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Letter to the Editor

Water resistance profile as a marker of skin barrier damage in atopic dermatitis patients

Keywords

Barrier function
Depth-dependency
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Environmental insults
Non-invasive measurement



The stratum corneum (SC) is the main barrier of the skin, offering a first line of defence against external pathogens and insults. The SC of patients with atopic dermatitis (AD) has underlying barrier deficiencies, even in non-lesional sites [1]. A defective SC barrier is more vulnerable to environmental insults, which trigger immune reactions, further deteriorating the barrier [2].

Transepidermal water loss (TEWL) is a commonly used measurement to assess SC barrier function, but does not provide information about the depth-dependent characteristics. In this study, we attempted to establish a method to visualise the depth-dependent barrier profiles and evaluate barrier deficiency by applying a recently proposed mathematical method [3] to clinical datasets from AD and healthy skin. The method calculates the resistance of SC to water diffusion along the SC depth, and thereby quantifies and visualises the depth-dependent barrier function, through integration of data of TEWL and water concentration profiles measured by confocal Raman microspectroscopy [4]. In order to evaluate the efficiency of the proposed method, we addressed the hypotheses that the subclinical damage in AD non-lesional skin can be quantified and visualised by a depth-dependent barrier resistance profile, and that the degree of the barrier deficiency in AD non-lesional skin is similar to that in mechanically- or chemically-insulted healthy skin by abrasion or exposure to sodium lauryl sulphate (SLS).

We first calculated the depth-dependent resistance from data collected in our previous studies in which Raman spectra and TEWL were measured in AD patients (Supplementary data, from Kezic et al., in preparation) and healthy controls [5] (Fig. 1a). We also calculated the SC thickness from the water concentration profiles by the method described previously [6]. The AD individuals were divided into three groups with high (>20.8), mid (16.8–20.8) and low (<16.8) TEWL values ($\text{g m}^{-2} \text{h}^{-1}$) to reflect interpersonal differences in severity of AD barrier deficiency. All three groups show a significantly lower resistance and a lower SC thickness compared to the healthy control, which has a TEWL value of 7.8. The differences in the peak resistance value (R_{max}) between the control and each of the AD groups are significant (Student's *t*-

test, $p < 0.0001$), indicating the effectiveness of the proposed method in characterising the disease status of the SC.

Next, we evaluated the effects of skin insults by SLS exposure and abrasion on healthy skin. Resistance profiles were calculated from TEWL values and water concentration profiles measured after application of a patch with 1% SLS solution to the forearm for 24 h [7] and after abrasion [8]. Abrasion was accomplished by passing 320J emery paper (3 M Company, Maplewood, MN, USA) over forearm skin ten times until TEWL increased by ≥ 20 units [8]. The obtained resistance profiles clearly demonstrate the spatio-temporal effects of the damage and recovery incurred by different insults on the depth-dependent SC barrier function (Fig. 1b and c).

The resistance profile immediately after the SLS insult (Day 0) demonstrates an overall decrease throughout the SC, with a shift of the peak towards the SC surface due to thinning of the SC (Figs. 1b and 2a), followed by gradual increase in both the resistance values and SC thickness during the recovery phase (Days 1–10). A similar overall reduction in the resistance profile throughout the SC is observed after abrasion.

The SLS exposure showed a slower recovery of water resistance profile than abrasion, with time constants $\tau = 9.5$ vs 5.5 calculated by fitting the recovery curves of R_{max} to a negative exponential function ($1 - ae^{-t/\tau}$) (Fig. 2b). SLS structurally damages the main constituent barrier components, including alteration of SC lipid organisation [9] and a significant reduction of natural moisturizing factor (NMF) components [7] throughout the SC. Repair of the damaged barrier requires replenishment of its components, for example by increased expression of involucrin, transglutaminase 1, and profilaggrin [10]. Recovery of water resistances from SLS damage therefore takes longer than the recovery from the removal of only the top layers of the SC by abrasion, via increased cornification.

Finally, resistance profiles of AD skin were compared to those of healthy individuals following insults. The conformity between the different data sets was confirmed by the correspondence of the resistance profiles for the healthy control (Fig. 1a) and pre-insult (Fig. 1b and c).

The resistance profiles were compared quantitatively by three indices; the peak resistance values (R_{max} , Fig. 2b), the rate of production of the resistance from the bottom of the SC (*proR*, Fig. 2c), and that of degradation towards the top (*degR*, Fig. 2d) [3]. There is a striking resemblance in the resistance profiles for the AD high TEWL group and those for the healthy individuals immediately after SLS exposure and after abrasion (Fig. 1), demonstrated by similar values for all the calculated indices (Fig. 2b–d). Agreement between all three indices suggests that the physical property of the barrier of AD patients' non-lesional skin is similar to that of healthy skin affected by SLS or abrasion, despite differences in the underlying mechanisms of barrier deficiency. For example, mechanisms of barrier formation might be altered by a feedback regulation that is activated upon barrier damage in skin

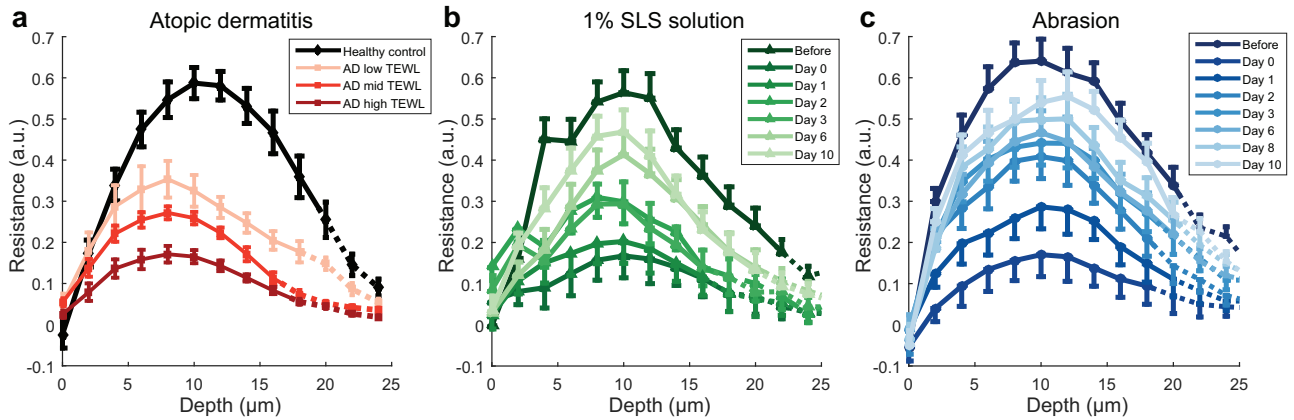


Fig 1. Resistance profiles for non-lesional skin from AD patients and for healthy skin following insults. Mean resistance profiles for (a) AD non-lesional skin ($n = 37$) with high (>20.8), mid (16.8 – 20.8) and low (<16.8) TEWL ($\text{g m}^{-2} \text{h}^{-1}$) and healthy controls ($n = 13$), and for healthy skin before and after (b) SLS exposure ($n = 20$) and (c) abrasion ($n = 18$). Resistances within and outside of the SC thickness are shown by solid and dotted lines, respectively. Error bars indicate the standard error of the mean. In (b) and (c), error bars are shown either above or below the mean.

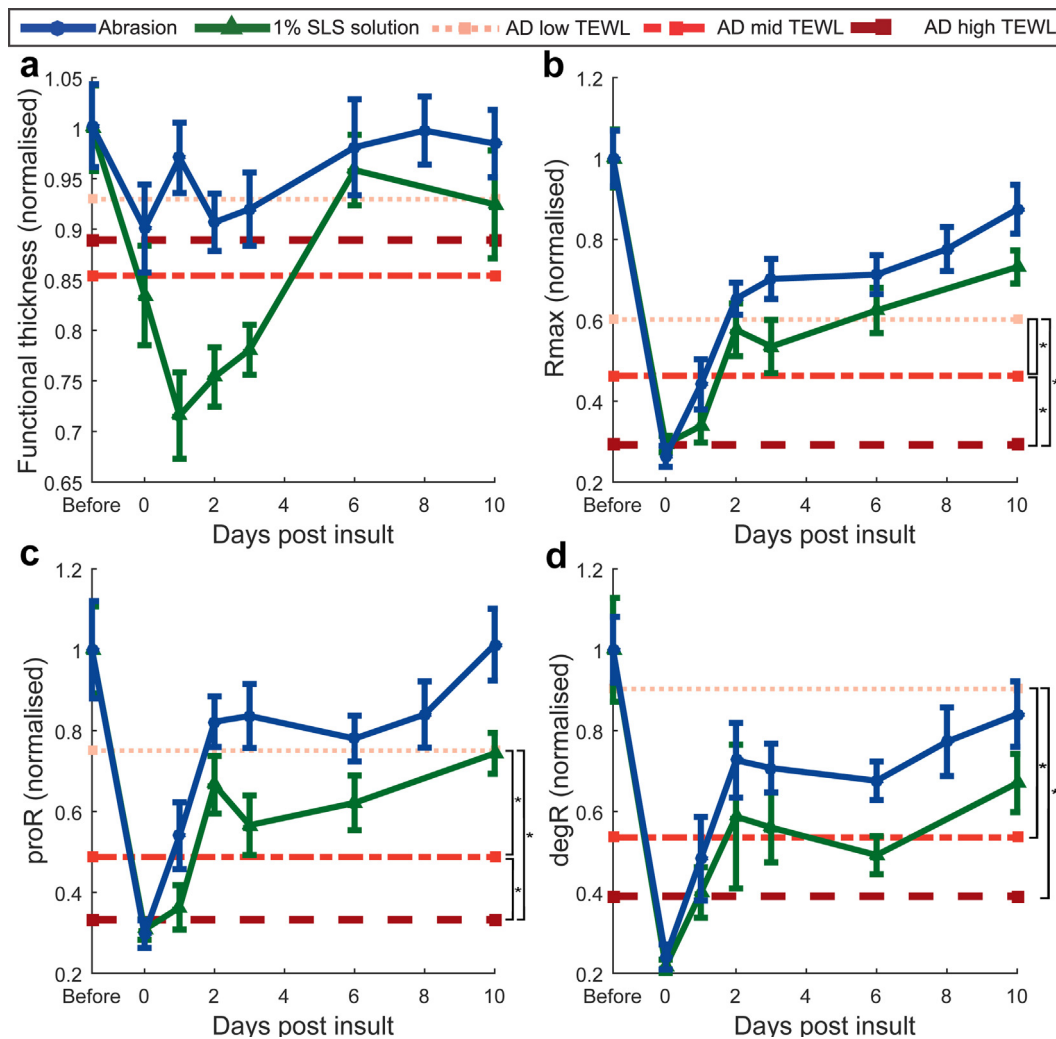


Fig 2. Quantification of damage and recovery of barrier function from insults on healthy skin and its comparison to AD non-lesional skin. Four indices are used to compare AD non-lesional skin ($n = 37$), healthy controls ($n = 13$), healthy skin before and after SLS exposure ($n = 20$) and abrasion ($n = 18$): (a) the functional SC thickness, (b) the maximum resistance value (R_{max}), (c) the spatial rate of resistance production (proR) and (d) the spatial rate of resistance degradation (degR). The indices are normalised to the values before the insults, or normalised to healthy controls for the AD groups. Significance between the AD group indices (student t -test, $p < 0.05$) is indicated by *. Error bars indicate the standard error of the mean.

following SLS exposure or abrasion, while the mechanisms of barrier formation are known to be altered in AD patients, for example by an impaired lamellar body secretion or a filaggrin deficiency, limiting the formation of the lipid envelope and NMF [2]. The indices for the mid and low TEWL groups are similar to those in the recovery phase from abrasion and SLS exposure, suggesting that the three AD groups have different abilities to repair and maintain the barrier, possibly due to variability in the magnitude of deficiency in filaggrin levels or lamellar body secretion.

In conclusion, we demonstrated that the resistance profiles successfully quantified and visualised the depth-dependent barrier deficiency using clinical datasets, confirming the efficiency of the proposed method. Interestingly, the resistance profiles of non-lesional skin of AD patients were similar to those of healthy individuals following chemical (SLS) or mechanical (abrasion) insults, suggesting that these different pathological conditions may have similar structural abnormalities, although the underlying mechanisms and the recovery kinetics are different.

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Conflict of interest

GNS is an employee of Johnson & Johnson Santé Beauté France, which funded this work. PJC is an employee of RiverD International BV, which manufactured the Raman spectroscopy instruments used in this work. DRH and DWK are employees of Kimberly-Clark Corporation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jdermsci.2015.10.017>.

References

- [1] M. Suárez-Fariñas, S.J. Tintle, A. Shemer, A. Chiricozzi, K. Nogales, I. Cardinale, et al., Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities, *J. Allergy Clin. Immunol.* 64 (2011) e1–e4.
- [2] M.J. Cork, S.G. Danby, Y. Vasilopoulos, J. Hadgraft, M.E. Lane, M. Moustafa, et al., Epidermal barrier dysfunction in atopic dermatitis, *J. Invest. Dermatol.* 129 (2009) 1892–1908.
- [3] M.D.A. Van Logtestijn, E. Domínguez-Hüttinger, G.N. Stamatas, R.J. Tanaka, Resistance to water diffusion in the stratum corneum is depth-dependent, *PLoS One* 10 (2015) e0117292.

- [4] P.J. Caspers, G.W. Lucassen, E.A. Carter, H.A. Bruining, G.J. Puppels, In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles, *J. Invest. Dermatol.* 116 (2001) 434–442.
- [5] S. Kezic, P.M.J.H. Kemperman, E.S. Koster, C.M. de Jongh, H.B. Thio, L.E. Campbell, et al., Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum, *J. Invest. Dermatol.* 128 (2008) 2117–2119.
- [6] S. Bielfeldt, V. Schoder, U. Ely, A. Van Der Pol, J. De Sterke, K. Wilhelm, Assessment of human stratum corneum thickness and its barrier properties by in-vivo confocal Raman spectroscopy, *IFSCC Mag.* 12 (2009) 9–15.
- [7] D.R. Hoffman, L.M. Kroll, A. Basehoar, B. Reece, C.T. Cunningham, D.W. Koenig, Immediate and extended effects of sodium lauryl sulphate exposure on stratum corneum natural moisturizing factor, *Int. J. Cosmet. Sci.* 36 (2014) 93–101.
- [8] D.R. Hoffman, L.M. Kroll, A. Basehoar, B. Reece, C.T. Cunningham, D.W. Koenig, Immediate and extended effects of abrasion on stratum corneum natural moisturizing factor, *Skin Res. Technol.* 1 (2015) 7.
- [9] P. Saad, C.R. Flach, R.M. Walters, R. Mendelsohn, Infrared spectroscopic studies of sodium dodecyl sulphate permeation and interaction with stratum corneum lipids in skin, *Int. J. Cosmet. Sci.* 34 (2012) 36–43.
- [10] H. Törmä, M. Lindberg, B. Berne, Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo, *J. Invest. Dermatol.* 128 (2008) 1212–1219.

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