



## Ectoine disperses keratin and alters hydration kinetics in stratum corneum

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### ABSTRACT

Moisturizing compounds are commonly applied topically to human stratum corneum (SC). Many types of molecular species are employed, most commonly including humectants and occlusives. We find new evidence of keratin dispersion caused by the moisturizing compound ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinocarboxylic acid), and provide the first characterization of its impacts on the hydration kinetics and biomechanics of SC. A second compound, 2-(2-hydroxyethoxy)ethylguanidine succinate (HEG) was investigated for comparison. A suite of biomechanical and biochemical assays including FTIR, drying stress, and cellular cohesion were used. Studies were conducted on normal, lipid-extracted, and lipid plus natural moisturizing factor extracted SC. Ectoine was found to improve the dispersity and hydration of keratin bundles in corneocytes. It also decreased rates of stress development in lipid extracted SC when exposed to a dry environment by ~30% while improving stress reduction during rehydration by ~20%. Peak stresses were increased in harsh drying environments of <5% RH, but SC swelling measurements suggest that water retention was improved in ambient conditions. Further, changes up to ~4 J/m<sup>2</sup> were seen in cohesion after ectoine treatments, suggesting corneodesmosome interactions. HEG was tested and found to disperse keratin without impacting corneodesmosomes. These results indicate that keratin dispersants produce beneficial effects on SC hydration kinetics, ultimately resulting in higher SC hydration under ambient conditions.

### 1. Introduction

Proper hydration is a key component of healthy stratum corneum (SC). Hydration impacts visual and sensory perception of skin, and our ability to detect tactile signals transmitted through the SC. These effects are largely mediated by alterations to the biomechanical properties of the SC [1–5]. Keratin, which comprises the largest volume fraction of the SC and is its major load-bearing component, is especially sensitive to changes in moisture content, though corneodesmosome (CD) binding structures and intercellular lipids are also affected.

Previous work has explored the impacts of topical treatments on the barrier function and intercellular cohesion of SC, which are largely governed by the lipids and CDs (respectively) [6–18]. The intercellular lipid bilayers and corneodesmosome protein junctions have been particularly emphasized. However, the interactions of active compounds with the keratin bundles within the corneocytes from a mechanical perspective remain largely unexplored. This is surprising given that keratin makes up the largest volume fraction of the SC and is its major

load-bearing component. Compounds which improve the hydration and distribution of keratin within corneocytes are becoming increasingly important in skin-care technologies. As such, understanding the biomechanical effects of these ingredients is critical, especially if secondary interactions with other SC components exist.

We applied a suite of biomechanical and biochemical assays to probe the effects of the moisturizing compound ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinocarboxylic acid, Fig. 1a) on the kinetics of water transport through human stratum corneum (SC). This resulted in marked changes in biomechanical properties and perception. Studies were conducted on normal, lipid-extracted, and lipid plus natural moisturizing factor (NMF) extracted SC. Ectoine was found to improve the dispersity and hydration of keratin bundles in corneocytes, to a higher degree than water hydration alone (Fig. 1b). It also decreased the rate at which mechanical stresses in lipid extracted SC developed when exposed to a dry environment by ~30% while improving stress reduction rates due to rehydration by ~20%. Ectoine increased peak drying stress in harsh drying environments of <5% RH, but SC swelling measurements

*Abbreviations:* HDX, hydrogen-deuterium exchange; DCB, double cantilever beam; AE, acetone/ether; AE/W, acetone/ether followed by water; HEG, 2-(2-hydroxyethoxy)ethylguanidine succinate.

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suggest that water retention is improved in ambient conditions. Further, changes of up to  $\sim 4 \text{ J/m}^2$  were seen in corneocyte cohesion after ectoine treatments, suggesting that this compound interacts with the corneodesmosome binding proteins of the SC. The compound 2-(2-hydroxyethoxy)ethylguanidine succinate (HEG) was tested for comparison and found to disperse keratin without impacting corneodesmosomes.

## 2. Materials and methods

### 2.1. Tissue preparation

Abdominal human cadaver tissue for cohesion, drying stress, and SC swelling measurements was acquired from Caucasian female donors aged 47–90 years. Tissue samples were stored at  $-80^\circ\text{C}$  until ready for processing. Tissue specimens from single donors were used for comparative tests in order to reduce variability within test sequences. To isolate the SC, we first separated the epidermal tissue from the dermis via a 10-min immersion in a  $35^\circ\text{C}$  water bath followed by a 1 min  $60^\circ\text{C}$  soak. This allows for the removal of the epidermis by mechanical separation with a flat-tipped spatula. Next, the SC was isolated from the underlying epidermis using a trypsin digest procedure previously documented [19–21]. Once isolated, the SC was allowed to dry in a low-humidity chamber at ambient temperatures ( $\sim 10\%$  RH,  $20^\circ\text{C}$ ).

### 2.2. Lipid and NMF extraction

Each tissue sample in this study was exposed to either (1) a 20 min soak in distilled water in order to extract NMFs [22], (2) a 20 min soak in a 1:1 mixture of acetone/ether (AE) in order to extract mobile lipids [23], or (3) a 20 min soak in a 1:1 mixture of acetone/ether followed by a 20 min soak in distilled water (AE/W) in order to extract both lipids and NMFs.

### 2.3. Treatments and application

Ectoine is a well-known moisturizing ingredient which is believed to function as a compatible solvent within the corneocytes [24]. HEG is a proprietary salt of succinic acid developed by the Kao Corporation. They were applied to the SC before mechanical testing by soaking for 20 min in a 7% (w/w) aqueous solution; this concentration was chosen to insure an easily measurable response during mechanical testing.

### 2.4. Infrared microspectrometry

Fourier transform infrared spectroscopy (FTIR) coupled with a hydrogen-deuterium exchange (HDX) experiment was used to measure changes in keratin bundles due to some treatment. Specifically, dispersion of keratin bundles cause changes in the SC amide II band. SC samples were obtained by tape stripping (AS flon, AS ONE corporation, Japan) from human volar forearms (Japanese males, 30–33 years, N =

3). Before tape stripping, forearm sites were subjected to AE/W treatment using a previously reported technique [25]. After drying, tape strips were collected and subsequently soaked for 20 min in either a 50 mM (0.7% w/w) aqueous ectoine solution or a 50 mM (1.0% w/w) aqueous HEG solution. They were then allowed to dry under an  $\text{N}_2$  atmosphere for 3 min. IR spectra of the dried SC samples were obtained using infrared microspectrometry (Spotlight 400 FT-IR, PerkinElmer, Inc.), and the location of these measurements were noted. The samples were then stored in a desiccator at  $25^\circ\text{C}/50\%$  RH under a deuterium/dry  $\text{N}_2$  atmosphere for 10 min to induce a gas-phase HDX. SC samples were then measured again at the same spot as previously. All spectra were normalized by the amide I peak, and the degree of HDX was calculated using the amide II band.

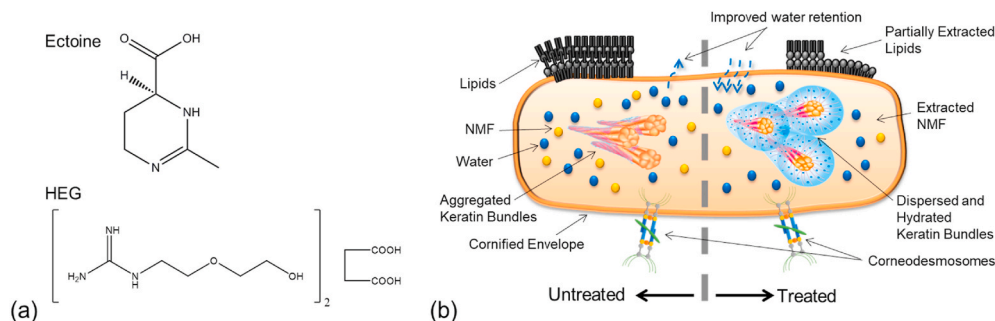
### 2.5. Change in SC thickness

Measurements were performed using a Bruker Dektak 150 profilometer. A tip force of 0.2 mg was used to create a profile map of a  $1 \times 2.5 \text{ mm}$  area of the top surface of the SC, from which an average thickness was extracted. Three samples were measured, and average changes in thickness  $\pm$  SD were reported. Samples were first measured after equilibrating to ambient humidity and temperature. They were then soaked for 20 min in distilled water, thickness was measured, and the percent change in thickness was determined. Samples were then allowed to dry overnight in ambient conditions and measured again. Next, samples were subjected to a second 20 min soak in either distilled water or a 7% (w/w) aqueous solution of ectoine, and the soaked and dried measurements were repeated.

### 2.6. Drying stress profiles

Substrate curvature tests were carried out as previously reported [9, 10, 17, 26]. In brief, square SC samples of dimension  $22 \times 22 \text{ mm}$  were hydrated via floating on DIW for 25 min and subsequently adhered to a  $178 \mu\text{m}$  thick glass cover slip (Fisher Scientific, Rochester, NY, U.S.A. 12-541-B) with a Young's modulus of 69 GPa and a Poisson's ratio of 0.2. Adhesion was achieved by allowing the SC to settle on the slide while excess water was removed by pipette. No adhesive was required for good adhesion. The SC was then trimmed to size using razor blades (VWR, 55411-050). The change in substrate curvature during drying of these samples was then measured, and used to calculate the biaxial stress state of the films using Stoney's equation  $\sigma = \left(\frac{E_{\text{sub}}}{1-\nu_{\text{sub}}}\right) * \frac{h_{\text{sub}}^2}{6h_{\text{sc}}} K$ , where  $E_{\text{sub}}$ ,  $\nu_{\text{sub}}$ ,  $h_{\text{sub}}$ , and  $h_{\text{sc}}$  are the Young's modulus, Poisson's ratio, and thickness of the substrate and the SC film, respectively.

All samples were allowed to dry in a  $<5\%$  RH, ambient temperature environment for 12 h. Next, they were allowed to re-hydrate by exposure to a high humidity ( $\sim 60\%$  RH) environment until stress was fully relaxed. Samples were then removed from the measurement system and exposed to relevant treatments, after which the drying/rehydration cycle was repeated.



**Fig. 1.** Keratin dispersing agents ectoine and HEG, including (a) their molecular structure and (b) a schematic representation of the state of a corneocyte before and after treatment with these dispersants and lipid/NMF extractions.

## 2.7. Corneocyte cohesion

Double cantilever beam (DCB) specimens were prepared such that linear elastic fracture mechanics could be employed to calculate strain energy release rates. To achieve this, polycarbonate substrates of dimension  $40 \times 10 \times 3$  mm were chosen to ensure that purely elastic substrate deformation occurred. After treatment application, isolated SC was adhered between two substrates using a cyanoacrylate adhesive. A small length of SC ( $\sim 4$  mm) was left unglued from one substrate to serve as a crack initiation site. The specimens were mounted at this end using loading tabs into an adhesion test system detailed in previous work [20, 21, 26–29]. For depth testing, subsequent measurements were made by re-adhering a fresh beam to the top of the SC after each delamination. Depth into the SC was measured using a commercial micrometer at fixed points along the length of the sample in order to eliminate the effects of adhesive layer roughness and variations in tissue thickness. These experiments allow us to measure the critical intercellular cohesion energy ( $G_c$ ) of the stratum corneum.

## 2.8. Statistical analysis

Data are presented as mean values plus or minus 95% confidence intervals except where otherwise noted. On average, four duplicates of each condition were tested for DCB testing. An average of 15 measurements were collected per sample, so that  $n \sim 60$  per test condition. Each substrate curvature and tissue thickness experiment was repeated three times.

## 3. Results

### 3.1. Keratin dispersion and hydration

The previously detailed FTIR-HDX method was used to estimate the level of keratin dispersion and hydration (Fig. 2a) [30,31]. In these experiments, aggregated keratin fibers strongly interact with each other via hydrogen bonding; this inhibits water access to the keratin bundles, and thus reduces deuterium substitution onto the amide groups. Dispersed keratin bundles allow for easier water access, resulting in higher levels of HDX. Thus, HDX measurements are a good indicator of

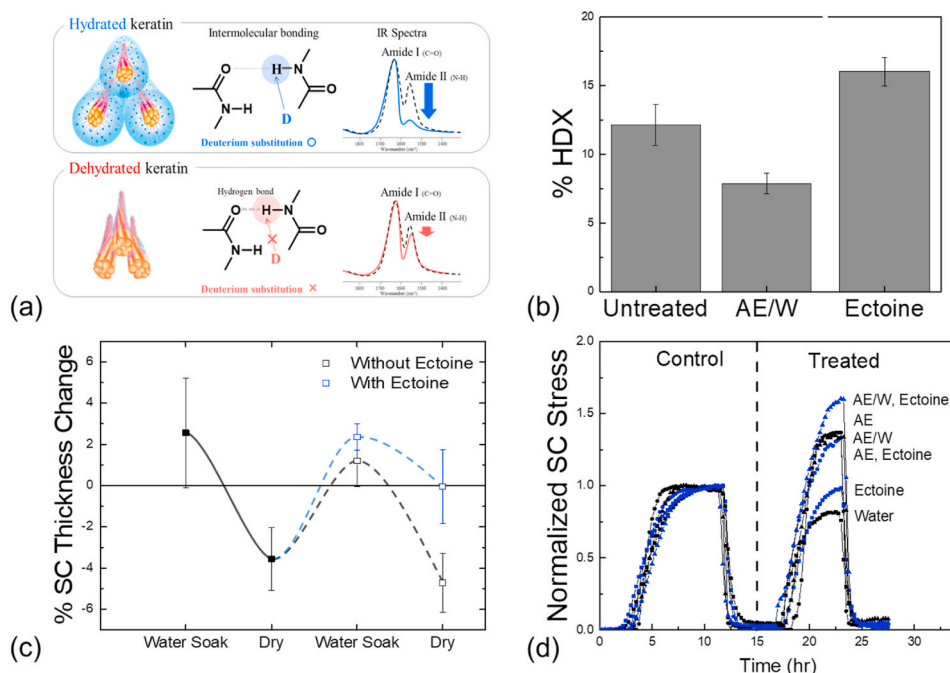
both keratin hydration and dispersity. It has been previously reported that AE/W treatment leaches NMF from the SC, leading to dehydration *in vivo* [25]. Therefore, the decrease in keratin hydration after AE/W exposure was evaluated quantitatively by measuring the level of HDX (Fig. 2b). Next, an ectoine treatment was applied as above, and the measurement was repeated. After AE/W exposure, a marked drop in HDX was observed. Subsequent ectoine treatment increased the level of HDX compared to the control tissue. This experiment was then repeated using HEG in place of ectoine, and similar trends were observed (Fig. S1a).

The effect of ectoine on tissue swelling and thinning due to hydration and dehydration was also measured (Fig. 2c). The tissue swelled and increased in thickness after soaking in water for 20 min. After drying overnight in ambient conditions, the tissue thinned to below its initial unsoaked value. Repeating a distilled water soak/dry cycle with no extra treatment resulted in a very similar profile, with swelling followed by substantial thinning. When the tissue was instead exposed to a 20 min soak in a 7% (w/w) aqueous solution of ectoine, it swelled to similar levels as for water-soaked SC. However, it thinned significantly less after drying overnight (a  $\sim 2\%$  reduction in thickness, compared to a  $\sim 6\%$  decrease previously).

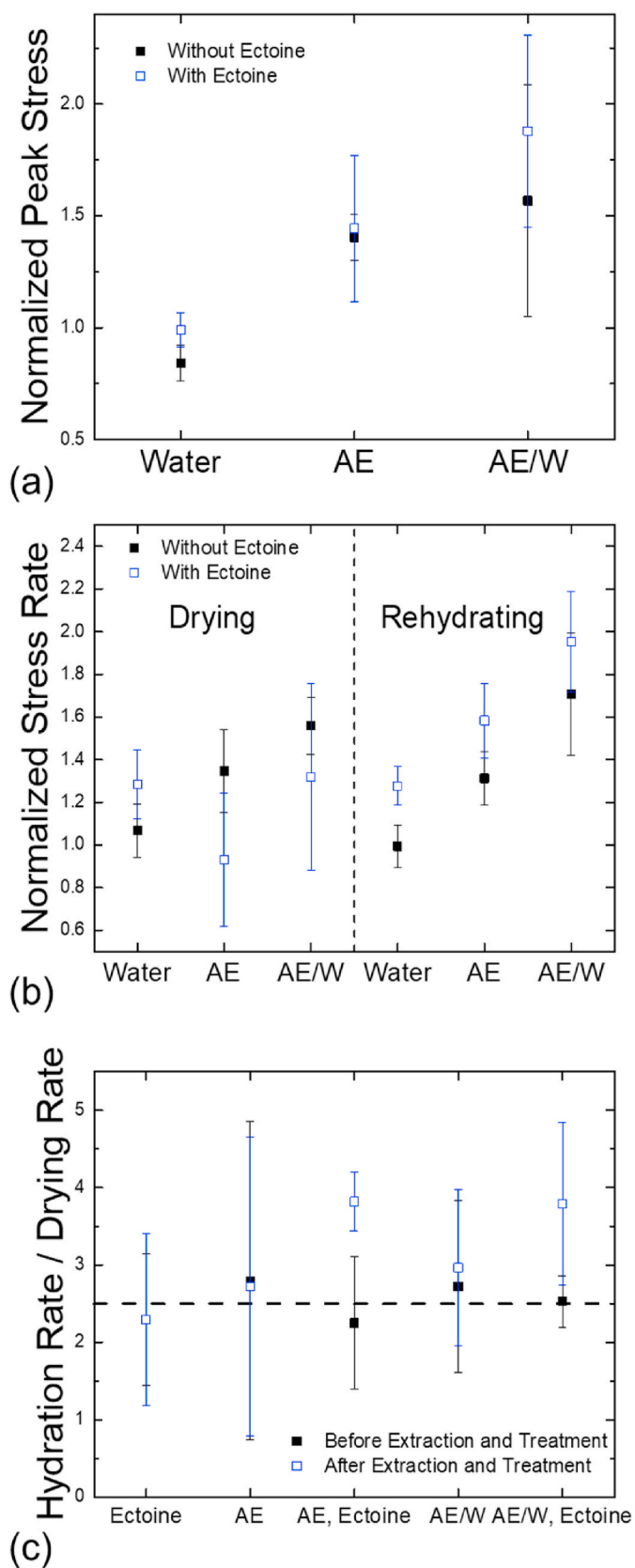
### 3.2. Drying stress

When subject to drying conditions, fully hydrated tissue developed drying stress profiles shown as “control” curves in Fig. 2d. These profiles were characterized by an initial lag time in which no stresses develop followed by a rapid increase in stress over  $\sim 4$  h before a plateau was observed. After humid air was substituted for dry air, stress quickly decreased to a near-zero plateau.

When subjected to subsequent lipid/NMF extractions and ectoine exposure, changes in drying and rehydrating stress rates, as well as peak stress values, were observed. Fig. 3a shows the change in peak stress with each treatment, both with and without ectoine. These are normalized to the peak stress from the control curves for each condition. The conditions are ordered from least to most aggressive extraction; i.e. water soaks weakly extract NMFs, AE soaks aggressively extract mobile lipids, and AE/W soaks extract both lipids and NMFs. For both ectoine exposed and non-exposed samples, peak stress development increased



**Fig. 2.** (a) Illustration of the method for measuring keratin hydration and dispersity via hydrogen-deuterium exchange (HDX). (b) Bar graph of the degree of HDX for untreated, lipid plus NMF extracted, and 0.7% w/w ectoine treated human SC (tape-stripped). (c) Average change in cadaver SC thickness  $\pm$  SD after soaking in water, either with or without 7% w/w ectoine. (d) Drying stress profiles of the cadaver SC obtained in order to study the effect of 7% w/w ectoine on stress relaxation during humidification processes. Each curve was normalized to the peak stress attained by untreated SC in order to facilitate comparison.



**Fig. 3.** (a) Change in peak drying stress after various exposures, normalized to control values. (b) Change in stress development or reduction rate compared to untreated control rates. (c) Ratio of stress rate during hydration to stress rate during drying.

with more aggressive extractions. Ectoine exposed samples tended to develop slightly higher peak stresses, though these differences were small. This behavior is notably different than prior observations of humectant compounds such as glycerin [17] which tend to reduce peak stress.

The rates of stress development and relaxation were also measured (Fig. 3b), again normalized to control rates. Increasingly aggressive extractions increased both drying and rehydrating rates in much the same way as for peak stresses. However, ectoine exposure tended to increase rehydration rates while decreasing the drying rates of both the AE and AE/W condition. The drying rate in water soaked tissue showed a slight increase after ectoine exposure.

Another interesting effect can be seen in Fig. 3c, where the ratio of the hydration stress rate to the drying stress rate is plotted. For normal SC (i.e. before any extraction or ectoine exposure), the SC loses stress by rehydrating  $\sim 2.5$ x faster than it develops stress during drying. Most extractions and exposures maintain this ratio; however, samples which had been lipid extracted and also exposed to ectoine showed a marked increase, suggesting improved hydration properties. Similar measurements do not yet exist for humectant compounds such as glycerin (an exogenous compound) or pyrrolidone carboxylic acid (PCA, an endogenous compound found in NMF), but would be interesting points of comparison for future work.

### 3.3. Intercellular cohesion

Fig. 4 shows the intercellular cohesion energy as a function of depth into the SC after application of ectoine for each of the extraction conditions. Untreated control tissue exhibited a previously observed increase in cohesion with depth into the tissue, resulting in a positive cohesion energy gradient. This is attributed to increased CD density in deeper tissue layers. Fig. 4a shows a slight decrease in cohesion gradient after a water soak as has previously been reported [20,27], with lower cohesion values at every depth. Subsequent ectoine treatment increased this gradient, producing higher cohesion values, especially in deeper tissue layers. This is suggestive of an interaction with CDs.

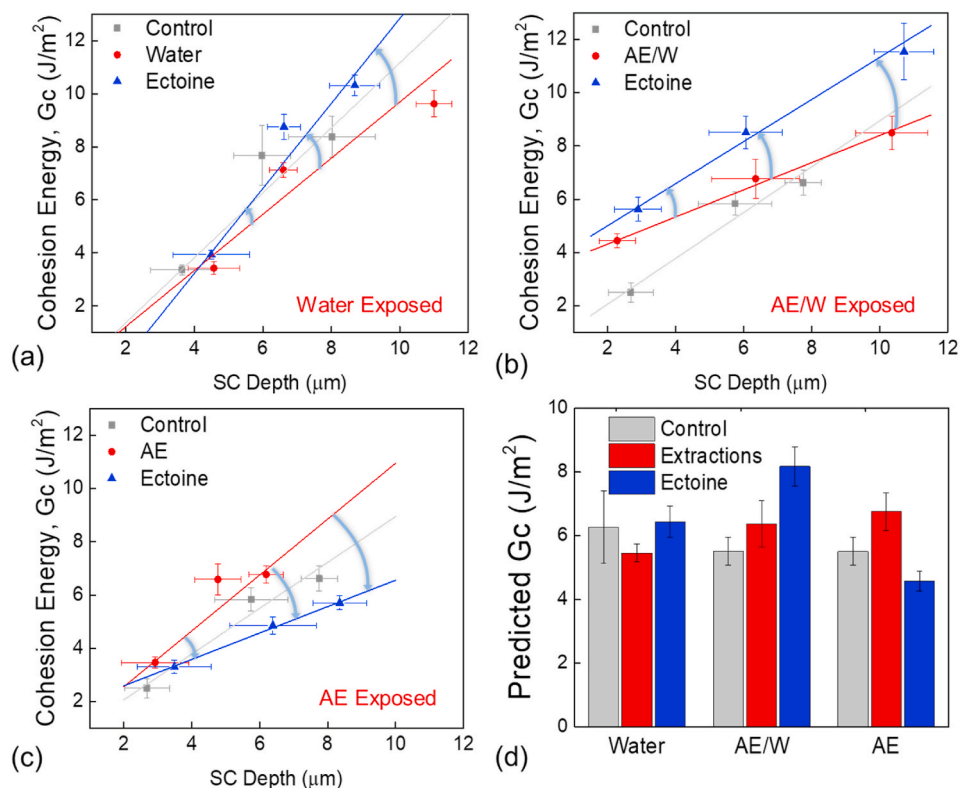
The effect of lipid and NMF extraction can be seen in Fig. 4b. Removal of mobile lipid bilayers cause bound lipids to directly interact, resulting in increases in cohesion which are especially prominent in the lower tissue layers. Ectoine treatment again increases cohesion energies and the cohesion gradient. In contrast, when ectoine is applied after an AE extraction with no subsequent water exposure, the trend is reversed; cohesion energies are reduced at all depths, and the gradient decreases. Fig. 4d plots the predicted  $G_c$  for each condition at a depth of 6  $\mu$ m, illustrating these trends.

These experiments were repeated using HEG as an active ingredient rather than ectoine. These results are shown in Figs. S1b–d. In this case, HEG produces  $G_c$  values and gradients which are very similar to the extracted tissue, suggesting that HEG does not engage in significant CD interactions. On the other hand, it does still interact with keratin to cause dispersion and hydration (Fig. S1a).

## 4. Discussion

### 4.1. Ectoine disperses keratin

HDX experiments were used to determine the level of keratin hydration and dispersity in the presence of ectoine (Fig. 2b) and HEG (Fig. S1a). The AE/W treatment results in dehydration due to damage to the SC barrier lipids and removal of NMF; this causes substantial aggregation of the keratin bundles, resulting in a marked decrease in the amount of HDX. Subsequent treatment re-hydrates and disperses the keratin. Ectoine treatment improves dispersion compared to untreated control tissue. This is in keeping with reports of reduced *trans*-epidermal water loss (TEWL) after ectoine treatment [24]. The HEG compound acts as a slightly less aggressive keratin dispersion agent than ectoine



**Fig. 4.** Cadaver SC graded delamination behavior following treatment with 7% (w/w) aqueous solution of ectoine after (a) NMF extraction via distilled water exposure, (b) lipid + NMF extraction via AE/W exposure, or (c) lipid extraction via AE exposure. Note in each of these cases the substantial change in cohesion energy gradient after treatment with ectoine. (d) The predicted  $G_c$  for each condition at a depth of 6  $\mu\text{m}$  into the tissue, based on linear fits for the data in a-c.

(Fig. S1a).

Additional indirect evidence of improved hydration induced by ectoine is provided by measurements of SC thickness as illustrated by Fig. 2c. In brief, ectoine may slightly increase tissue swelling after a water soak, but more substantially reduces the amount of thinning experienced during drying. This suggests that more water is being retained by the tissue after equilibrating to ambient humidity due to the higher number of sites available for water interaction after keratin is dispersed.

#### 4.2. Dispersants improve SC hydration kinetics

Use of solvents to strip lipids is a widely-used procedure [5,12,32–34], and some studies have probed the degree to which solvent species and exposure duration impact the quantity of lipids extracted [35,36]. Similarly, water soaks extract NMFs from isolated SC [22]. Extraction of either NMFs or lipids tend to increase drying rates and peak stress development (Fig. 2d). When NMFs are removed by a water soak there is a reduction in hygroscopic compounds within the SC. Lipid extractions aggressively disrupt and reduce the primary lipid barriers to water transport [10,12–14,16,37]. Further evidence of increased water transport after lipid extraction has been observed in studies on TEWL in solvent-damaged SC [38–42]. Thus, both treatments increase the rate at which water is able to pass through the tissue, and allow for greater total water loss in extreme drying environments. The result is a higher peak stress due to drying and an increase in the rate at which that stress is achieved (Fig. 3a and b). Unsurprisingly, the highest rates and peak stresses develop when both NMFs and lipids are extracted with the AE/W treatment.

The same trend is seen in rehydration rates, and for similar reasons. When barriers to water transport are removed, moisture re-enters the tissue more easily in highly humid conditions. Notice that NMF stripping does not by itself increase the rehydration rate. This is because when

NMFs alone are removed, no additional driving force for water uptake is introduced, and no significant water transport barrier is disrupted.

These changes are augmented by the introduction of ectoine. Peak stress development is slightly increased after the addition of ectoine (Fig. 3a). This suggests that in harsh drying conditions of <5% RH, a greater amount of water is driven out of the tissue after keratin dispersion. It is likely that the new water interactions enabled by keratin dispersion are of the “partially bound” variety [11]; additional spectroscopic studies may shed light on these questions. In contrast, recall that when ectoine-treated tissue was allowed to dry under ambient humidity conditions it showed less thinning (suggestive of increased water retention). Thus, while highly aggressive drying conditions will eventually drive out all available non-bound water, ectoine does seem to improve total hydration under standard conditions, as previously reported [24,43]. Interestingly, this behavior stands in contrast to that observed in prior work for the humectant glycerin [17] where reductions in peak drying stress were observed. This suggests that ectoine’s action is not due purely to its own humectant properties but is rather the result of its keratin interactions. However, it is unlikely that ectoine’s humectant properties play no role in these results. Further work needs to be done to fully disentangle these interactions; in particular, a detailed comparison of the biomechanical and biochemical impacts of ectoine compared to those of exogenous humectants such as glycerin and NMF compounds will shed valuable light on the mechanisms involved.

Rehydration rates are universally increased by keratin dispersion (Fig. 3b). As more partially bound water interactions sites are made available, a greater driving force for water uptake exists, which increases the rate of stress reduction. Drying rates exhibit more nuanced effects. After the primary barriers to water transport are removed (i.e. after lipid extraction by AE or AE/W treatment), it can be seen that ectoine suppresses the drying rate. This effect is driven by the same increase in partially bound water interactions as for the rehydration rate increase; now, there exists a greater energetic barrier to water loss,

reducing the rate of drying stress development. However, when only NMFs are removed, a slight increase in drying rate due to ectoine is observed. It is likely that the presence of the lipid water transport barrier is masking the dispersion effect of ectoine on drying rate, and instead the removal of the NMF species dominates. These effects are further highlighted by plotting the ratio of the hydration rate to the drying rate for each condition both before and after extractions/treatments (Fig. 3c). Before extractions, normal SC rehydrates ~2.5 times faster than it dries. Extraction of NMFs, lipids, or both all maintain this ratio. When ectoine is applied in the absence of the primary lipid water barriers, this ratio substantially increases.

Previous *in-vivo* studies have established that ectoine treatments increase water content of the SC [43]; our data supports this observation. Improved water transport, and especially rehydration rates, allows for greater water uptake from underlying moisture reservoirs *in-vivo*. This, coupled with the higher base level of keratin hydration at ambient humidity conditions caused by ectoine exposure, results in higher total SC water content.

#### 4.3. Ectoine alters corneodesmosome binding properties

The impact of extracting treatments on SC cohesion energy has also been explored in previous studies [18,27]. In brief, lipid extracting solvent exposures tend to drastically increase  $G_c$  in SC, sometimes increasing the cohesion energy by as much as 100%. It was postulated that this is due to bound lipids on adjacent corneocytes coming into close proximity due to the removal of intermediate mobile lipid layers. Bound lipid interactions are thought to be stronger than those between mobile lipids, resulting in higher cohesion between cells. This trend is confirmed in these studies; in every experiment solvent extraction resulted in increased cohesion energies compared to untreated controls. NMF extracting water soaks have the opposite effect, producing reduced cohesion energies.

Ectoine induced changes to cellular cohesion in for each extraction condition. Previous studies have shown that the majority of intercellular cohesion energy is provided by the corneodesmosomes [28], and unpublished observations suggest that mobile lipids contribute only on the order of ~100 mJ/m<sup>2</sup> in unextracted tissue. Because of this, and because for each extraction condition the ectoine effect was increasingly prominent with depth into tissue, we propose a CD related mechanism.

Note that ectoine caused marked increases in  $G_c$  compared to extracted tissue for both water and AE/W exposed samples (Fig. 4a and b). On the other hand, a decrease in cohesion was observed when ectoine was applied to AE extracted tissue. In other words, samples whose penultimate exposure before ectoine treatment was to water exhibited increases in  $G_c$ , whereas a penultimate solvent soak resulted in a decrease to  $G_c$ . This observation suggests that ectoine may be acting on the CDs to increase cohesion preferentially if the corneocytes have already been exposed to water. This exposure would result in keratin fibers which are pre-hydrated before ectoine is introduced, which may change the energetics or kinetics of the ectoine-keratin interaction, possibly freeing up ectoine to engage in CD interactions. In contrast, a penultimate AE soak increases cohesion by facilitating bound lipid interactions (raising  $G_c$ ), which are then disrupted by the subsequent aqueous ectoine solution. Since the keratin is not pre-hydrated in this case, ectoine may interact with these fibers more preferentially and quickly, reducing the impact on cell cohesion. Note that this is further indirect evidence of direct keratin-ectoine interactions; these behaviors are not explained by the presence of simple humectants. Additional experiments and modeling should be undertaken to further explore these effects.

A second compound, HEG, was tested to examine the effect of the structure of ectoine on cohesion and dispersion (Figs. S1b–d). This compound acts as a slightly less aggressive keratin dispersion agent than ectoine (Fig. S1a). However, it does not induce the same changes in cell cohesion. Notice in Figs. S1b–d that very little change in cell cohesion

energies or cohesion gradient is observed after treatment by HEG.

## 5. Conclusion

We used a combination of biochemical and biomechanical assays to probe the effect of two moisturizing compounds, ectoine and HEG on human stratum both *ex* and *in-vivo*. Using IR spectroscopy, it was shown that treatment with ectoine increased the degree of HDX with keratin proteins in the tissue, indicating dispersion of the keratin fibers. SC thickness measurements exhibited less tissue thinning during drying for ectoine treated tissue, suggesting improved water retention. We then probed the mechanical impacts of keratin dispersants, including both ectoine and HEG. It was found that keratin dispersants improved hydration kinetics in the SC. It is well established that the principal barrier to TEWL in intact SC consists of the mobile lipid bilayers; thus, it was unsurprising that the greatest improvements were observed when this barrier had disrupted, consistent with previous work investigating ectoine's role as a compatible solvent in human SC.

It was further observed that ectoine interacted with the corneodesmosome protein structures in the SC to alter the cohesion energy of the tissue. Given that many undesirable skin conditions, especially those which produce scaly buildups, involve abnormally strong and lasting SC cell cohesion, increases in SC  $G_c$  are generally undesirable. HEG was tested as a new compound which induced keratin dispersion in the SC but did not induce higher cell cohesion.

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## Statement of ethics

Full-thickness samples of *ex-vivo* cadaveric human skin were obtained from Caucasian female donors through the National Disease Research Interchange (NDRI). NDRI is a 501(c) (3) not-for-profit, National Institutes of Health (NIH)-funded organization that provides project-driven human biospecimen service to academic and corporate scientists. According to standard international guidelines, research with cadaveric samples does not involve human subjects and IRB ethics approvals are not required.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2021.101134>.

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