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Letter to the Editor

Effect of glycerin on drying stresses in human stratum corneum

To the Editor,

Dry skin conditions are accompanied by significant changes in the stratum corneum (SC) biomechanical properties including the "so-called" drying stress, σ_{sc}, which leads to the perception of skin stiffness/tightness and provides a mechanical driving force for skin damage processes like cracking and chapping (Fig. 1a) [1]. Surprisingly, the effects of moisturizers on these properties as well as their role in reducing the mechanical driving force for dry skin damage are not well characterized.

In this letter, we demonstrate the effects of glycerin (GLY) formulations (Table 1) on the σ_{sc} developed in abdominal SC procured from a 60-year-old Caucasian female using the substrate curvature method [1,2]. The σ_{sc} measured has the same biaxial *in vivo* stress state and moisture exchange with the environment. Also, moisture cannot be replenished by the underlying epidermal layer, which provides an opportunity to isolate treatment effects on SC components and moisture exchange with the environment.

Following GLY treatment, both the drying stress rate, $d\sigma_{SC}/dt$, and the final σ_{SC} values significantly decreased compared to control (distilled water, DIW) with increasing GLY concentration (Fig. 2a). To understand the effect of glycerin on SC components, attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) was employed and the symmetric C–H stretching (Fig. 2b) frequency was probed as a function of tissue depth ($N = 3$ correspond ~1/2 the SC thickness) using a delamination technique (Fig. 2c) [3]. The 30% and 100% GLY treatments resulted in peak locations above the control suggesting glycerin penetration into the mid-SC and increased lipid fluidity. No lipid extraction was apparent as evidenced by no significant change in the symmetric C–H stretching/amide II peak height ratio compared to the control.

Glycerin has high humectancy, it can attract water from the viable skin layers to the SC and from the environment if the ambient RH exceeds 70% [4]. In our study, the humectants could neither attract water from the underlying substrate as they would *in vivo* nor could they draw from the external dry environment. Therefore, the σ_{SC} is explained solely in terms of humectant effects on SC water loss.

Using a recent biomechanics model generated to accurately predict the relationship between SC water loss and measured σ_{SC} values [1], the predicted reduction in water lost compared to the control was ~2, 4 and 8% after 10, 30 and 100% GLY treatments, respectively (Fig. 1b). Only a small reduction in the water lost is needed to fully account for the reduced σ_{SC}, not surprising given σ_{SC} sensitivity to water content. The other possible reason for lower σ_{SC} values with increasing glycerin concentration may be increased lipid fluidity. This would reduce the σ_{SC} by viscous flow and increased movement of the corneocytes.

Glycerin effectiveness was further compared to occlusive PET. Following PET application, σ_{SC} values increased to a peak value lower than all GLY treatments, remained relatively constant for ~2 h and then slowly relaxed to ~2.25 MPa (~37% lower than the control and ~20% lower than the 100% GLY treated SC) (Fig. 2a). ATR-FTIR measurements on delaminated SC surfaces somewhat surprisingly suggested PET penetration into the SC along with increased lipid fluidity (Fig. 2c).

The principal effect of PET as an occlusive emollient [5,6] on the σ_{SC} is achieved through control of the SC water content. Using our model, we determined the reduced water loss following PET treatment to be ~13% (~5% higher than the pure GLY) (Fig. 1b). This demonstrates that PET is more effective in maintaining a

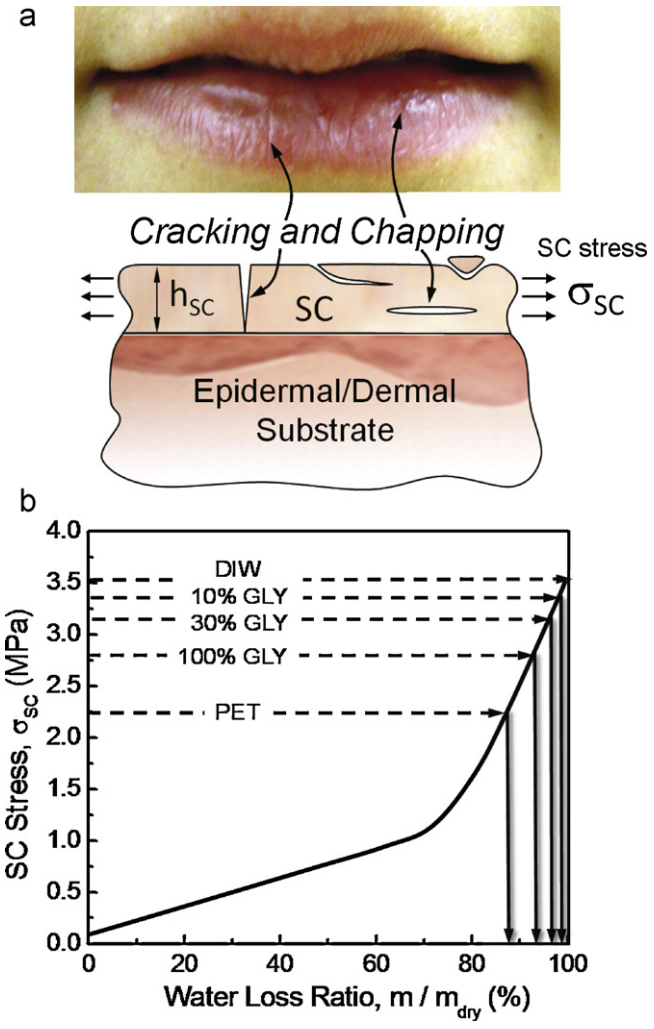

Fig. 1. (a) A schematic illustration showing typical dry skin cracking and chapping processes that result from the development of drying stresses in SC. (b) SC drying stress as a function of water loss ratio in the drying environment.

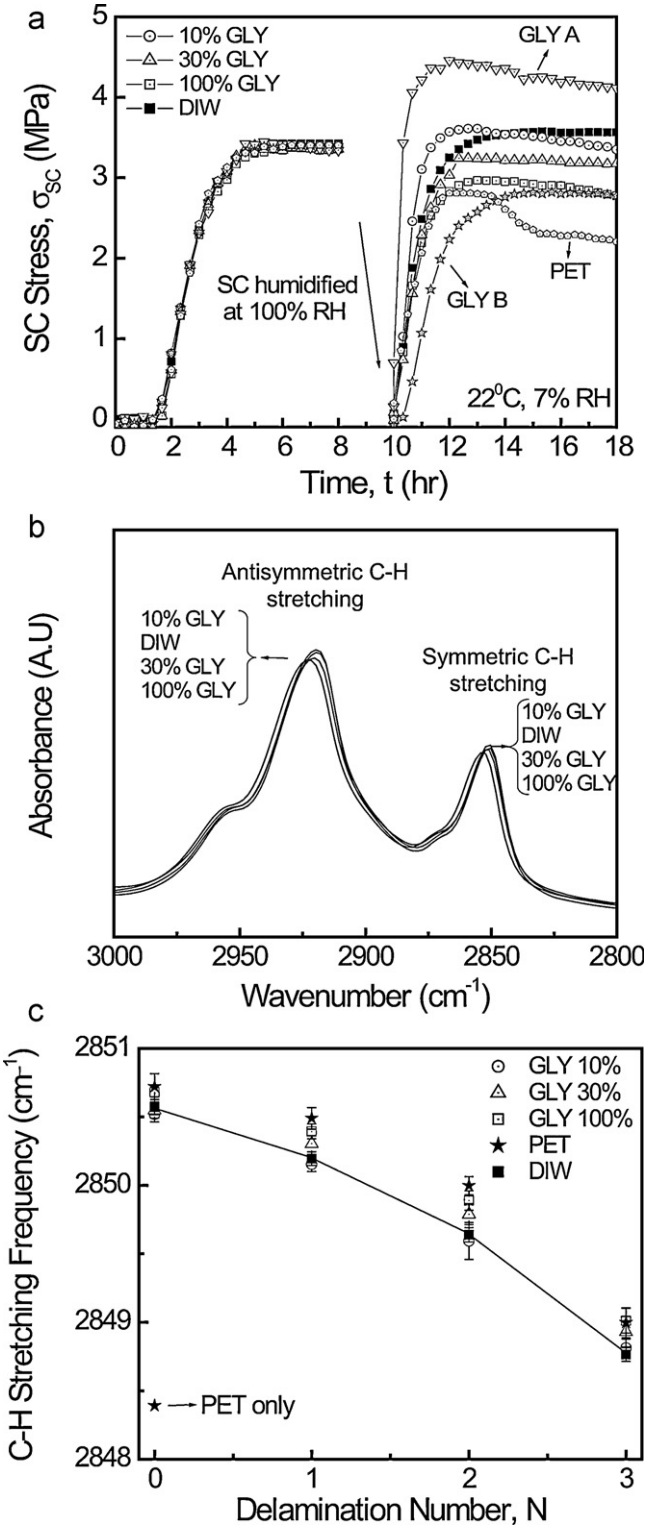

Fig. 2. (a) SC drying stress as a function of drying time for SC treated with the GLY and PET coatings and exposed to 7% RH and 22 °C air. (b) ATR-FTIR spectra of SC showing asymmetric and symmetric C–H bond stretching absorbances after humectant treatment. (c) The location of the symmetric C–H stretching peak as a function of the delamination number for SC specimens treated with the GLY and PET coatings and exposed to the 7% RH and 22 °C drying environment for 8 h.

higher hydration state in the tissue than GLY. The gradual stress relaxation after the peak stress is likely associated with increased fluidity of the lipid matrix and gradual softening of the tissue due to increased PET penetration. This is similar to previously observed behavior where emollient diffusion into the SC caused significant σ_{SC} relaxation [7].

Table 1
The treatments used in this study, their compositions, physical states and their mechanism of action to moisturize skin are listed above.

Treatment	Composition	Physical state	Type
GLY	10%, 30% and 100% (v/v) in DIW	Liquid	Humectant
GLY-A	40–50% (v/v) Glycerin, 40–50% (v/v) Water, 1–5% (v/v) Glyceryl Polyacrylate	Solid (gel)	Humectant
GLY-B	40% Glycerin (v/v), cetearyl alcohol, stearic acid, sodium cetearyl sulfate, methylparaben, propylparaben, dilauryl thiodipropionate, sodium sulfate	Solid (cream)	Oil/lamellar gel/water emulsion

We then measured σ_{SC} following GLY-A and GLY-B formulations. σ_{SC} values of GLY-A treated specimens which contained 40–50% glycerin increased rapidly to a peak and then decreased to a final σ_{SC} value higher than that of the control. In contrast, the σ_{SC} values of specimens treated with GLY-B increased and stabilized at σ_{SC} values lower than that of the control after ~2 h in the drying environment (Fig. 2a).

The high σ_{SC} and $d\sigma_{SC}/dt$ values following GLY-A treatment suggest that the SC was more sensitive to water loss following exposure despite the high glycerin content and humectancy of GLY-A. This formulation does not readily release water even under severe drying conditions by trapping water molecules in its cage-like glyceryl polyacrylate clathrate matrix [8]. Therefore, the high σ_{SC} and $d\sigma_{SC}/dt$ values are likely associated with the strong affinity of glyceryl polyacrylate to absorb water from the SC in this isolated SC experimental model. On the other hand, *in vivo*, the presence of an infinite water sink in the epidermis would provide the source of water to GLY-A resulting in a fully hydrated GLY-A layer that then behaves more effectively. A contributing effect to the resulting high σ_{SC} and $d\sigma_{SC}/dt$ values may be the glyceryl polyacrylate penetration into the SC and its effect on the SC lipids. Penetration of emollients, which results in increased lipid fluidity in the SC has been shown to impart high σ_{SC} and $d\sigma_{SC}/dt$ values in the tissue [7]. Finally, the significantly larger stress relaxation observed for GLY-A compared to GLY formulations suggests that the penetration of glyceryl polyacrylate into the SC may be increasing lipid fluidity.

σ_{SC} values for the GLY-B treated tissue were similar to the tissue treated with 100% GLY although the formulation contained only 40% glycerin. The lower σ_{SC} observed with GLY-B may be due to the increased glycerin penetration in the presence of the emulsifiers used in the treatment. The formulation also contains an oil/lamellar gel/water type emulsion that forms a hydrophobic film on SC that further reduced water loss.

We conclude by noting that this study provides the basis from which biomechanical models can be employed to evaluate the efficacy of moisturizers in alleviating the potential for dry skin damage. Such evaluations may have significant clinical implications. As we have shown elsewhere, the mechanical driving force for dry skin damage, G, is particularly sensitive to the σ_{sc} since it scales with the square of σ_{sc} [1]. The decrease in the final σ_{sc} values following GLY or PET treatments is therefore likely to be a major factor in reducing the mechanical driving force for dry skin damage.

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Letter to the Editor

The 3′-UTR AACCins5874 in the stratum corneum chymotryptic enzyme gene (*SCCE*/*KLK7*), associated with atopic dermatitis; causes an increased mRNA expression without altering its stability

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with cutaneous hyper-reactivity to environmental triggers innocuous to normal, non-atopic individuals, caused mainly by changes in the cutaneous and systemic immune responses [1]. However increasing evidence highlight the speculation of a primary genetic defect in the skin barrier which could affect the levels of protease activation within the skin, leading to the development of AD [2]. The stratum corneum chymotryptic enzyme (*SCCE*/*KLK7*) is believed to be an important player in regulating the epidermal homeostasis of the normal skin barrier [3,4]. We previously reported a 4-bp insertion (AACCins5874) at the 3′-UTR of the *SCCE* gene to be significantly associated with AD [5].

In this study we analysed five more SNP's throughout *SCCE* gene (Fig. S1). All SNPs were analyzed by PCR-RFLP (Table S1) in 120 AD patients and 203 matched-controls [5]. Allelic distribution analysis showed no significant difference between cases and controls. However, a dose effect was detected for *SCCE* AACCins5874 [$OR_{Heterozygote}$, odds ratio (OR) = 1.09 (0.62,1.92) and $OR_{Homozygote}$ = 2.73 (1.52,4.91) respectively]. Therefore, a χ^2-test trend was carried out for *SCCE*-AACCins5874 insertion [OR = 2.62 (1.58–4.35); P_c = 0.009], suggesting that SCCE-AACCins5874 allele confers more than 2–fold risk for disease in dose-dependent manner under a recessive model of inheritance (Table S2). Haplotype analysis using the programs EHPLUS [6] and SNPtagger (Table S3) confirmed the single-marker analysis (Table 1). *SCCE* C3224-5713C-AACCins5874 haplotype showed a strong association with AD (P_c = 0.00001). Moreover, haplotypes containing AACCins5874 when grouped together also showed significant association with disease (P_c = 0.0001). In contrast, a weak but significant negative association was revealed with the *SCCE* 3224G-G5713-AACC haplotype (P_c = 0.02), suggesting a protector effect.

As variations in the 3′-UTR of genes may affect the stability and/or translation of the mRNA [7,8], we analysed the effect of AACCins5874 in the 3′-UTR of *SCCE* on gene expression and stability using a destabilised luciferase reporter vector. The latter was based on the pGL3-Basic Vector (Promega, Madison, WI) modified so that the *luc*-SV40poly(A) fragment was replaced with a fragment, downstream of the synthetic poly(A) signal site, encompassing the IL-8 promoter, the destabilised luciferase gene (*luc*-PEST) and the IL-1β 3′-UTR sequence. The IL-8 promoter was used to induce the production of the luciferase-PEST RNA by activation upon IL-1β stimulation through the Nfkβ pathway. Addition of the synthetic fragment encoding the proteolytic "PEST" signal to the firefly luciferase coding sequence aimed to destabilise

Table 1
SCCE haplotypes distribution in 120 AD and 203 controls. Haplotypes have been created using EH program and the comparison of frequencies of each haplotypes in cases and controls has been performed. Remaining corresponds to the pooling of haplotypes with frequency less than 5% in the same group. Three SNPs have been selected by the SNPtag software. Those are at positions 3224, 5713 and 5874 respectively. "AACCins5874-all together" means that all haplotypes, which contains AACCin5874 have been grouped together. P_c, corrected *p*-value.

SCCE haplotypes (C3224G-G5713C-AACC/AACCins5874)	Control	Freq	AD cases	Freq	P_c
C-G-AACC	168	0.413	81	0.338	0.432
C-G-AACCins5874	76	0.188	37	0.155	0.228
C-C-AACCins5874	21	0.052	40	0.165	0.00001
G-G-AACC	59	0.146	16	0.068	0.02
G-G-AACCins5874	38	0.093	37	0.155	0.162
G-C-AACCins5874	32	0.079	21	0.088	0.558
AACCins5874-all together	167	0.411	135	0.563	0.0001
Remaining	12	0.029	8	0.031	–
Total	406	1.00	240	1.00	–