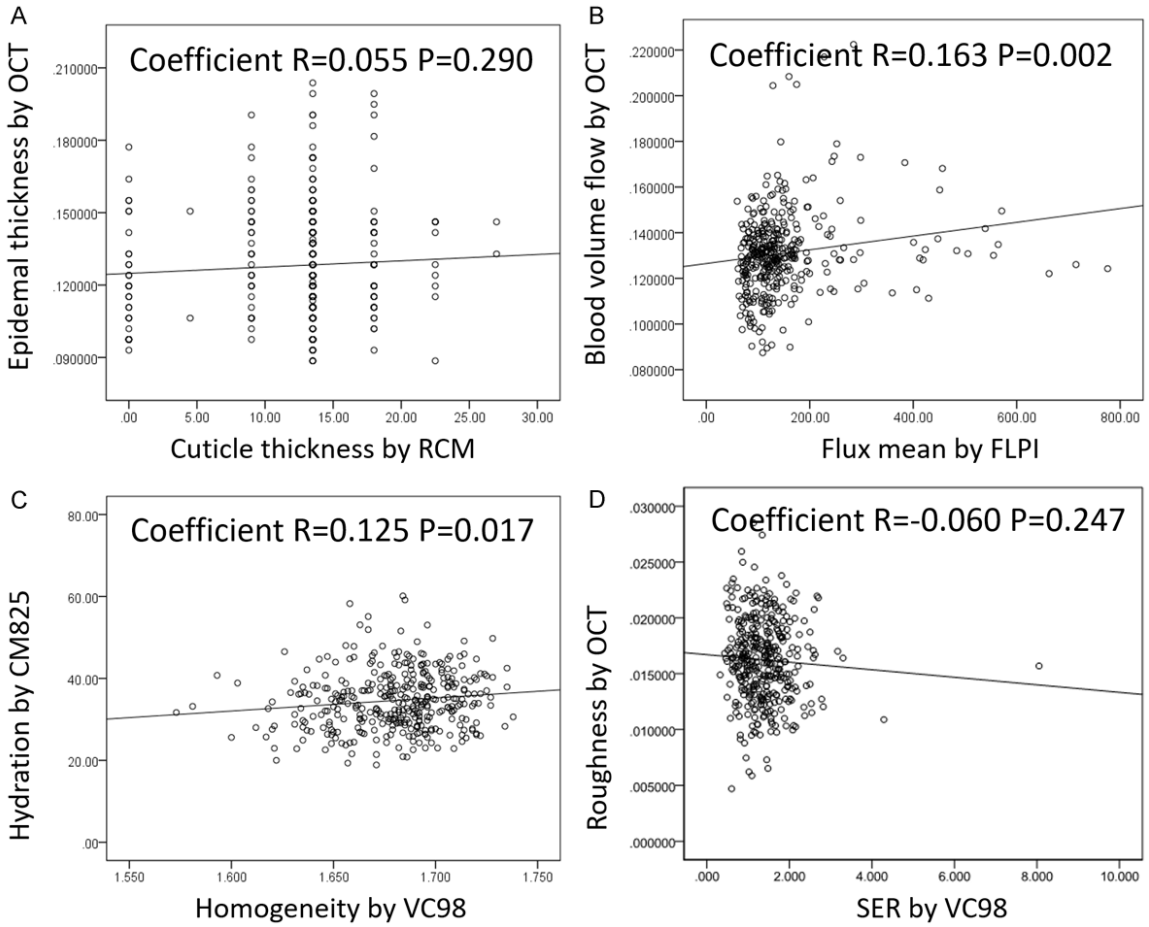

Figure 8. Correlation between different parameters by various devices. Pearson test was used to assess the correlation of similar parameters by different devices. A. No correlation between epidermal thickness by OCT and cuticle thickness by RCM was found. B. Blood volume flow by OCT was positively correlated with Flux mean by FLPI. C. Hydration by CM825 was positively correlated with homogeneity by VC98. D. No correlation between roughness by OCT and SER by VC98 was observed. P<0.05 was considered as significant.

Table 1. Correlation between epidermal thickness by OCT and cuticle thickness by RCM

	D0	D1	D2	D3
SLS group				
R	-	-0.389	-0.046	0.091
P value	-	0.067	0.835	0.680
TAPE group				
R	-0.139	-0.017	-0.015	-0.152
P value	0.526	0.940	0.944	0.489
Control group				
R	-0.133	-0.061	0.029	-0.033
P value	0.544	0.781	0.894	0.881
Filter group				
R	-0.248	-0.088	-0.238	0.485
P value	0.254	0.691	0.274	0.019

A Pearson correlation was used to determine the relationships between the variables. P<0.05 was considered significant.

underlying epidermis was stimulated, which may be visualized or quantified by devices. TEWL is a major index reflects the activity of SC barrier, and TM 210 is used in our study to measure TEWL through evaluating the passively diffused vapor via SC, thus the ability to keep moisture of skin is estimated [10]. Application of RCM is based on the focal point illumination and it allows visualization of different layers [11-13]. OCT possesses the capacity to generate 2D images of structure and collagen content in tissue as well as blood flow of skin, in which infrared light is introduced and echo time is assessed by interferometry [6]. Visioscan® VC 98, in which a camera and UV-A light video were used, provides images of skin and quantifies skin conditions such as roughness and smoothness by special evaluating technology [14, 15]. FLPI method obtains a distribution of

Table 2. Correlation between blood flow volume by OCT and Flux mean by US

	D0	D1	D2	D3
SLS group				
R	0.006	0.135	0.354	0.168
P value	0.978	0.539	0.097	0.444
TAPE group				
R	0.086	0.117	0.340	0.260
P value	0.696	0.594	0.113	0.230
Control group				
R	-0.251	0.105	-0.183	0.192
P value	0.248	0.634	0.404	0.380
Filter group				
R	-0.044	0.146	-0.009	0.161
P value	0.842	0.506	0.968	0.462

A Pearson correlation was used to determine the relationship between the variables. P<0.05 was considered significant.

Table 3. Correlation between hydration by CM825 and homogeneity by VC98

	D0	D1	D2	D3
SLS group				
R	0.034	-0.147	0.199	0.320
P value	0.878	0.503	0.361	0.137
TAPE group				
R	-0.235	0.051	-0.124	0.367
P value	0.281	0.817	0.574	0.085
Control group				
R	-0.006	-0.189	-0.145	0.485
P value	0.980	0.388	0.510	0.019
Filter group				
R	-0.060	0.074	-0.363	-0.103
P value	0.784	0.737	0.089	0.641

A Pearson correlation was used to determine the relationship between the variables. P<0.05 was considered significant.

blood flow speed in superficial surface through scanning and calculating blood flow in a few seconds [4]. Corneometer CM 825® is able to assess hydration of skin through determining capacitance with no direct galvanic contact [16]. Chromameter® CR400 is usually used to evaluate skin color [17].

A single blind, randomized and open study conducted by M. Paye et al. evaluates effect of cosmetics on skin with Reviscometer which is a non-invasive tool for skin evaluation, and it displays increased TEWL, elevated erythema as

Table 4. Correlation between roughness by OCT Ra and SER by VC98

	D0	D1	D2	D3
SLS group				
R	-0.049	-0.053	-0.099	-0.124
P value	0.825	0.881	0.654	0.573
TAPE group				
R	-0.139	-0.147	-0.480	-0.231
P value	0.527	0.504	0.021	0.289
Control group				
R	0.011	-0.301	0.419	-0.083
P value	0.960	0.163	0.047	0.707
Filter group				
R	0.088	0.006	-0.111	0.037
P value	0.689	0.978	0.614	0.869

A Pearson correlation was used to determine the relationship between the variables. P<0.05 was considered significant.

well as decreased hydration values at 8 days after intervention [18]. In another study in which eight techniques are applied, SLS irritation results in an increased TEWL as well as increased blood flow within 48 h followed by reduction [8]. Consistent with the previous study, raised TEWL, blood flow and erythema were also observed in the SLS group of our study. Interestingly, the maximum of these parameters in our study appeared at 24 h after intervention, the same as those in the previous study. Tape stripping is used in a study conducted by D. Falcone et al. which explores protein biomarkers on skin surface, and it leads to an immediate increase with the highest TEWL value occurring within 0-20 mins after tape stripping, while a continuous decline was observed from 24 h to 72 h [19]. Paulo R. Bargo et al. performed a study applying RCM to quantitatively determine rates for barrier recovery after tape stripping in six healthy participants, which displays that SC thickness elevated until 3 days after intervention and continually thickened to baseline level in the remaining observation period [3]. Partially in line with these studies, the TAPE group in our study also presented a decrease of TEWL from 24 h to 72 h, while SC thickness showed no change after tape stripping in our study. These results might own to the followings: (1) The barrier disruption by tape is due to the mechanical removal of corneocyte layers, thus diffused vapor via SC temporarily increase, while the recovery of SC turns to be prominent during longer observa-

tion and subsequently declines the TEWL value gradually; (2) Tape stripping in study of Paulo R. Bargo et al. was performed by average 45 times (range: 25-69) which is more than 25 times of our study, and it is revealed that the degree of irritant depends on the times of tape stripping. Thus more violent striping is conducted in previous study that might lead different response process during recovery.

Few studies explore the correlation between parameters detected by different non-invasive devices. A study conducted by M. Peppelman et al., which applies RCM with tape stripping model to evaluate skin recovery, displays a positive correlation of epidermal thickness data between RCM images and histology images, and this result confirms our hypothesis that analogous indexes from different devices may have strong correlations [20]. Different with the previous study, our study investigated correlations between parameters from different non-invasive techniques, and it disclosed a positive correlation between blood volume flow by OCT and flux by FLPI as well as the positive correlation between hydration by CM825 and homogeneity by VC98. These results in our study might be a result of insufficient water content in corneocytes leading to larger intercellular spaces and poor plumpness of corneocytes. Retaining moisture in SC attributes to improve homogeneity of skin.

Our study explored changes and recovery of skin post irritations, while it still has some limitations: (1) Sample size was relatively small; (2) Data for longer observation was unavailable; (3) Tolerance to tape stripping might differ in participants, while the tape group in our study served as a reference and this difference was minimized. The similar flushing skin might be used as a criterion of acute irritant in future study instead of uniform times of stripping.

In conclusion, our study disclosed changes of skin conditions after SLS and tape stripping irritations through various non-invasive detection methods, and disclosed a positive correlation between blood volume flow by OCT and flux by FLPI as well as a positive correlation between hydration by CM825 and homogeneity by VC98.

Acknowledgements

This study was supported by Shanghai Municipal Commission of Health and Family Planning Fund (No. 201440374).

Disclosure of conflict of interest

None.

Address correspondence to: Ou Qin, Department of Skin and Cosmetic Research, Shanghai Skin Disease Hospital, Shanghai 200443, China. Tel: +86-21-61833073; Fax: +86-21-61833062; E-mail: drqinou@163.com

References

[1] Welzel J, Reinhardt C, Lankenau E, Winter C and Wolff HH. Changes in function and morphology of normal human skin: evaluation using optical coherence tomography. Br J Dermatol 2004; 150: 220-225.

[2] Gladkova ND, Petrova GA, Nikulin NK, Radenska-Lopovok SG, Snopova LB, Chumakov YP, Nasonova VA, Gelikonov VM, Gelikonov GV, Kuranov RV, Sergeev AM and Feldchtein FI. In vivo optical coherence tomography imaging of human skin: norm and pathology. Skin Res Technol 2000; 6: 6-16.

[3] Bargo PR, Walston ST, Chu M, Seo I and Kollias N. Non-invasive assessment of tryptophan fluorescence and confocal microscopy provide information on skin barrier repair dynamics beyond TEWL. Exp Dermatol 2013; 22: 18-23.

[4] Du Z, Zan T, Li H and Li Q. A study of blood flow dynamics in flap delay using the full-field laser perfusion imager. Microvasc Res 2011; 82: 284-290.

[5] Wassef C and Rao BK. Uses of non-invasive imaging in the diagnosis of skin cancer: an overview of the currently available modalities. Int J Dermatol 2013; 52: 1481-1489.

[6] Silver R, Helms A, Fu W, Wang H, Diaconu D, Loyd CM, Rollins AM and Ward NL. Using optical coherence tomography for the longitudinal non-invasive evaluation of epidermal thickness in a murine model of chronic skin inflammation. Skin Res Technol 2012; 18: 225-231.

[7] Ashtikar M, Matthaus C, Schmitt M, Krafft C, Fahr A and Popp J. Non-invasive depth profile imaging of the stratum corneum using confocal Raman microscopy: first insights into the method. Eur J Pharm Sci 2013; 50: 601-608.

[8] Fluhr JW, Kuss O, Diepgen T, Lazzerini S, Pelosi A, Gloor M and Berardesca E. Testing for irritation with a multifactorial approach: comparison of eight non-invasive measuring techniques on five different irritation types. Br J Dermatol 2001; 145: 696-703.

[9] Slotosch CM, Kampf G and Loffler H. Effects of disinfectants and detergents on skin irritation. Contact Dermatitis 2007; 57: 235-241.

[10] Schliemann S, Schmidt C and Elsner P. Tandem repeated application of organic solvents and sodium lauryl sulphate enhances cumulative skin irritation. Skin Pharmacol Physiol 2014; 27: 158-163.

[11] Robertson K and Rees JL. Variation in epidermal morphology in human skin at different body sites as measured by reflectance confocal microscopy. Acta Derm Venereol 2010; 90: 368-373.
[12] Fan C, Luedtke MA, Prouty SM, Burrows M, Kollias N and Cotsarelis G. Characterization and quantification of wound-induced hair follicle neogenesis using in vivo confocal scanning laser microscopy. Skin Res Technol 2011; 17: 387-397.
[13] Haroon A, Shafi S and Rao BK. Using Reflectance Confocal Microscopy in Skin Cancer Diagnosis. Dermatol Clin 2017; 35: 457-464.
[14] Ali A, Akhtar N and Chowdhary F. Enhancement of human skin facial revitalization by moringa leaf extract cream. Postepy Dermatol Alergol 2014; 31: 71-76.
[15] Wakeman MP. An open-label forearm-controlled pilot study to assess the effect of a proprietary emollient formulation on objective parameters of skin function of eczema-prone individuals over 14 days. Clin Cosmet Investig Dermatol 2017; 10: 275-283.
[16] Momota Y, Shimada K, Minorikawa N, Gin A, Matsubara T, Nakamura Y, Katayama M and Sako T. Application of three hygrometers under different skin conditions in dogs: dry, moist and haired skin. Vet Dermatol 2017; 28: 554-e131.
[17] Xia MN, Wang XM, Liu YQ, Yuan C, Zou Y and Fan GB. Relationship between skin parameters (darkness or thickness) and photoreaction of Chinese Han skin. Cutan Ocul Toxicol 2012; 31: 306-311.
[18] Paye M, Mac-Mary S, Elkhyat A, Tarrit C, Mermet P and Humbert PH. Use of the reviscometer for measuring cosmetics-induced skin surface effects. Skin Res Technol 2007; 13: 343-349.
[19] Falcone D, Spee P, Salk K, Peppelman M, van de Kerkhof PCM and van Erp PEJ. Measurement of skin surface biomakers by transdermal analyses patch following different in vivo models of irritation: a pilot study. Skin Res Technol 2017; 23: 336-345.
[20] Peppelman M, van den Eijnde WA, Jaspers EJ, Gerritsen MJ and van Erp PE. Combining tape stripping and non-invasive reflectance confocal microscopy : an in vivo model to study skin damage. Skin Res Technol 2015; 21: 474-484.