Letter to the Editor

Topical niacinamide enhances hydrophobicity and resilience of corneocyte envelopes on different facial locations

The stratum corneum (SC) undergoes a variety of catabolic and anabolic reactions towards its outer surface layers in preparation for its external assault from the terrestrial environment [1]. These events are essential for the formation of a healthy barrier. Key is the maturation of the corneocyte envelope (CE) (Fig. 1). Morphologically, CE's appear fragile in the deeper layers of the SC and more rigid in the outer layers, the mechanics of which have been confirmed with several biomechanical approaches [2]. Early methods to assessing the maturity of the CE's were based upon staining with tetramethylrhodamine isothiocyanate (TRITC) where rigid CEs stain more intensely than fragile ones [3]. Later, their protein content was assessed by involucrin immunochemistry and their lipid content by Nile red staining which was then expressed as a ratio [4-9]. Using these approaches, improvements in CE maturity of the volar forearm and the legs have been observed with moisturizers containing glycerol [3,10] and niacinamide [11].

We have recently published on the regional complexity of facial skin using a variety of bio-instrumental techniques [12,13] and also developed new CE maturation assays to further probe the corneocyte lipid envelope (CLE) hydrophobicity and corneocyte protein envelope (CPE) structural integrity as independent parameters [14]. These findings prompted us to assess the effect of niacinamide in a 4-week placebo-controlled, randomized topical study on these biochemical and physical parameters on several facial skin sites in young ($n = 24$, 20.8 ± 1.7 years old) and old Caucasian study participants ($n = 24$, 57.5 ± 2.9 years old) living in Pretoria, South Africa. The study was approved by the School of Health Care Sciences Research Committee (SRC) together with the Medunsa Campus Research and Ethics Committee (MREC), South Africa, and took place from July to September 2016. All participants provided written informed consent before start of the study.

After a three day wash-out phase, either the vehicle or the test cream including 3% of niacinamide (Table 1) were applied twice daily (morning and evening) onto the face after cleansing. The subjects were randomly assigned to use either the vehicle or the test cream. A simple but panellist-acceptable vehicle was chosen with a suitable penetration enhancer, in this case 1–2 propanediol. 2%

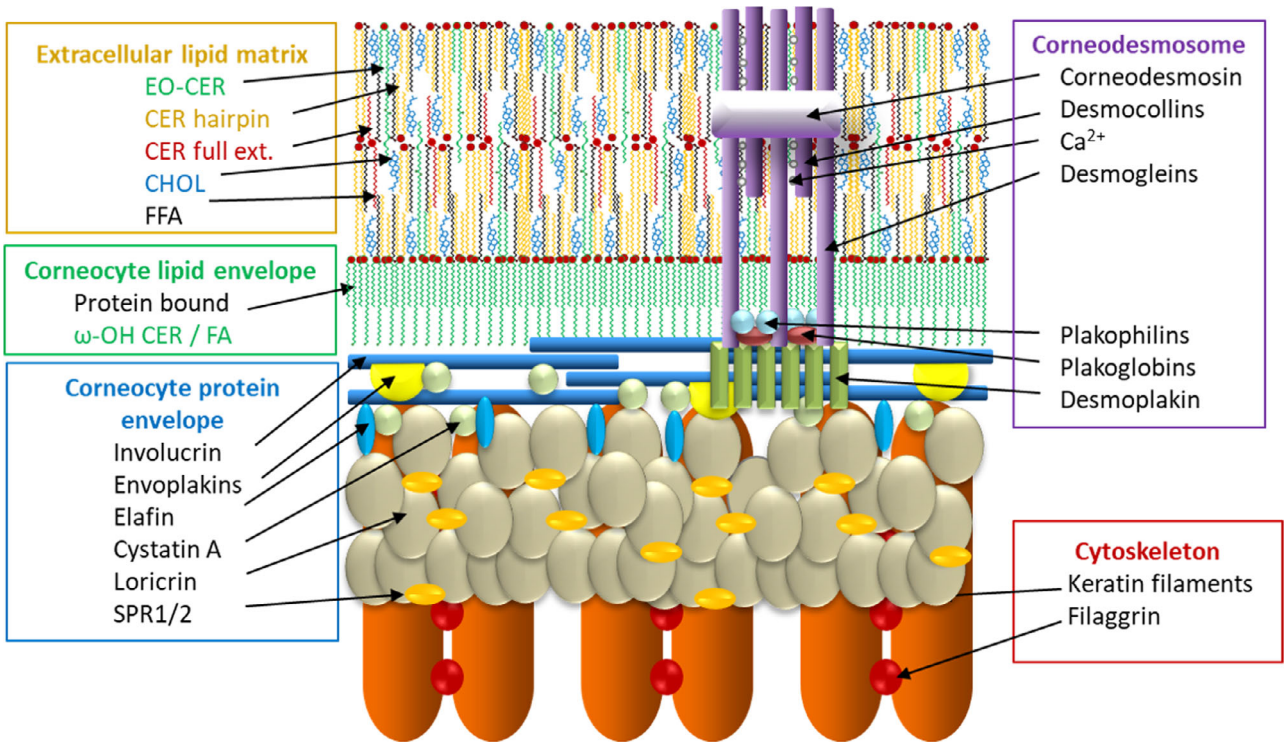

Figure 1 Model of the corneocyte envelope (CE). An early step in the cornification process is the formation of the intercellular cytoskeleton mainly composed of keratin filaments and filaggrin. Cross-linking of proteins, mainly of loricrin and involucrin form the rigid inner corneocyte protein envelope (CPE). Then, a lipid monolayer, the corneocyte lipid envelope (CLE), is covalently attached to the CPE. The CLE serves as a scaffold for the lamellar organization of the extracellular lipid matrix. Courtesy of Thomas Schmitt, Halle University, DE.

Table 1 INCI list of test creams

Vehicle (pH 5.7): Aqua, caprylic/capric triglyceride, coco-caprylate, decyl cocoate, propanediol, glyceryl stearate, stearic acid, cetearyl glucoside, polyacrylamide, C13-14 isoparaffin, laureth-7, phenoxyethanol, ethylhexylglycerin, xanthan gum, parfum, sodium hydroxide.
Active (pH 5.8): Aqua, caprylic/capric triglyceride, coco-caprylate, decyl cocoate, propanediol, niacinamide, glyceryl stearate, stearic acid, cetearyl glucoside, polyacrylamide, C13-14 isoparaffin, laureth-7, phenoxyethanol, ethylhexylglycerin, xanthan gum, parfum, sodium hydroxide.

[15] and 5% [11] concentrations of niacinamide have been used in the past to evaluate its clinical efficacy. 3% was selected as an intermediary concentration in these studies.

The application dosage was approximately 2 mg cm^{-2} of skin. Single tape-strippings (D-Squames) were taken on four pre-defined facial sites (central forehead (CF), cheek, 3 cm vertically beneath the outer edge of the eye (CH), top nasolabial sulcus (NT) and midpoint nasolabial sulcus (NM)) before the first product application and after the four weeks treatment phase (Fig. 2) as previously reported [14]. The CLE and CPE were assessed using our recently published methods [14].

Microsoft Excel Office 2016 was used to analyse the data. Normality was evaluated using the Kolmogorov–Smirnov statistical test. Paired *t*-test was used to compare means and investigate differences between sites, and probability of $P < 0.05$ was considered statistically significant. All results are presented as mean and standard error of the mean (SEM).

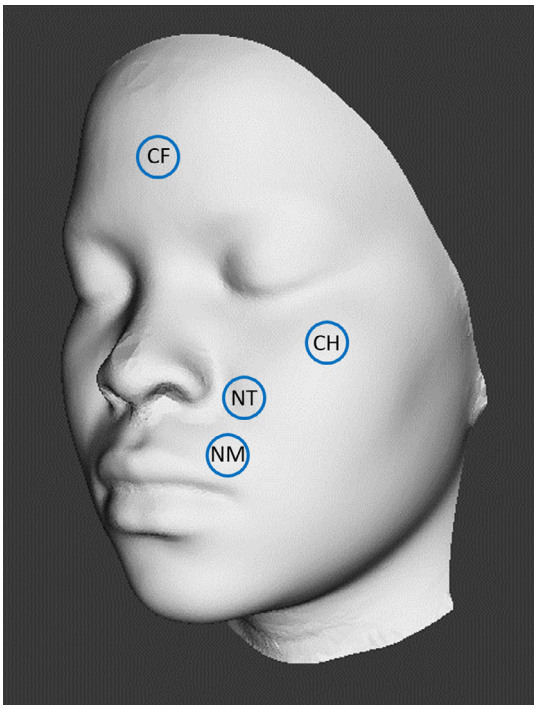

Figure 2 Facial test sites, central forehead (CF), cheek (CH) (3 cm vertically beneath the outer edge of the eye), top nasolabial sulcus (NT), midpoint nasolabial sulcus (NM).

We first performed baseline comparisons of the effect of ageing on CE maturation parameters (size, hydrophobicity, rigidity and relative CE maturity (RCEM)). Others have observed an ageing-related increase in corneocyte size that we also observed except for the NT site (Fig. 3a). More pronounced changes were shown for hydrophobicity (Fig. 3b) and sonication-stress resilience (Fig. 3c). Interestingly for the former, increases were observed on the CF and CH areas with age whereas the other two sites were less hydrophobic (Fig. 3b). These results further demonstrate the complexity in facial ageing and the sensitivity of our assays as previous studies have found no age-related changes in the Nile red to involucrin comparison [16]. A decrease in hydrophobicity was expected as reduced levels of covalently bound lipids have been observed on forearm SC as well as of substrate and associated enzymes necessary for their attachment in photoaged skin sites. This could be the reason for the decline in the hydrophobicity in the aged nasolabial areas as these facial sites are those recently reported to have the highest levels of oxidative stress [17]. The ageing-related decreases in CE rigidity were consistent across all four sites (Fig. 3c). When considering all three measures as the RCEM (Fig. 3d), only the cheek had a small increase in maturation with ageing whereas the nasolabial areas were consistently immature. These results highlight the complexity of facial site CE maturation status and the impact of ageing. The changes we observe with ageing relative to the lack of change in other studies may highlight the increased sensitivity of our methodology.

Next, we examined the effect of niacinamide on these parameters. Only a small increase in corneocyte size was found following treatment on the NT site (not shown). Numerical increases in size were observed on the other test sites and are in agreement with other studies [11]. Hydrophobicity and CE rigidity increased in all facial sites and both age groups which was then reflected in the RCEM (Fig. 4a-c). Nevertheless, there was a marked vehicle effect suppressing CE maturation that has also been demonstrated in previous studies [11]. Despite the vehicle effect, the biggest changes in hydrophobicity were observed on the CF for the young subjects and the NT for the older subjects. The NM had the least change in both age groups. The biggest changes in CE rigidity were found in the older subjects probably reflecting their lower starting levels. Clearly, niacinamide treatment improved CE maturation as measured by our methodology but CE hydrophobicity is not improved as much in the NM area.

Our data on the effects of niacinamide on facial skin are largely consistent with another publication using the Nile red/involucrin ratio following treatment of the volar forearm [11]. However, using our new methods, we specifically demonstrated the differences in increased CE hydrophobicity and increased mechanical resilience following treatment with niacinamide.

Increase in CE hydrophobicity from ceramide attachment results from a variety of enzyme processing steps on intercellular ceramide EOS-linoleate and one of the key enzymes in that processing is 12R-lipoxygenase (12R-LOX) [18]. 12R-LOX is the first maturation enzyme and acts as a primer for the enzyme cascade forming the CLE. We have previously reported its reduced mass and activity levels in photodamaged facial SC [19,20]. Also, transglutaminase (TG) activity which is believed to be involved in the final attachment of ceramides to the CE is also known to be decreased in facial SC [19]. Other enzymes are possibly involved in lipid attachment in this cascade such as short-chain dehydrogenase/reductase family

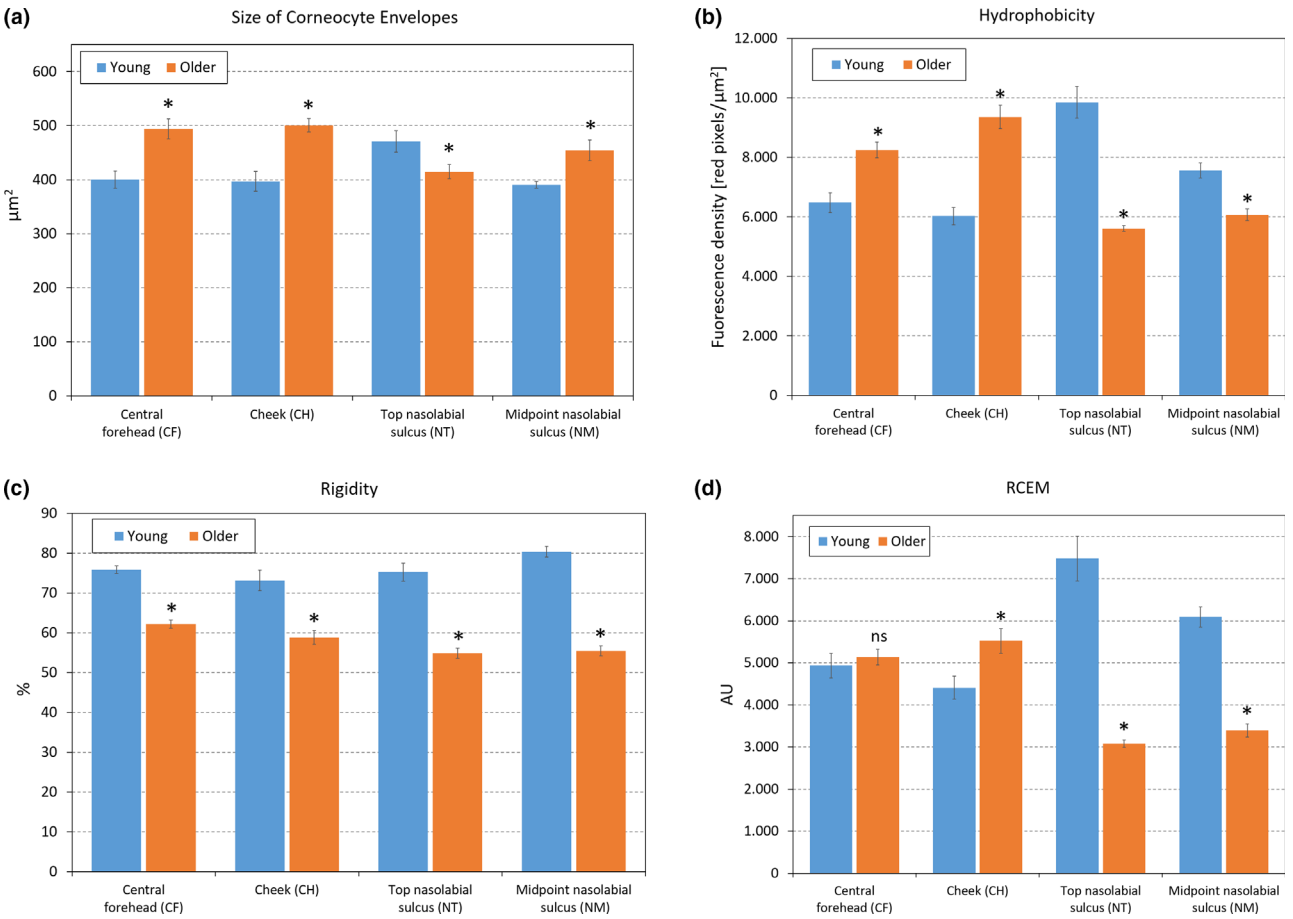

Figure 3 Baseline data, differences of CE maturation parameters (size (a), hydrophobicity (b), rigidity (c), RCEM (d)) between young and aged facial SC. Data are mean ± SEM, statistical comparison young vs. old, * $P < 0.05$, ns not significant.

9C member 7 (SDR9C7). However, our proteomic work showed no deficiency in its mass levels in facial SC but we have not measured activity levels [19,21]. Niacinamide has been reported to increase keratinocyte ceramide biosynthesis [15] and, although it is speculative, it is highly likely that it also increases the expression of 12R-LOX and TG especially as niacinamide is reported to increase the gene expression of peroxisomal proliferator activated receptor-alpha (PPARα), a key nuclear hormone receptor involved in keratinocyte differentiation and corneocyte formation [22].

The development of a healthy SC involves a well-orchestrated series of enzymatic steps enabling lipid, natural moisturizing factor and CE formation and maturation [1]. The effects of niacinamide on CLE hydrophobicity and CPE integrity are consistent with improved CE maturation, a key event in healthy skin. However, using the forearm as a surrogate testing site does not reveal the full picture of the effect of niacinamide on different facial skin sites [11-13]. The negative effect of the vehicle in this study and previous studies, most likely caused by its 1-2 propanediol content, shows many similarities to the effects of aqueous cream and its use in cosmetic products should be minimized [9,23]. It was demonstrated that 1-2 propanediol can increase the activity of the desquamatory serine protease kallikrein 7 (KLK7) [24] and the negative effect of increasing protease activity on CE maturation has been shown to be mitigated with protease inhibitors [25]. However, these study results support further investigations into the effect of niacinamide on ceramide EOS-linoleate levels together with enzymes involved in its processing and attachment to the CE. It is possible that the regional differences in skin permeability may reflect differences in barrier function as facial barrier function is complex [26].

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Conflicts of interests and disclosures

RV is an employee of DSM, AVR is a consultant to DSM and MC is an employee of Newtone Technologies. BS, DG and MEL have no conflict of interest.

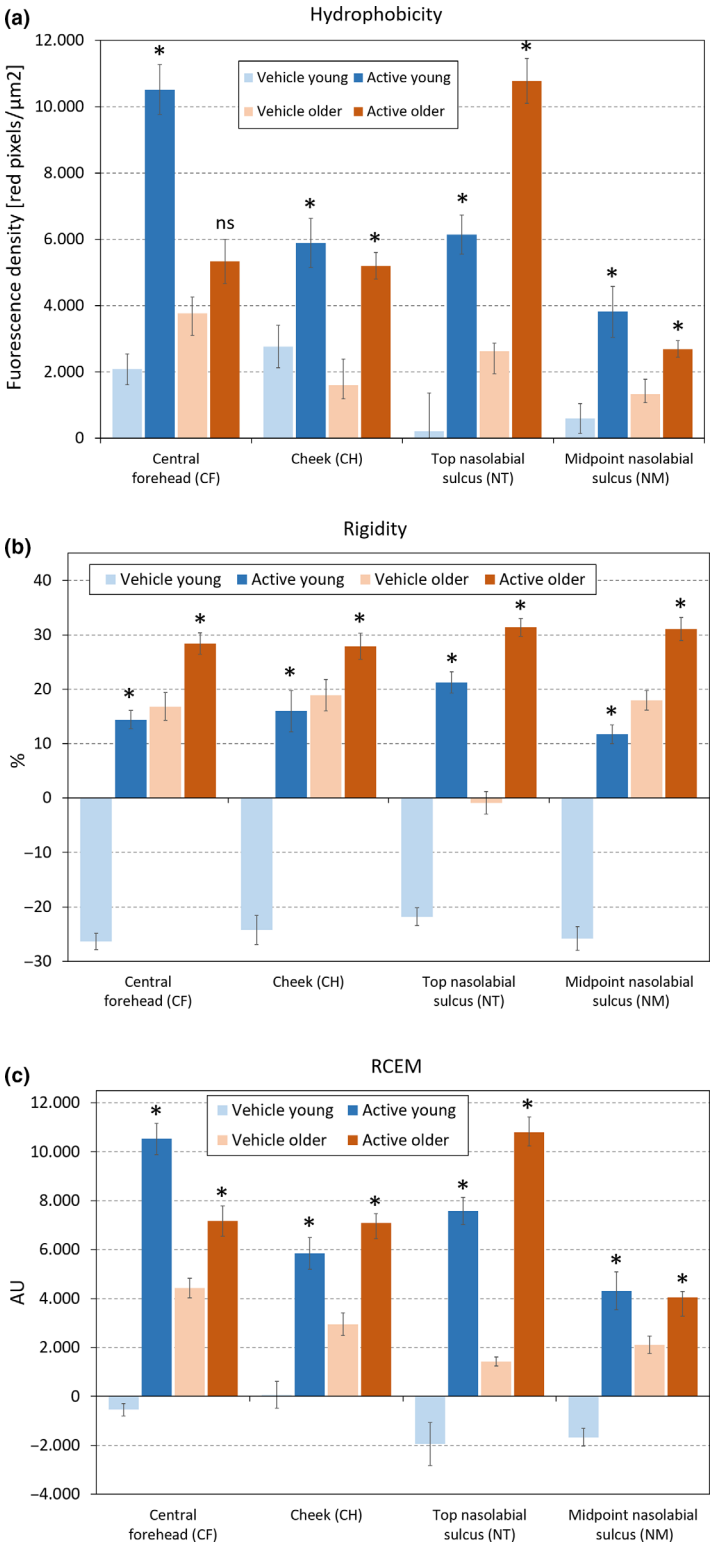

Figure 4 Baseline corrected data of the impact of topically applied niacinamide on CE maturation parameters (hydrophobicity (a), rigidity (b), RCEM (c)) in young and aged facial SC. Data are mean ± SEM, statistical comparison baseline vs. treatment, * $P < 0.05$, ns not significant.

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