I certify that I have read this dissertation and that, in my opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy.

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Abstract

The outermost layer of human skin, the stratum corneum (SC), is subject daily to variable ambient moisture and temperature conditions as well as application of potentially damaging cleansing agents. The inevitable results of these exposures are “tightness” of the skin which is directly related to the buildup of tensile residual drying stresses in the SC layer. In this work, we first describe the application of the substrate curvature technique to quantitatively measure the magnitude of these stresses and their relationship to selected drying environments and times. The SC drying stresses were observed to be very sensitive to the relative humidity and temperature of the drying environment as well as harshness of the chemical treatment. There was a strong correlation with the SC drying stresses and the chemical potential of water in the drying environment. The evolution of drying stresses in SC is discussed in relation to the effects of hydration and damage caused by chemical treatments on the underlying SC structure.

We also describe the application of the substrate curvature technique to characterize stresses in occlusive topical coatings. We then extend the substrate curvature technique to measure the combined effects of the coating applied to human stratum corneum (SC) where the overall drying stresses may have contributions from the coating, the SC and the interaction of the coating with the SC. We show how these separate contributions in the coating and SC layers can be differentiated.

Using this methodology, we characterize the effect of a range of moisturizing treatments on the drying stresses in human stratum corneum. Following moisturizer treatment, the SC was observed to have distinctive stress profiles with drying time depending on the effectiveness of the treatment. The stress values of specimens treated with the humectant moisturizers were observed to increase and stabilize after a few hours in the drying environment where they remained relatively constant until the end of exposure to the drying environment whereas the stress values of specimens treated with the emollient treatments were observed to rise rapidly to a peak stress value and relax to a final
stress value. The effect of moisturizing treatments on the SC drying stresses was rationalized in terms of SC water loss and the chemical state of the SC components.

Finally, we employ a fracture mechanics approach to understand the implications of the drying stresses in SC as a mechanical driving force for damage propagation (e.g. cracking and chapping) in the tissue. The crack driving force $G$ was found for several cracking configurations and compared with the intercellular delamination energy, $G_c$, which is a property of the tissue that provides a measure of the resistance to cracking. Using this approach, we demonstrate how damaging treatments enhance and moisturizing treatments alleviate the propensity for dry skin damage.
Acknowledgements

When I was a child, my grandmother used to tell me that time passes really quickly. At that point in my life, hating to go to school every day, time seemed to be stuck somewhere. When academic work became much more interesting in college, my grandmother’s statement became all too true – time was passing by very quickly. During my undergraduate work at Washington and Lee University, I had the great opportunity to work one to one with a professor whose specialty was on polymer rheology. At that time, I started falling in love with the concept of research. It almost became a hobby, a lifestyle for me by my senior year in college. As a very junior researcher, I was fascinated by all research opportunities out there and decided to pursue graduate research at one of the best programs in the country.

When I stepped into the good old Peterson building five years ago, I knew that this was not going to be an easy experience. Coming from a liberal arts school, I was at a disadvantage compared to my peers coming from the best engineering programs across the country and the world. While struggling with my classes and bad news related to my dad’s deteriorating health condition, there was only one thing that made me happy – my research project. This was almost the ideal project for someone who once wanted to get into the medical school but chose interdisciplinary research instead. I’d like to thank my advisor, Prof. Reinhold Dauskardt, for offering me a position in the skin research program.

I feel very lucky to have had Prof. Dauskardt as an advisor. He kept me in balance in bad times and good times and prepared me for real life. I greatly appreciate all the time and commitment he invested in my academic and personal growth. Aside from the many things he has taught me, I will never forget his great sense of humor. I am looking forward to our future work and interactions.

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Finally, I would like to dedicate this thesis to my wonderful parents, who have raised me to be the person I am today and strive to be. My dad will always continue to live in my life, and I will never forget his teachings. Mom & dad – you have been with me every step of the way, through good times and bad. Thank you for the unconditional love, guidance, and support, instilling confidence in me to pursue anything I put my mind to and helping me to succeed. Thank you for everything. I love you!
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Chapter 1 Introduction

1.1 Background and Significance

The outermost layer of skin, the stratum corneum (SC), provides protection and a controlled permeable barrier to the external environment while subject to highly variable conditions including changing temperature, humidity, mechanical and abrasive contact (Figure 1.1). In addition, the SC must withstand daily application of topical cleaning agents and potentially damaging acute and chronic chemical exposure. The mechanical properties of the SC are crucial not only for its mechanical and biophysical function [1], but are also a vital factor in the cosmetic aspects of skin appearance, “feel” and “firmness,” and play a central role in skin chapping and cracking associated with dry skin conditions [2].

Mechanical behavior of the SC is also important for wound healing, adhesive dressings, and emerging biosensor and drug delivery technologies that must have a reliable mechanical interface with skin. Residual stress in the SC affects the growth and healing of skin. Fibrosis, scar formation and various tissue responses including inflammation and expression of growth factors may be affected by mechanical loading [3,4,5,6,7]. As the outermost layer of skin, the SC acts as the principal mechanical interface between the epidermal and dermal layers and the exterior environment and thus plays a crucial role in determining the stress state in skin.

Figure 1.1 Layered structure of human skin. The outermost layer of human skin, stratum corneum (SC), is exposed to variable environmental temperature and chemistry, solar radiation, bacteria and metabolism products, as well as mechanical loads. Illustration source: Skin Care Forum, Cover picture of Issue 36, http://scf-online.com.
Surprisingly, current understanding of even basic mechanical behavior of SC remains incomplete and largely limited to the simple measurements of in-plane properties [8,9,10,11,12,13]. As a consequence, the relationship between the stress state of SC and tissue condition and treatment has not been established.

The intent of the SC research in the Dauskardt Group over the past eight years has been to develop and validate novel thin-film mechanical methods to assess the mechanical properties of human SC [14,15,16,17]. Prior to this research, the essential aspects of the mechanical function of skin were explored. The strength, stiffness and viscoelasticity of the SC, as well as its intercellular delamination energy, were characterized as a function of environmental and chemical conditioning, and the resulting trends were discussed in relation to the hydration and chemical damage of SC components.

The research presented in this dissertation builds on the success of the past SC research in the Dauskardt group and provides a significantly new and mechanics and materials approach to quantitatively characterize the drying stresses in SC and understand their role in enhancing or alleviating skin damage processes.

1.2 BIOMECHANICS OF DRY SKIN AND TISSUE DAMAGE

In this dissertation, we address one of the most ubiquitous and chronic skin conditions that generally results from the variable humidity and temperature conditions to which our skins are exposed. The inevitable results of these exposures are “tightness” of the skin and skin damage in the form of chapping and cracking (Figure 1.2). The skin tightness is directly related to contraction of the SC and the buildup of tensile stresses in the SC layer. These are referred to as SC drying stresses. The skin chapping and cracking is directly related to intercellular delamination and fracture of the SC layer.

The basic premise for the approach is shown in Figure 1.2 which shows schematically a number of well known SC cracking and chapping configurations associated with dry skin conditions. The past SC research had mainly
concentrated on the quantitative measurement of the “resistance” to cracking, quantified by a critical value of the strain energy release rate, $G_c$, and its relationship to tissue structure, condition and treatment. However, it is well known that dry skin conditions are accompanied by an increase in SC stress, $\sigma_{SC}$. Indeed, it is the increased SC stress that provides the mechanical driving force for cracking. From a mechanics viewpoint, the stressed SC layer can be treated as a thin (15-30 $\mu$m) film on a thick (1-3 mm) substrate consisting of epidermal and dermal layers, and the driving force $G$ for any of the cracking and delamination configurations shown in Figure 1.2 must have the mathematical form shown in the figure. $G$ depends on the SC stress squared divided by the SC plane strain Young’s modulus, $E_{SC}$, which provides a measure of the elastic strain energy density stored in the film, the SC thickness, $h_{SC}$, and a non-dimensional parameter $Z$ which provides information about the specific cracking type (peeling, channel cracking, delamination, etc). The condition for SC cracking and delamination can then be expressed as $G \geq G_c$.

Such dry skin conditions and damage are considered to be one of the fundamental problems in skin science [18,19,20]. Given the chronic and widespread nature of dry skin damage and the extensive research conducted in the biophysics and biochemical aspects of this skin condition, it is shocking that the underlying mechanics and fracture processes that directly determine this thin-film cracking process have not been quantified and understood. Part of the reason is the lack of understanding of the mechanical and fracture properties of the SC layer that have been exacerbated by the difficulty in making measurements in this very thin tissue. Another reason is a simple lack of attention from the materials and mechanics community.

Building on the success of prior SC research, the research presented in this dissertation explains the use of thin film techniques to characterize the driving force for SC cracking and provides a fundamental scientific and clinically relevant basis from which cracking and chapping associated with dry skin conditions can be understood and characterized.
Figure 1.2 Schematic illustration of typical cracking and intercellular delamination processes resulting from “dry skin” conditions in human stratum corneum. The mechanics condition for cracking or delamination is shown as a balance between the crack driving force, $G$, and the tissue resistance to cracking $G_c$. When $G \geq G_c$ the SC will crack or delaminate.

$$G \geq G_c \left[ \frac{J}{m^2} \right]$$

We used the substrate curvature method to characterize the drying stress from the curvature of an elastic substrate onto which the SC has been adhered. This technique is widely used in thin film materials science to quantify the evolution of stress in thin films on elastic substrates [21], yet it had never been attempted in SC. The biaxial drying stresses measured with this technique were compared with uniaxial drying stresses measured with micro tension experiments.

The focus of the presented research will be to characterize not only the value of $G_c$ for normal and dry skin conditions, but also to determine the crack driving force $G$ for the same tissue conditions. With this information we will be able to validate the above criterion for cracking by direct comparison with observed SC damage during drying or other treatments known to produce dry and chapped skin. This demonstration will have profound implications for a
quantitative understanding of the conditions for damage of dry skin. Furthermore, we will use this approach to demonstrate the efficacy of moisturizing treatments in reducing skin damage. Finally, we will establish a fundamental biomechanics understanding of the mechanical function of human SC and its dependence on cellular and intercellular components that will have implications for other clinical conditions and emerging technologies that interface with skin.

The mechanics are presented here in simple form and more complex forms modified to account for viscoelastic behavior of the SC or substrate may be required as described in Section 8.3. In addition, note that small-scale yielding conditions in the SC are most likely easily satisfied based on our SC research demonstrating that cracking in the SC is confined to intercellular delamination involving a limited plastic zone size [15].

1.3 IN VIVO COMPLEXITIES AND RELEVANCE

The research presented in this dissertation does not presume to address all in vivo complexities, which would be unrealistic with current understanding of the mechanics of SC cracking and the more complex affects of tissue variation and body location. Rather, our approach is to initially establish the fundamental mechanics principals from which cracking can be quantitatively understood and rationalized in SC tissue itself. With this understanding in place, it will be possible to rationalize the role of tissue condition on in vivo cracking and optimize tissue treatments quite independent of complexities such as tissue location.

We also note that SC is not metabolically inactive, and possesses limited forms of metabolic activity particularly involving the enzymatic degradation of corneodesmosomes which is crucial in the normal process of desquamation [1]. Hydration gradients through the SC thickness can also affect properties and are partly determined by underlying skin layers [22]. However, unlike most other soft tissues in the body, the SC is not vascularized and does not undergo rapid
apoptosis and structural damage after harvesting. Consequently, SC tissue can be isolated and appropriately stored without major change for extended periods. Extensive research in the skin science community is performed on such isolated tissue.

1.4 STRATUM CORNEUM STRUCTURE

Stratum corneum (SC) is the thin outermost layer of epidermis, which is the top layer of human skin. It is comprised of 10 – 20 cell layers giving an average thickness of ~ 10 – 25 μm over most body sites with even greater number of layers and larger thicknesses of ~ 200 – 600 μm for callus such as on the palm and sole [23,24]. The tissue has a composite structure consisting of heavily keratinized, disk-shaped corneocyte cells bound together by intercellular lipids and degraded desmosomal protein junctions, or corneodesmosomes (Figure 1.3). To maintain full coverage and effective barrier properties, SC renewal occurs by the continual replacement of mature exterior cells with younger interior cells. While the entire epidermis renews itself on a cycle of between 45 – 75 days, the SC has a faster turnover rate with a complete regeneration roughly every 14 days [25]. The detachment of individual corneocytes governing this renewal rate is dictated by the state of the SC and its components.

Proper SC cell detachment and renewal, or desquamation, has been associated with the progressive degradation of corneodesmosomes toward the outer skin surface [1,2,26]. Evidence suggests a strong link between this degradation process and the many gradients within SC tissue including water content, pH, natural moisturizing factors, lipids, and desquamatory compounds [18,27]. Normal desquamation has been linked to a variety of factors including appropriate hydration and the presence within the SC of specific molecular compounds such as cholesterol sulfate, which inhibits corneodesmosome degradation [18]. Other factors such as increased hydration have been shown to accelerate corneodesmosome degradation and intercellular lipid disruption, which also affect mechanical integrity as well [1,2,20].
From a mechanics viewpoint, the disk-shaped SC cells, or corneocytes, composed largely of aligned keratin filaments, have been likened to bricks bound together by a lipid-rich mortar [28]. While different values for corneocyte dimensions can be found in the literature, typical dimensions suggest that corneocytes, typically hexagonal in shape, are ~ 0.3 – 0.8 μm in thickness and ~ 30 – 35 μm in diameter with the extracellular matrix comprising ~ 10% wt/wt of the SC and intercellular lamellae being at most 0.1 μm thick [29,30]. Functionally, intercellular lipids have been identified as the primary pathway for chemical diffusion and as the barrier to water permeability through the SC layer [31,32]. The keratin filaments inside the corneocytes terminate in the cell membrane, or corneocyte envelope, that consists of 15 nm of protein with 5 nm monolayer of lipid covalently bound to the cell exterior [1,24].

**Figure 1.3** Schematic illustration of stratum corneum and its microstructural components. The SC renewal process involving intercellular debonding of corneocytes as they move to the outer skin surface is indicated. Illustration source: Skin Care Forum, Cover picture of Issue 35, http://scf-online.com.
components of the proteinaceous aspect of the corneocyte envelope are highly cross-linked loricrin, involucrin, and proline-rich proteins that make this shell highly insoluble [1]. These cell units are embedded within the lamellar lipid matrix formed during the epidermal maturation process. Corneodesmosomes, provide further mechanical linkage between SC cells and must degrade prior to corneocyte detachment in the upper layer of the SC [25].

Of the all SC components, we expect the intracellular keratin and the cellular protein envelope to have the largest effect on the driving force for cracking due to their expected effects on both the SC drying stress and elastic modulus. From a mechanics perspective, the total volume fraction of intercellular components (lipids (5-30% of total tissue volume) and corneodesmosomes) is limited and cannot have a dominant effect on SC elastic and drying stress values. The prior SC research presented in the next section has also reached similar conclusions with respect to the in-plane stress and elastic property measurements.

**1.5 Prior Stratum Corneum Research**

The prior SC research developed new and unique mechanics-based techniques to study both the in-plane and particularly the out-of-plane mechanical properties of human SC tissue as a function of temperature, hydration and chemical treatment [14,15,16,17]. The stress-strain and viscoelastic properties of free standing SC tissue was studied as a function of temperature, hydration and tissue treatment producing some of the most precise and accurate mechanical properties yet reported for human SC. With increasing hydration from equilibration in higher RH environments, tissue strength and modulus were observed to decrease (Figure 1.4a). SC was noted to be remarkably linear elastic for a soft tissue although its viscoelastic behavior became more pronounced with increasing hydration as seen in the obvious hysteresis loops that form with loading and unloading (Figure 1.4b).

Transient testing involving stress relaxation and creep recovery were used to better understand the time-dependent properties inherent within the SC.
Phenomenological models adapted to include multiple time scales by using a stretched-exponential form were employed to describe the time-dependent stress or strain response of the tissue. The associated retardation time scale, $\tau_s$, and the $\beta$ parameter characterize the stretched-exponential component of the strain response. Examining data for SC in both dry (45% RH conditioned) and highly hydrated (100% RH conditioned) states revealed significant differences in creep behavior. However, to gain a better insight into the distribution of time scales, a spectral decomposition method [33] was used to directly determine individual relaxation time scales in SC.

Using this technique, unique and distinct relaxation time scales important for deformation in human SC were found. Results shown in Figure 1.5 include discrete relaxation time scales for human SC in increasingly moist conditions. The physical basis for the effect of increasing hydration and relaxation times is associated with the highly keratinized nature of the corneocytes in which the keratin fibrils, aligned in the planar direction of the SC, can more easily slide and accommodate tissue deformation. As keratin-based systems hydrate, the

**Figure 1.4** Stress-strain data for ~20 $\mu$m thick free standing films of human SC showing (a) the pronounced effect of hydration on SC mechanical properties with SC modulus decreasing orders of magnitude over the RH conditioning examined, and (b) obvious hysteresis indicative of viscoelastic behavior in stress-strain testing for 100% RH conditioned SC [17].
hydrogen bonding between keratin fibrils becomes disrupted by the presence of water allowing the fibrils to have more degrees of freedom [34].

The first quantitative intercellular delamination energies, $G_c$ [J/m²], of human SC were reported, and the measured delamination energy was shown to be sensitive to tissue conditioning and treatments including selected surfactants and pH treatments [15]. Fracture mechanics-based cantilever-beam specimens were used to determine SC intercellular delamination energy. Novel stress-separation tests were also performed to measure the out-of-plane mechanical behavior of the SC. The delamination energy for debonding of cells within the stratum corneum layer was found to be sensitive to the moisture content of the tissue and to the test temperature (Figure 1.6a).

The delamination energy of intercellular lipid-extracted specimens using a chloroform-methanol treatment (CMT) was substantially higher than untreated controls. Stress-separation tests produced similar results. We should emphasize that these results represent the first reported quantitative studies of the out-of-plane mechanical properties of SC perpendicular to the skin surface. The delamination energy and strength property dependence on temperature and moisture were rationalized in terms of the underlying SC cell structure and intercellular lipids [14]. Additionally, time-dependent intercellular delamination experiments were performed to

Figure 1.5 Discrete creep retardation time spectra for human SC hydrated in 10, 45, 100% RH showing distinct changes in retardation spectra for highly hydrated (100% RH conditioned SC) compared to drier specimens [17].
understand the rate of intercellular separation processes at both short and long times [16]. Such behavior appears more sensitive to tissue condition and treatment as shown in Figure 1.6b.

**Figure 1.6** Effects of SC tissue treatments on intercellular delamination behavior measured (a) under critical loading conditions showing the effect of selected buffered pH, surfactant, and delipidization treatments [15], and (b) under subcritical conditions showing the sensitive nature of these time-dependent delamination properties to treatment conditions (SDS: sodium dodecyl sulfate (10% wt/wt); CMT: chloroform-methanol (2:1 v:v) treatment; APG: alkyl polyglucoside (10% wt/wt); AAS: alkyl amidosulfosuccinate (10% wt/wt)) [16].

In another first, the intercellular delamination energy of SC was characterized as a function of tissue depth [15] and was observed to increase with increasing depth into the tissue as shown in Figure 1.7. This was a remarkable finding considering that the graded character of SC had been long recognized, but only few attempts had been made to provide quantitative measurements of mechanical properties as a function of tissue depth.

The techniques developed above were employed to benchmark “good” and “bad” skin conditions with treatments known to be damaging or beneficial to tissue condition. Among many others, the treatments assessed have included
both harsh surfactant sodium dodecyl sulfate formulations and milder formulations containing sodium lauryl ether sulfate. We have even demonstrated that subsequent treatments for possible “repair” of the tissue using glycerol, sunflower seed oil, and other possible molecules can be made and quantified.

**Figure 1.7** Schematic of SC showing aligned keratin filaments, corneocyte envelopes, intercellular lipids, and corneodesmosomes. The variation of intercellular delamination energy through the thickness of the \( \sim 20 \, \mu \text{m} \) thick

### 1.6 SYNOPSIS

The presented research in this dissertation involves synergistic experimental and mechanics modeling work to provide a fundamental scientific and clinically relevant basis from which cracking and chapping associated with dry skin conditions can be quantitatively characterized and understood.

Chapter 1 provides a brief introduction to the topical matter addressed in this dissertation. The structure and function of SC as the outermost layer of skin
is discussed together with the potential effect of SC components on the drying stresses in the tissue. A biomechanics framework to account for the SC drying stress as a mechanical driving force for dry skin damage is presented. The previous SC research is discussed briefly to motivate the present study.

Chapter 2 describes the theory behind the mechanics and fracture mechanics techniques used in subsequent chapters to describe SC behavior.

Chapter 3 provides a detailed description of the tissue used for these studies as well as its preparation. The methods used to probe the mechanical properties of SC are detailed as well.

Chapter 4 describes the application of the substrate curvature method to accurately characterize SC drying stresses as a function of time following environmental pre-conditioning and chemical treatment in a range of drying environments. A unique relationship between the SC stress and water in the drying environment is established.

Chapter 5 presents the application of the substrate curvature method to differentiate drying stresses in topical coatings and SC.

Chapter 6 describes the application of the substrate curvature method to characterize the effect of a range of moisturizing molecules on the drying stresses developed in human stratum corneum (SC). Following moisturizing treatment, the SC is observed to have distinctive stress profiles depending on the effectiveness of the treatment. The role of moisturizing molecules on the drying stresses in SC is discussed in relation to their effects on the underlying SC structure. The efficacy of moisturizing treatments in alleviating dry skin damage is determined by comparing the intercellular delamination energy, $G_c$, with the strain energy release rate, $G$.

Chapter 7 describes the effect of corneodesmosome degradation on SC intercellular delamination energy. The study has potential implications to better understand the mechanics behind SC desquamation.

Chapter 8 serves to summarize the significant results obtained from this work as well as present some ideas for future studies in this area.
1.7 References


Chapter 2 Theoretical Mechanics Background

Thin film materials are widely used in all industries to fulfill a wide variety of applications [1]. These materials are subject to appreciable residual stress, which can cause cracking of the films [2]. In this dissertation, we focus on the residual drying stresses that occur in our body’s primary chemical, diffusional, and mechanical barrier, stratum corneum (SC), as a result of its exposure to variable temperature and moisture conditions as well as application of cleansing agents and potentially damaging chemicals. In vivo, the SC layer can be treated as a thin (15 – 30 μm) stiff film on a thick (1-3 mm) compliant viscoelastic substrate consisting of epidermal and dermal layers as shown in Figure 2.1. From a mechanics perspective, the SC drying stresses provide a mechanical “driving force” for dry skin damage such as cracking and chapping of the SC. To better understand the damage processes in human skin, we will review the commonly observed fracture patterns in a pre-tensioned film on a substrate, together with a discussion of the governing parameters. The fracture mechanics theory based on the Griffith fracture criteria, which provides the foundation for modern fracture mechanics, is first outlined to contextualize the results and discussion presented in subsequent chapters.

![Figure 2.1](image)

**Figure 2.1** Given the very different mechanical properties of SC compared to the underlying substrate, SC can be thought of as a stiff and stressed elastic film on a compliant skin substrate.
2.1 Griffith Energy Balance

In the 1920s, using a simple thermodynamics approach, Griffith reasoned that a crack can form and grow only if such processes are energetically favorable, meaning the creation of a crack in a material would cause its total system energy to decrease or to remain constant [3]. Accordingly, this theory dictates whether a crack in a body will grow or shrink according to an energetic balance. Considering an infinitely wide elastic plate with a through-crack of length 2a subject to a constant tensile stress as illustrated in Figure 2.2, the total energy of the system is expressed as the sum of the total strain energy of the plate, \( \Pi \), and the energy of the surfaces associated with the crack, \( W_s \):

\[
U = \Pi + W_s \tag{Eq. 2.1}
\]

The total strain energy of the plate in Figure 2.2 can be further broken down:

\[
\Pi = \Pi_o + \Delta \Pi_{2a} + \Delta \Pi_w \tag{Eq. 2.2}
\]

where \( \Pi_o \) is the strain energy of the uncracked plate, \( \Delta \Pi_{2a} \) represents the change in strain energy with the introduction of a crack, and \( \Pi_w \) is the external work.
done on the system. For fixed grip conditions, Griffith showed that the presence of the crack decreases the total strain energy of the plate according to:

\[ \Pi = \Pi_o - \frac{\pi \sigma^2 a^2 B}{E} \]  
(Eq. 2.3)

where \( \sigma \) is the applied stress, \( a \) is the half crack length, \( B \) is the plate thickness and \( E \) is the Young’s modulus of the material. The energy associated with the creation of two new surfaces, \( W_s \), is given by:

\[ W_s = 4aB \gamma_s \]  
(Eq. 2.4)

where \( \gamma_s \) is the surface energy with units of energy per unit area.

Combining Equations 2.1 – 2.3, yields the following expression for the system energy:

\[ U - \Pi_o = -\frac{\pi \sigma^2 a^2 B}{E} + 4aB \gamma_s \]  
(Eq. 2.5)

The total energy of the system minus the strain energy of the initial uncracked plate, \( U-\Pi_o \), the change in strain energy with the introduction of a crack, \( \Pi - \Pi_o \), and the surface energy of the crack, \( W_s \), are plotted as a function of the crack length in Figure 2.3.

**Figure 2.3** In the Griffith thermodynamic fracture analysis, the balance between strain energy released (\( \Pi - \Pi_o \)) and energy required for surface creation (\( W_s \)) yields a critical crack length.
With increasing crack length, the surface energy of the crack increases linearly due to the creation of new surfaces whereas the $\Pi - \Pi_0$ component decreases in a quadratic fashion due to the release of stored strain energy. Looking at the total energy of the system, we observe that there exists some critical crack length, $a_c$, beyond which crack growth will be energetically favorable. This can be better understood by examining the extremum of the variation of the system energy with crack area, $A$:

$$\frac{d(U)}{dA} = \frac{d(U)}{d(aB)} = 0 = \frac{\pi \sigma^2 a}{E} - 2\gamma_s$$  \hspace{1cm} (Eq. 2.6)

This analysis would predict that for the given geometry and applied stress, when $a < a_c$ crack growth is inhibited because the system energy would increase as the crack grew ($dU / dA > 0$); and when $a \geq a_c$ crack growth becomes energetically favorable ($dU / dA \leq 0$). Solving the equilibrium condition above, we arrive at the Griffith condition, which reports the predicted fracture stress in terms of the flaw size:

$$\sigma_c = \sqrt{\frac{2E\gamma_s}{\pi a_c}}$$  \hspace{1cm} (Eq. 2.7)

Griffith observed that the equation above accurately described the fracture behavior of glass specimens containing flaws of varying sizes. While this model was shown to be applicable to capture the fracture behavior of ideally brittle solids, it largely underestimated the fracture strength of metals and other engineering materials [4]. The discrepancy between predicted and observed behavior was attributed to plastic deformation and energy dissipation mechanisms such as ligament bridging between crack faces and micro-cracking in the specimen which may all impede crack extension by dissipating energy that would be utilized in crack extension (Figure 2.4).

After Griffith’s initial work, other researchers (Irwin and Orowan) modified the Griffith condition defined above and included another term, $\gamma_{pl}$, to account for additional dissipative mechanisms [5,6]:

$$\sigma_c = \sqrt{\frac{2E(\gamma_s + \gamma_{pl})}{\pi a_c}}$$  \hspace{1cm} (Eq. 2.8)
Irwin defined the strain energy release rate, $G$, for a material:

$$G \equiv -\frac{d\Pi}{dA}$$  \hspace{1cm} (Eq. 2.9)

The strain energy release rate (also referred as the crack driving force in this dissertation) represents a measure of the energy available for crack extension. It has units of energy per unit area ($J/m^2$). Irwin took into account that a material’s resistance to fracture can include energy dissipation mechanisms other than the crack tip bond rupture leading to creating of new surfaces, and defined the material’s resistance to fracture, $G_c$:

$$G_c = 2\left(\gamma_s + \gamma_{pl}\right)$$  \hspace{1cm} (Eq. 2.10)

Then, for the given loading conditions, $\sigma$, and specimen crack geometry, $a$, shown in Figure 2.2, fracture can occur when $G$ reaches a critical value $G_c$:

$$G \geq G_c = \frac{\pi\sigma_c^2a_c}{E}$$  \hspace{1cm} (Eq. 2.11)

where $\sigma_c$ and $a_c$ are the far-field stresses and the crack length at incipient crack growth. The definition of $G$ presented in Eq. 2.9 is equivalent to:

$$G = \frac{P^2}{2B} \frac{dC}{da}$$  \hspace{1cm} (Eq. 2.12)

where $P$ is the applied load, $B$ is the specimen thickness as before, $C$ is the specimen compliance, and $a$ is the crack length.

Figure 2.4 Schematic of a cracked body under tensile loading showing different components that work against crack growth including surface formation (bond rupture) and other dissipation mechanisms such as plasticity in the specimen.
2.2 THE STRESS INTENSITY FACTOR

The stress intensity factor, $K$, is also commonly used to quantify the material’s resistance to fracture. While $G$ is the thermodynamic crack driving force, the stress intensity factor $K$ is a parameter characterizing the process zone ahead of the crack tip. Near the crack tip, the stress state approaches a singularity that is scaled by the stress intensity factor. Considering the crack tip in a conventional polar coordinate system shown in Figure 2.5, the resulting stress field near the crack tip for pure opening loading in the $y$-direction with a crack propagating in the $x$-direction (similar to the double-cantilever beam (DCB) geometry used in this dissertation) is given by:

$$
\sigma_{xx} = \frac{K_I}{\sqrt{2\pi r}} \cos\left(\frac{\theta}{2}\right) \left[1 - \sin\left(\frac{\theta}{2}\right) \sin\left(\frac{3\theta}{2}\right)\right]
$$

$$
\sigma_{yy} = \frac{K_I}{\sqrt{2\pi r}} \cos\left(\frac{\theta}{2}\right) \left[1 + \sin\left(\frac{\theta}{2}\right) \sin\left(\frac{3\theta}{2}\right)\right]
$$

$$
\tau_{xy} = \frac{K_I}{\sqrt{2\pi r}} \cos\left(\frac{\theta}{2}\right) \sin\left(\frac{\theta}{2}\right) \cos\left(\frac{3\theta}{2}\right)
$$

$\sigma_{zz} = 0$ for plane stress

$\sigma_{zz} = v(\sigma_{xx} + \sigma_{yy})$ for plane strain

$$
\tau_{xz} = \tau_{yz} = 0 \quad \text{(Eq. 2.13)}
$$

Figure 2.5 Crack tip at the origin of a conventional polar coordinate system.
where $K_I$ is the Mode I stress intensity factor, with typical units of $MPa\sqrt{m}$, that defines the amplitude of the stress field ahead of the crack tip. Note that a crack can be subject to three basic loading modalities as shown in Figure 2.6. These modes known as opening, in-plane shear and out-of-plane shear are designated as Mode I, Mode II, and Mode III, respectively. Similar expressions to Eq. 2.13 are available to describe the stress states in other loading modes, and the stress state for mixed-mode loading is the superposition of the contribution of each of the individual modes.

![Figure 2.6](image)

**Figure 2.6** Schematic showing different modes of loading: a) Mode I loading b) Mode II loading c) Mode III loading.

Closed-form solutions of $K$ have been derived for many sample configurations, and $K$ can often be estimated through experimental and numerical analysis techniques for more complicated configurations [4]. In general, the stress intensity factor has the following form:

$$K_{(I,I,I)} = Q\sigma\pi a$$  \hspace{1cm} (Eq. 2.14)

where $Q$ is a dimensionless constant that depends on the geometry and mode of loading. For example, the stress intensity factor for a through plate subjected to a remote stress, $\sigma$, as shown in Figure 2.2 is given as:

$$K_I = \sigma\sqrt{\pi a}$$  \hspace{1cm} (Eq. 2.15)

As in the discussion of $G$ in the previous section, fracture occurs when the stress intensity factor $K$ exceeds some critical value of stress intensity, $K_c$. 


Fracture toughness is usually reported as the critical Mode I stress intensity factor, \( K_{IC} \).

As the formulation in Eq. 2.13 is developed for ideally linear elastic isotropic materials, there are certain criteria that must be met to ensure that a linear elastic approach is valid and that \( K_I \) can be used to determine material properties independent of specimen size. The formulation predicts that the stresses become infinite at the crack tip \( (r \to 0) \) which is physically not possible. In reality, materials that yield begin to form a plastic deformation zone ahead of the crack tip once the stresses exceed the material yield stress. The radius of this zone is:

\[
r_p = \frac{1}{2\pi} \left( \frac{K_I}{\sigma_{ys}} \right)^2
\]

(Eq. 2.16)

where \( \sigma_{ys} \) is the material’s yield stress. To ensure that a linear elastic fracture is valid, first small scale yielding requirements must be met: the size of plastic deformation must be limited in size such that:

\[
r_p \leq (a,b)/15
\]

(Eq. 2.17)

where \( a \) is crack length and \( b \) is the remaining length of uncracked specimen. Additionally, plane strain conditions which stipulate that \( r_p \leq B/15 \) must be satisfied. If these two conditions are met, \( K_I \) can be used as a fracture criterion and defined as \( K_{IC} \) at incipient crack growth.

### 2.3 Equivalence of G and K

We have reviewed two methods for determining fracture criteria. One used a thermodynamics approach and the other one used a mechanics approach. Both criteria can be shown to be equivalent methods for predicting the onset of material failure. A comparison of the expressions for \( G \) and \( K \) for a crack in an infinite plate loaded with a remote tensile stress shows that the relationship between \( G \) and \( K \) is simply:

\[
G = \frac{\pi \sigma^2 a}{E}
\]
\[ K_I = \sigma \sqrt{\pi a} \]
\[ G = \frac{K_I^2}{E'} \]  
(Eq. 2.18)

where \( E' = E \) for plane stress and \( E / (1-\nu^2) \) for plane strain. Similar relationships between \( G \) and \( K \) can be derived when shear loading is present as well. Lastly, it should be noted that \( G \) is a scalar, but \( K \) is not.

### 2.4 Fracture In Film–Substrate Systems

As stated previously, thin film materials are widely used in all industries to fulfill a wide variety of applications and are typically subject to appreciable residual stress, which can cause cracking of the films [2]. Often films are deposited at temperatures greater than the ambient environment and have thermal expansion coefficients that are different from the substrate material. This mismatch is thermal expansion creates a compressive or tensile residual film stress that can lead to delamination or cohesive fracture [2,7,8]. In addition to thermal stress, residual stresses may also rise from the actual deposition process or as a result of lattice mismatch for epitaxial films.

![Figure 2.7 Schematic of a thin film on a substrate.](image)

For example, in the thin film-substrate system shown in Figure 2.7, if the thermal expansion coefficient of the film, \( \alpha_f \), differs from that of the substrate, \( \alpha_s \), the misfit strain is biaxial, having magnitude:
\[ \varepsilon_o = - (\alpha_s - \alpha_f)(T_f - T_i) \]  
(Eq. 2.19)

where \( T_f \) and \( T_i \) are the final and initial temperatures, respectively. The resulting residual stress will also be biaxial, with magnitude:

\[ \sigma_f = (\alpha_s - \alpha_f)(T_f - T_i) \frac{E_f}{1-v_f} \]  
(Eq. 2.20)

where \( E_i \) is the Young's modulus and \( v_f \) is the Poisson's ratio. The film-substrate is stress-free at a high temperature \( T_i \). Upon cooling, the residual stress will be tensile when the thermal expansion coefficient for the film is larger than that of the substrate and will be defined explicitly with Eq. 2.20 when the film width, \( w \), is very large compared to the thickness of the film, \( h_f (w >> h) \). For a narrow strip \( (w \leq h) \), the residual stress will be:

\[ \sigma_f = (\alpha_s - \alpha_f)(T_f - T_i)E_f \]  
(Eq. 2.21)

Using the energetic approach presented in Section 2.1, one can determine the crack driving force, \( G \), for the thin film-substrate system studied in this section from the differentiation of stored strain energy with crack area, \( A \). Note that the stored strain energy for a wide \( (w >> h_f) \) and a narrow \( (w \leq h_f) \) strip will be:

\[ \Pi_{w>>h_f} = \frac{1-v_f}{E_f} \sigma_f^2 h A \]

\[ \Pi_{w<h_f} = \frac{1}{2} \frac{\sigma_f^2}{E_f} h A \]  
(Eq. 2.22)

respectively. Finally, \( G \) for a wide and a narrow strip will be:

\[ G_{w>>h_f} = \frac{1-v_f}{E_f} \sigma_f^2 h \]

\[ G_{w<h_f} = \frac{1}{2} \frac{\sigma_f^2}{E_f} h \]  
(Eq. 2.23)

For the discussed system, the thin film will delaminate when \( G \) attains the fracture resistance, \( G_c \). The relationship between \( G \) and \( G_c \) defines a critical film thickness, \( h_c \), below which film cracking will not occur. For the wide strip, this thickness is given by:
In general, any film debonding or cracking associated with only film stresses have the form:

\[ G = Z \frac{\sigma_f^2 h}{E_f} \]  

(Eq. 2.25)

where \( E_f \) is the plane stress or plane strain modulus. \( Z \) is a constant that depends on the precise geometry and the elastic mismatch between film and substrate through Dundurs’ parameters:

\[ \alpha = \frac{E_f - E_s}{E_f + E_s} \]

\[ \beta = \frac{E_f (1 - \nu_f)(1 - 2\nu_s) - E_s (1 - \nu_s)(1 - 2\nu_f)}{2(1 - \nu_f)(1 - \nu_s)(E_f + E_s)} \]  

(Eq. 2.26)

When the film and substrate have identical elastic moduli, \( \alpha = \beta = 0 \) and \( Z = 1.976 \). The value of \( Z \) decreases slightly for a compliant film on a relatively stiff substrate (\( E_f < E_s \) and \( \alpha < 0 \)). However, when the substrate is more compliant than the film (\( \alpha > 0 \)), there is less constraint against film cracking. Thus, \( Z \) increases as \( \alpha \) increases. For very compliant substrates, \( Z \) increases rapidly, with \( Z > 30 \) for \( \alpha > 0.99 \) [9,10,11]. The effect of \( \beta \) is secondary and often ignored.

Commonly observed cracking patterns (surface cracking, channeling, substrate damage, spalling, debonding) are sketched in Figure 2.8, together with their \( Z \) values, where the film-substrate system is taken to be elastically homogenous. The surface crack originates from a flaw and then arrests by the interface [Figure 2.8a]. The stresses are not high enough to cause channeling through the film. They can be detrimental for some application such as corrosion protection coatings. Another possible cracking mode for thin film under tension is channel cracking [Figure 2.8b], where through-film cracks propagate in the film. The channeling process does not arrest until it encounters another channel or an edge, creating a connected channel network [2].
As the cracks in the film propagate further towards the interface between the film and the substrate, they may bifurcate along the interface causing debonding of the film [Figure 2.8e] or cross the interface and cause substrate damage [Figure 2.8c]. Those cracks that lead to substrate damage can be stabilized at a certain depth in the substrate or keep growing in the substrate parallel to the interface causing spalling [2] [Figure 2.8d].

2.5 CRACK DRIVING FORCE OF SC

Using the mechanics background in the preceding sections, we calculated the crack driving force, G, of SC for through and delamination cracking configurations as shown in Figure 2.9. Considering that the drying stresses in SC
are typically on the order of a few MPa, G value of a through crack can increase up to several hundred J / m² for a 1 mm through crack whereas G values of a delamination crack are much lower. We would like to note that these G values can lead to cracking in the tissue since the in-plane fracture energy of the tissue is ~200 J / m² [12] and the intercellular delamination energy of the tissue is ~1-8 J / m² [13].

**Figure 2.9** The crack driving force of SC for through and delamination cracking configurations.

### 2.6 Conclusion

In this chapter, the theoretical basis of thin film fracture has been explored to better understand damage processes in human SC. This theory will be applied in later chapters to characterize the intercellular delamination energy and the
crack driving force of the tissue and to predict which treatments can enhance or alleviate dry skin damage.

2.7 References


Chapter 3 Experimental Procedures

The methods used to explore the mechanical properties of stratum corneum (SC) are reviewed in this chapter. The thin film nature of SC poses a unique challenge when handling the tissue, particularly given its high compliance when hydrated. Techniques which have been used in other applications to examine thin film structures, particularly as related to microelectronics have been adapted and refined for use with this biological tissue [1,2].

3.1 ISOLATION OF HUMAN STRATUM CORNEUM

The thin film nature of human SC (~20 μm) poses a unique challenge when handling the tissue, particularly given its mechanical fragility and high compliance when hydrated. The research presented in this dissertation was conducted exclusively on human cadaver SC obtained from female Caucasian donors, 30 – 70 years of age, from the abdomen, back or thigh. Female donors and body sites were selected to reduce tissue variability and the presence of hair, which can introduce holes and tears in the tissue.

The SC isolation process is shown in Figure 3.1. Donor tissue was received from the National Disease Research Interchange (NDRI, Philadelphia, PA) as full thickness skin from which subcutaneous fatty tissue was removed with scissors. Epidermal tissue was separated from isolated dermis after immersion in a 35°C water bath for 10 min followed by a 1 min soak at 60°C. The epidermis was mechanically separated from the dermis. Subsequently, the SC was isolated from the underlying epidermis by soaking the tissue in a trypsin enzymatic digest solution (0.1% wt/wt in 0.05 M, pH 7.9 Tris buffer) at 35°C for 120 min. The orientation of the outer SC surface was noted and recorded. After treatment, detached epidermal cells were rinsed off the SC with distilled water.

After processing, the SC was allowed to dry on filter paper then removed and stored in ambient conditions of ~ 22°C and ~ 45% RH. Please see Appendix A for a more detailed procedure.
Uniaxial tensile testing was performed using a MTS Bionix 200 tensile testing apparatus (Bionix 200, MTS Systems Corporation, Eden Prairie, MN) equipped with a 1 kN load cell. Specimens were enclosed in a custom acrylic box built around the testing area to enable relative humidity (RH) control. The RH inside the chamber was controlled by placing open containers of saturated salt solutions or water in the enclosed space. Test environment was monitored using a temperature and RH sensor (TM325, The Dickson Company, Addison, IL). The experimental setup is shown in Figure 3.2. To set specimen gage length, the grips were brought into contact as detected by observing a compressive force on the load cell output, next the displacement reading was set to zero, and the fixtures were moved apart using computer control to the desired grip spacing / gage length which was typically 10 mm.

**Figure 3.1** Schematic illustration of the methods used to separate and produce free standing films of the stratum corneum layer with minimal tissue damage.

### 3.2 MICRO-TENSION

Uniaxial tensile testing was performed using a MTS Bionix 200 tensile testing apparatus (Bionix 200, MTS Systems Corporation, Eden Prairie, MN) equipped with a 1 kN load cell. Specimens were enclosed in a custom acrylic box built around the testing area to enable relative humidity (RH) control. The RH inside the chamber was controlled by placing open containers of saturated salt solutions or water in the enclosed space. Test environment was monitored using a temperature and RH sensor (TM325, The Dickson Company, Addison, IL). The experimental setup is shown in Figure 3.2. To set specimen gage length, the grips were brought into contact as detected by observing a compressive force on the load cell output, next the displacement reading was set to zero, and the fixtures were moved apart using computer control to the desired grip spacing / gage length which was typically 10 mm.
Figure 3.2  Tensile testing apparatus showing (a) the actual test grips (without a specimen), the clear acrylic environmental enclosure, the temperature and humidity sensor (*), and the 1 kN load cell (**). Schematics of the grips are shown in (b) and (c) illustrating the grooved aluminum gripping surfaces and cellophane tape-attached paper to prevent cutting of the SC by the metal grips.

Specimens of SC were prepared by placing SC on a sheet of paper, seating a polycarbonate beam of known width over the tissue, then cutting around the beam template. Specimens 6 mm in width and approximately 25 mm in length were prepared in this manner. Given the highly compliant nature of SC, particularly when wet, a special mounting procedure was performed. As seen in Figure 3.2(b,c), the aluminum mounting grips were fabricated with diagonal grooves to prevent sample slip during testing. However, to prevent specimen failure due to tissue tearing at the grips, pieces of paper were placed between the SC and the gripping surface. Specifically, pieces of paper were adhered to the grip fixtures attached to the loading system as illustrated in Figure 3.2(b). Next SC was adhered to a separate square piece of paper using cellophane tape, and this
combined unit was adhered with an additional piece of tape to the upper free grip fixture as shown in Figure 3.2(c). The upper grip was then secured to the upper test fixture shown in Figure 3.2(b). A piece of paper was taped to the lower free grip as well and that grip was attached to the lower grip fixture to adhere the SC in place. During this procedure, the load applied to the SC was monitored to ensure that the specimen was not loaded excessively during mounting. After mounting, grip separation was adjusted to remove any residual loads, and any change in gage length was noted. The environmental enclosure was then placed around the testing grips, and the environment and specimen were allowed to equilibrate prior to testing.

The uniaxial drying stress was measured by initially loading the specimen until a minimum load of 10 mN was observed to straighten but not significantly strain the specimen between the loading fixtures. The displacement of the grips was then fixed and the load recorded as a function of time for a 8 hr drying period under temperature and humidity controlled conditions. The tissue thickness, $h_{SC}$, was measured in situ every 20 minutes with the long working distance optical microscope. Using the measured loads and $h_{SC}$, the true stress in the SC during drying was determined.

To measure SC modulus, the treated or untreated SC was displaced at a rate of 100 $\mu$m /s. The true stress and strain was calculated from the measured loads and grip displacements together with the SC specimen thickness, $h_{SC}$, measured using a long working distance optical microscope (TT23012, Questar, New Hope, PA). The SC modulus was found from the slope of the true stress vs. true strain curve.

### 3.3 Substrate Curvature

In our curvature experiments, 22 x 22 mm borosilicate glass cover slips, 177 $\mu$m thick (Fisher Scientific, 12-541-B) with a Young’s modulus of 69 GPa and a Poisson’s ratio of 0.2, were used as the substrate. This substrate was preferred over other elastic substrates because of the good adherence of the wet tissue (explained later in this section). Cr / Au films (35 Å / 465 Å) were deposited by
evaporation onto one side of the glass substrate opposite to the side containing the SC to improve reflectivity. A scanning laser substrate curvature instrument (FLX-2320, Tencor Instruments, Mountain View, CA) was used to measure the substrate angle of deflection, \( \alpha \), in terms of the dimensions \( L_s \) and \( L_b \) as a function of position, \( y \) as defined in Figure 3.3. The average curvature was subsequently calculated from a linear regression analysis of \( \alpha \) versus position data [2]. An initial curvature measurement was taken before the tissue was adhered to the substrate to detect any residual curvature of the substrate not associated with subsequent SC stresses. In what follows, the curvature represents the change in curvature from the initial curvature of the substrate.

The relationship between the biaxial SC film stress, \( \sigma_{sc} \), and the elastic curvature, \( K \) was expressed with the Stoney’s equation [3]:

\[
\sigma_{sc} = \left( \frac{E_{sub}}{1 - \nu_{sub}} \right) \frac{h_{sub}^2}{6h_{sc}} K 
\]

(Eq. 3.1)

where \( E_{sub} \), \( \nu_{sub} \), \( h_{sub} \) and \( h_{sc} \) are the Young’s modulus, Poisson’s ratio, thickness of the substrate and SC specimen thickness, respectively.

Square (25 x 25 mm) SC specimens were prepared by placing SC on a sheet of paper, seating a 25 x 25 mm polycarbonate beam over the tissue, then cutting around the beam template. SC was subsequently submersed in 50 mL of DIW or any desired treatment inside a petri dish for 25 minutes followed by a 5 minute rinse in DIW prior to testing. To prepare the specimens, a glass cover slip on filter paper was submersed and positioned under the treated SC prior to the end of 5 minute rinse in DIW. As water was removed with pipettes, the SC settled onto the cover slip, fully coating the side of the cover slip opposite to the Cr/Au films without forming any visible wrinkles in the SC. Excess SC at the edges of the cover slip was removed with a razor blade (VWR, 55411-050). Initially, we attempted to adhere SC to the glass substrates using the established tissue bonding methods we employ for intercellular delamination energy measurements. This procedure was discontinued when wet SC was observed to adhere to the glass without any adhesive. Thus, the SC stresses were measured based solely on the curvature of the glass substrate.
Figure 3.3 The experimental arrangement for the substrate curvature technique showing the SC mounted on a glass substrate. A scanning laser equipped with detector measures the angle of deflection, $\alpha$, vs. position, $y$, on the substrate. The average curvature is calculated from a linear regression of the deflection angle.

The specimen was positioned Au-face towards the laser in the substrate curvature instrument in the same orientation as the initial curvature measurement. The temperature and RH of the air in the instrument was controlled and measured with a hygrometer (TM325, The Dickson Company, Addison, IL) at 5 minute intervals.

There were concerns that SC could be sliding on the substrate during drying since there was no adhesive between SC and the glass substrate. These concerns will be addressed in Chapter 4 and tissue adherence to the substrate will be confirmed with optical microscopy measurements.

In the moisturizer study, the coating stress during drying was confirmed by applying the solid or liquid moisturizing treatments directly on the glass substrate and then exposing them to a given drying environment. The liquid moisturizers were dipcoated on the specimens. The draw speed used was 200
mm/s. The solid moisturizers were applied as coatings using a precise casting knife applicator with a micrometer controller (3580/1, Elcometer, Rochester Hills, MI).

### 3.4 Double Cantilever Beam Fabrication

Using the double cantilever bending (DCB) fracture-mechanics based approach developed in previous SC research to quantitatively characterize the intercellular delamination energy of the SC [4,5,6,7], we measured the cracking resistance, $G_c$, of treated SC specimen before and after the drying exposures. This was intended to understand the effect of different treatments in enhancing or alleviating dry skin conditions.

The technique involves sandwiching the SC between two elastic polycarbonate substrates (Hyzod® GP, Sheffield Plastics Inc., Sheffield, Massachusetts) with cyanoacrylate adhesive (Instant Krazy Glue© Gel, Elmer’s Products Inc., Columbus, Ohio) to form fracture mechanics based DCB specimens (Figure 3.4). Cyanoacrylate adhesive polymerization is readily initiated by the presence of small amounts of water on the bonding surfaces limiting the adhesive to the SC exterior. The transparent polycarbonate beams facilitate optical inspection of the inner sandwich structure during specimen preparation and testing. To enable the use of linear elastic fracture mechanics to determine the strain energy release rates, substrate dimensions were chosen to ensure purely elastic deformation of the substrates during testing [8,9].

To fabricate the DCB specimens, a thin layer of cyanoacrylate adhesive was applied to one face of a nominally $40 \times 10 \times 2.88 \text{ mm}^3$ polycarbonate substrate leaving a $7 – 10 \text{ mm}$ region of the beam end uncoated. The substrate was pressed against the untreated or treated SC on the filter paper backing and a scalpel was used to cut around the substrate to detach the adhered SC from surrounding tissue. The SC orientation was noted to ensure that the outer SC surface was facing out.

In the moisturizer study, prior to treatment, specimens consisting of untreated SC glued to the polycarbonate beam were equilibrated at 100% RH and
22\degree C for 2 hrs. Specimens were then treated with liquid or solid moisturizing treatments. The liquid moisturizers were dipcoated on the specimens. The draw speed used was 200 \( \mu \text{m/s} \). The solid moisturizers were applied as coatings using a precise casting knife applicator with a micrometer controller (3580/1, Elcometer, Rochester Hills, MI). Following exposure to the moisturizing treatments, specimens were dried at 7\%RH and 22\degree C for 6 hrs, and blotted with filter paper (Grade 595 Filter Paper, Schleicher & Schuell MicroScience GmbH, Dassel, Germany) for 2 min to remove excess treatment.

![Double cantilever beam (DCB)](image)

**Figure 3.4** Schematic drawing of a double-cantilever beam (DCB) specimen and its cross sectional geometry.

To form the final sandwich structure, another substrate coated with adhesive in the same manner was pressed against the SC face of the complimentary beam with adhesive–free ends aligned. Excess adhesive along the sandwich edges was removed with a scalpel to ensure that the two halves of the sandwich structure were bound together by SC only. Aluminum loading tabs were subsequently adhered to the SC free ends of the DCB substrates using additional cyanoacrylate.

### 3.5 Delamination Testing Techniques

The DCB specimens were tested in a custom built mechanical test system with a computer controlled DC servoelectric actuator operated in displacement control. Tests were performed at a constant displacement rate of 2 \( \mu \text{m/s} \). Corresponding loads were measured using a 222 N load cell.
3.5.1 Intercellular Delamination Energy Measurements

The delamination length, \( a \), was measured from recorded load-displacement, \( P-\Delta \), and their elastic compliance relationship:

\[
C = \frac{\Delta}{P} = \frac{2}{3} \left( \frac{a + 0.64h}{EI} \right)^3
\]

(Eq. 3.2)

where \( I = bh^3/12 \), and \( C \) is the specimen compliance, \( P \) is the load, \( \Delta/2 \) is the corresponding displacement of each beam from its original position at the loading point, \( E' = E/(1-\nu^2) \) is the plane strain Young’s modulus for the polycarbonate, \( \nu \) is Poisson’s ratio, \( I \) is the area moment of inertia, \( b \) is the polycarbonate substrate width, and \( h \) is the height of each beam.

By measuring the critical load, \( P_c \), and the delamination length, \( a \), at incipient crack extension, the delamination resistance, \( G_c \), was determined from critical values of the strain energy release rate, \( G \) [8,9]:

\[
\frac{12P^2a^2}{b^2h^3E} \left( 1 + \frac{\sqrt{5}}{2} \frac{h}{a} + \frac{1}{2} \left( \frac{h}{a} \right)^2 \right)
\]

(Eq. 3.3)

Multiple delamination energies, \( G_c \), were measured for each DCB specimen by recording the critical loads, \( P_c \), and associated delamination lengths, \( a \), during delamination extension (Figure 3.5). For the present specimens, the values of \( E \) and \( \nu \) for the polycarbonate were 2.379 GPa and 0.38, respectively. Given the thin...
film nature of the SC compared to the massive polycarbonate substrate the contribution of the elastic strain energy in the SC layer can be ignored in the analysis [1].

3.5.2 Graded Intercellular Delamination Energy Measurements

To measure intercellular delamination energies through the thickness of the SC, successive DCB specimens were prepared from completely separated specimens by adhering new polycarbonate substrates onto the inner side of the previously tested specimen as schematically illustrated in Figure 3.6. In this manner, the inner side of the tissue was re-delaminated three times to systematically probe the delamination properties of the SC as a function of tissue depth.

![Figure 3.6](image)

**Figure 3.6** Illustration of through-thickness delamination measurements showing the (a) multiple delamination process in which substrates with delaminated SC attached are reused.

3.6 CONCLUSIONS

This chapter served to introduce the mechanical testing methods used to characterize the main components of the driving force for dry skin damage (e.g. SC modulus and drying stress) together with the cracking resistance to intercellular delamination. A means to probe through-thickness delamination
properties is detailed as well. The exact pre-conditioning and test conditions used for the various SC tests are detailed in the following chapters, while this chapter serves to explore the general methodologies and principles behind the tests performed.

3.7 REFERENCES


Chapter 4 Drying Stress and Damage Processes in Human SC

4.1 Abstract

The drying stresses that develop in stratum corneum (SC) are crucial for its mechanical and biophysical function, its cosmetic feel and appearance, and play a central role in processes of dry skin damage. However, quantitative methods to characterize these stresses are lacking, and little understanding exists regarding the effects of drying environment, chemical exposures and moisturizing treatments. We describe the application of a substrate curvature technique adapted for biological tissue to accurately characterize SC drying stresses as a function of time following environmental pre-conditioning and chemical treatment in a range of drying environments. SC stresses were observed to increase to stress levels of up to ~ 3 MPa over periods of 8 hours depending on pre-treatment and drying environment. A unique relationship between the SC stress and water in the drying environment was established. The effect of glycerol on lowering SC stresses and damaging surfactants on elevating SC stresses were quantified. Extensions of the method to continuous monitoring of SC stresses in response to changes in environmental moisture content and temperature are reported. Finally, a biomechanics framework to account for the SC drying stress as a mechanical driving force for dry skin damage is presented. (This section is currently in press: K. Levi, R.J. Weber, J.Q. Do and R.H. Dauskardt. Drying Stress and Damage Processes in Human Stratum Corneum. International Journal of Cosmetic Science, 2009)

4.2 Introduction

The stratum corneum (SC) is exposed daily to variable temperature and moisture conditions as well as application of cleansing agents and other potentially damaging chemicals [1]. These often result in the perception of skin
“stiffness” and “tightness” although the connection to the mechanical properties and stresses in the skin remains elusive due in part to a paucity of mechanical properties of the skin layers following such exposures [2,3,4]. Skin tightness may be directly related to changes in the stiffness of the SC layer and the buildup of tensile biaxial SC drying stresses in the plane of the SC layer. We describe new methods to quantitatively measure their magnitude and their relationship to selected drying environments and times.

The presence of SC drying stresses and their role in skin damage processes have been postulated and connections have been suggested to water loss during drying of treated SC [2,3,5,6,7]. From a mechanics perspective, the SC drying stresses provide a mechanical “driving force” for dry skin damage such as cracking and chapping of the SC as shown in Figure 4.1. Such damage can ultimately lead to tissue responses including inflammation, scarring and abnormal desquamation and further exacerbate the effects of skin disorders such as atopic dermatitis, ichthyosis vulgaris and chronic xerosis [8,9,10,11,12,13,14].

Figure 4.2 A schematic illustration showing typical dry skin cracking and chapping processes that result from the development of drying stresses in SC. The driving force for these damage processes, $G$, can be quantified in terms of the SC stress, elastic properties and thickness. The parameter $Z$ is determined by the cracking configuration only. The resistance to SC damage is given in terms of the intercellular delamination energy, $G_c$. Damage occurs when $G \geq G_c$ as indicated.
In vivo, the SC layer can be treated as a thin (15 – 30 μm) stiff film on a thick (1-3 mm) substrate consisting of the epidermal and dermal layers. The driving force for crack propagation for the cracking configurations shown in Figure 4.1 can be quantified in terms of the strain energy release rate, $G$, [15]:

$$G = \frac{Z \sigma_{SC}^2 h_{SC}}{\tilde{E}_{SC}}$$  \hspace{1cm} (Eq. 4.1)

where $\sigma_{SC}$ is the SC drying stress, $h_{SC}$ the SC thickness, $\tilde{E}_{SC}$ the plane strain SC Young's modulus and $Z$ a non-dimensional parameter for the specific cracking configuration ($Z = 3.95$ for surface cracks, 1.98 for channel cracking, and 0.50 for delamination). Note that this formulation assumes that the SC is a linear elastic thin film on an elastic substrate, which is accurate for the low strains, hydration conditions and time scales important for dry skin damage processes. The viscoelastic nature of the epidermal and dermal substrate layers will likely result in a greater mechanical driving force for skin damage. Extensions of the model to include these viscoelastic effects have been computed for other layered systems [16]. However, since their effects would only increase the propensity for cracking already revealed by the simpler model, the added complexities of these extensions are not considered further in the present study.

Cracking and chapping will develop in the SC during drying when the value of $G$ exceeds the intercellular delamination energy, $G_c$, which is a property of the tissue and provides a measure of the resistance to cracking. We have previously reported on the values of $G_c$ for SC and its relationship to tissue structure, condition, and treatment [17,18,19]. What remains is to accurately establish the value of $G$ from the measured $\sigma_{SC}$ values from which dry skin damage can be inferred. We then develop a biomechanics framework in which skin stress and skin damage processes can be predicted and modeled in terms of environmental exposure, tissue conditioning, and treatment.

The development of drying stresses in the SC has been demonstrated with tension experiments where the uniaxial stresses that develop during drying of a porcine SC specimen clamped between fixed grips [20]. These experiments are experimentally challenging since the SC is mechanically fragile and difficult to
handle after isolation and treatment, and does not simulate the roughly equal in vivo biaxial stress state of SC. We describe a new method to characterize the biaxial drying stress, $\sigma_{SC}$, from the curvature of an elastic substrate onto which the SC has been adhered. The technique is widely used in thin-film materials science to quantify the evolution of stress in thin films on elastic substrates [21,22]. The resulting SC stress state more closely resembles the in vivo biaxial stress state, and moisture can only escape from the outer SC surface during drying, thus more closely approximating in vivo drying conditions.

The $\sigma_{SC}$ values were examined as a function of time following environmental pre-conditioning and chemical treatment in a range of drying environments involving selected environmental conditions. The treatments included a strong anionic surfactant, sodium dodecyl sulphate (SDS), and a moisturizing solution containing glycerol (GLY), widely reported in the skin science literature [5,23,24,25]. The $\sigma_{SC}$ values were observed to increase from zero to plateau stress levels in the range ~ 0.07 - 3.2 MPa after ~ 4 hrs depending on pre-treatment and drying environment.

The biaxial drying stresses were consistent with uniaxial drying stresses measured using the micro-tension technique when the difference in stress state is taken into account. A strong relationship was observed between the plateau $\sigma_{SC}$ level and the ambient chemical potential of water in the drying environment. The sensitivity of the substrate curvature method to easily distinguish $\sigma_{SC}$ values after selected damaging and moisturizing treatments is shown together with extensions of the method to measure $\sigma_{SC}$ values that increase and decrease in response to changes in the moisture content and temperature of the environment. Finally, the evolution of drying stresses in SC is discussed in terms of hydration and chemical damage of SC components. A biomechanics framework to understand the implications of the $\sigma_{SC}$ as a mechanical driving force for damage propagation is reviewed.
4.3 MATERIALS AND METHODS

4.3.1 Tissue Preparation

Human SC was acquired from the abdomen of a 65 year old deceased Caucasian female donor. The study was approved by the Stanford University Institutional Review Board and conducted according to Declaration of Helsinki Principles. All comparative testing was performed on specimens taken from adjacent regions of the tissue from this single donor to reduce variations. The epidermal layer was separated from the dermis by immersion in a 35°C water bath to remove adipose tissue and afterward 60°C water bath for 1 min followed by careful mechanical separation from the dermis using a flat-tipped spatula. The SC was then detached from the epidermal layers by soaking in a trypsin enzymatic digest solution (0.1% (w/w) in 0.05M, pH 7.9 Tris Buffer) at 35°C for 135 minutes. During separation, the orientation of the outer SC surface was recorded. The isolated SC was rinsed with distilled water (DIW) at room temperature and allowed to dry under atmosphere on filter paper (Grade 595 Filter Paper, Schleicher & Schuell MicroScience GmbH, Dassel, Germany) and then removed and stored under ambient conditions of ~ 45% RH and ~ 18°C. Unlike most other soft tissues in the human body, SC is not vascularized and does not undergo rapid apoptosis and structural damage after such harvesting. The trypsin digest only causes removal of “living” cells (uncornified cells) of the full thickness epidermis. It does not alter the structure of the cornified SC layer. SC isolated in this manner can be appropriately stored with minimal change for extended periods [26]. The thickness of the untreated tissue was measured with a digital micrometer (Digimatic Micrometer, Mitutoyo Corp., Japan) to be 15 ± 1 μm thick and, as expected, was fairly homogenous for localized regions in the human body [27].

4.3.2 Micro-Tension

Uniaxial tensile testing was performed using a tensile testing apparatus (Bionix 200, MTS Systems Corporation, Eden Prairie, MN) equipped with a 44.48N load cell. To prevent curling or folding of the wet tissue after treatment,
each specimen (10 x 25 mm) was removed from solution with filter paper. The specimen was then trimmed to 6 x 25 mm and mounted onto opposing pneumatic pressure grips with a 10 mm long gauge length. True stress versus strain curves were subsequently measured to determine the Young’s modulus, \( E_{SC} \), of the SC for drying periods of 0, 1, 2, 4 and 8 hrs in 15% RH and 25°C air and for 8 hrs in 30, 45 and 100% RH and 25°C air following the DIW rinse. The true stress and strain was calculated from the measured loads and grip displacements together with the SC specimen thickness, \( h_{SC} \), measured using a long working distance optical microscope (TT23012, Questar, New Hope, PA).

In separate experiments, the drying stress was measured by initially loading the specimen until a minimum load of 10 mN was observed to straighten but not significantly stress the specimen between the loading fixtures. The displacement of the grips was then fixed and the load recorded as a function of time for a 8 hr drying period under temperature and humidity controlled conditions. The \( h_{SC} \) was measured \textit{in situ} every 20 minutes with the long working distance optical microscope. Using the measured loads and \( h_{SC} \), the true stress in the SC during drying was determined. Note that the measured \( h_{SC} \) included Poisson’s contractions that develop in response to the uniaxial SC drying stress. These contractions are, however, very small and typically on the order of only 0.3%.

4.3.3 Substrate Curvature

In-plane tensile or compressive stresses that develop in a film adhered to an elastic substrate result in concave or convex elastic curvature of the substrate, respectively. The resulting curvature can be measured using optical, interferometric or capacitative methods to determine the film stress [21,28,29,30]. In our curvature experiments, 22 x 22 mm borosilicate glass cover slips 177 \( \mu \)m thick (Fisher Scientific, 12-541-B) with a Young’s modulus of 69 GPa and a Poisson’s ratio of 0.2 were used as the substrate. Cr / Au films (35 Å / 465 Å) were deposited by evaporation onto one side of the glass substrate opposite to the side containing the SC to improve reflectivity. A scanning laser substrate
curvature instrument (FLX-2320, Tencor Instruments, Mountain View, CA) was used to measure the substrate angle of deflection, $\alpha$, in terms of the dimensions $L_s$ and $L_b$ as a function of position, $y$ as defined in Figure 4.2. The average curvature was subsequently calculated from a linear regression analysis of $\alpha$ versus position data [21]. An initial curvature measurement was taken before the tissue was adhered to the substrate to detect any residual curvature of the substrate not associated with subsequent SC stresses. In what follows, the curvature represents the change in curvature from the initial curvature of the substrate.

![Figure 4.2](image)

**Figure 4.2** The experimental arrangement for the substrate curvature technique showing the SC mounted on a glass substrate. A scanning laser equipped with detector measures the angle of deflection, $\alpha$, versus position, $y$, on the substrate. The average curvature is calculated from a linear regression of the deflection angle.
The relationship between the SC film stress, $\sigma_{sc}$, and elastic curvature, $K$, may be expressed by the well known Stoney’s equation [31]:

$$\sigma_{sc} = \frac{E_{sub}}{1 - \nu_{sub}} \frac{h_{sub}^2}{6h_{sc}} K \quad \text{(Eq. 4.2)}$$

where $E_{sub}$, $\nu_{sub}$ and $h_{sub}$ are the Young’s modulus, Poisson’s ratio and thickness of the substrate, respectively. Initial and final $h_{sc}$ values were measured using a digital micrometer (Digimatic Micrometer, Mitutoyo Corp., Japan) and were assumed to vary linearly with time from the first detected drying stress until the plateau stress was reached. The relationship above is based on the “thin-film” assumption that generally requires the product of the film biaxial modulus and thickness to be $\leq 1/80$th of the equivalent product for the substrate to ensure an error less than ~ 5% in the resulting film stress. An important advantage of this assumption and Stoney’s equation is that the film elastic properties are not required to calculate the film stress. In the present study, the ratio of these film and substrate products was ~ $5 \cdot 10^{-4}$ and easily satisfied the thin-film assumption. Nevertheless, since the SC film thickness was relatively large compared to the substrate thickness, a more complete “thick-film” analysis was undertaken to validate the thin-film approach.

The relationship between film stress, $\sigma_{sc}$, and curvature, $K$, using a more complete elastic analysis with accounts for the film flexural rigidity is given by [32]:

$$\sigma_{sc} = \frac{E_{sub} h_{sub}^3}{12h_{sc} (h_{sub} - s)(1 - \nu_{sub})} K \quad \text{(Eq. 4.3)}$$

in which the position, $s$, of the neutral plane for bending

$$s = \frac{\frac{E_{sub}}{1 - \nu_{sub}} h_{sub}^2 + \frac{E_{sc}}{1 - \nu_{sc}} (h_{sc}^2 + 2h_{sub} h_{sc})}{2\left(\frac{E_{sub}}{1 - \nu_{sub}} h_{sub} + \frac{E_{sc}}{1 - \nu_{sc}} h_{sc}\right)} \quad \text{(Eq. 4.4)}$$

where $E_{sc}$ and $\nu_{sc}$ are the Young’s modulus and Poisson’s ratio of the SC, respectively, and the other variables remain as defined above. This analysis requires $h_{sc}$, $E_{sc}$ and $\nu_{sc}$ values during the drying period. $h_{sc}$ was measured using
the digital micrometer noted above and the value of \( E_{\text{SC}} \) was measured as described in the micro-tension section. For the wet SC, we used \( \nu_{\text{SC}} \approx 0.5 \) given the high hydration level and the incompressible nature of water. For dry SC, we used \( \nu_{\text{SC}} \approx 0.4 \) typical for polymeric materials and soft tissues. These values were assumed to vary linearly with time from the first detected drying stress until the plateau stress was reached. This assumption may result in inaccuracies for intermediate values of \( \sigma_{\text{SC}} \) where the \( h_{\text{SC}} \), \( E_{\text{SC}} \) and \( \nu_{\text{SC}} \) are not known explicitly but inferred from micro-tension tests. However, as demonstrated in the results, \( \sigma_{\text{SC}} \) values were not sensitive to \( E_{\text{SC}} \) and \( \nu_{\text{SC}} \) as anticipated by the thin-film assumption and the maximum possible error resulting from the \( h_{\text{SC}} \) assumption was less than 15% for the intermediate stress values.

### 4.3.4 Specimen Preparation and Treatments for Substrate Curvature Measurements

Square (25 x 25 mm) specimens were excised from the isolated SC for the substrate curvature measurements and subsequently submerged in 50 mL of the following solutions for 25 minutes followed by a 5 minute rinse in DIW prior to testing: 30% GLY (v/v) in DIW (Invitrogen Corporation, Carlsbad, CA), 10% SDS (w/v) in DIW at pH 6.4 (Invitrogen Corporation, Carlsbad, CA) and DIW (control). To prepare the specimens, a glass cover slip on filter paper was submerged and positioned under the treated SC prior to the end of the 5 minute rinse in DIW. As water was removed with pipettes, the SC settled onto the cover slip, fully coating the side of the cover slip opposite to the Cr/Au films without forming any visible wrinkles in the SC. Excess SC at the edges of the cover slip was removed with a razor blade (VWR, 55411-050). The specimen was then positioned Au-face towards the laser in the substrate curvature instrument in the same orientation as the initial curvature measurement. The temperature and RH of the air in the instrument was controlled and measured with a hygrometer (TM325, The Dickson Company, Addison, IL) at 5 minute intervals. Initial and final mass of the specimens were measured using an analytical balance (TR-204, Denver Instruments, Denver, CO).
The SC drying stress of DIW treated tissue was measured for a series of drying conditions with selected RH between 15 and 100% and temperatures between 25 and 34°C for 8 - 18 hours. The ability of the substrate curvature method to measure SC stresses during repeated changes in RH and temperature was studied by cycling either the RH between 15 and 100% or the temperature between 35 and 45°C after an initial drying period. Finally, the effects of the chemical treatments (30% GLY and 10% SDS) on SC drying stress were investigated at either 15% RH and 25°C or 27% RH and 32°C.

Assuming that the drying environment was a mixture of ideal gases, the chemical potential of water in the environment was defined by:

\[ \mu_w = \mu_o + RT \ln(a_{H_2O}) \]  

(Eq. 4.5)

where \( \mu_o \) is the standard chemical potential, R the universal gas constant, T the temperature in Kelvin and \( a_{H_2O} \) the activity of water in the drying environment given by [33]:

\[ a_{H_2O} = \frac{P_{H_2O}}{P_o} = \frac{610.78 \% RH}{101325} \frac{100}{100} \exp \left( \frac{17.27(T - 273.16)}{T - 35.86} \right) \]  

(Eq. 4.6)

where \( P_{H_2O} \) is the partial pressure of water and \( P_o \) the standard pressure (101325 Pa).

### 4.3.5 Confirmation of SC / Substrate Adherence

To ensure that the SC was not sliding on the substrate during drying, substrate curvature specimens were placed under an optical microscope equipped with a CCD camera (QM100, Questar, New Hope, PA) set to capture an image every 20 min at a resolution of 0.65 pixels / \( \mu \)m while exposed to identical drying conditions. Easily observable features on the SC of each specimen were selected and image analysis was employed to measure the changes in distance between the points as the specimen dried.

### 4.3.6 Statistical Analysis

SC drying stresses are presented as mean ± 1.96 x the standard error of the mean (SEM) in which the mean values reported are expected to fall within these
bounds with 95% confidence. Three to four specimens were tested for each condition yielding 8 – 13 final drying stress values per specimen for substrate curvature method and 15 – 20 final drying stress values per specimen for micro tension method. SC moduli are presented as mean ± SD with n = 4 for each test condition.

4.4 RESULTS

4.4.1 SC Thickness and Young’s Modulus

Optical microscope measurements of $h_{\text{SC}}$ together with $E_{\text{SC}}$ values of the DIW treated SC measured from micro-tension stress versus strain curves as a function of drying time in 15% RH and 25°C air are shown in Figure 4.3a. The $h_{\text{SC}}$ was observed to decrease from $23 \pm 1 \mu m$ before exposure to the drying environment to $15 \pm 1 \mu m$ after ~ 3 hrs exposure to the drying environment, after which no significant changes were observed. The resulting shrinkage strain of 34% corresponds well with published values for SC dried under the same conditions [34]. The $E_{\text{SC}}$ for the initially wet SC was $28.4 \pm 2.0$ MPa and increased markedly over ~ 4 hrs, eventually reaching a stable value of $272.0 \pm 4.6$ MPa after 8 hrs. The measured $E_{\text{SC}}$ values after 8 hrs of conditioning in selected RH environments are shown in Figure 4.3b. These values are consistent with reported values for human SC dried under similar conditions [35]

4.4.2 Drying Stress Evolution

The $\sigma_{\text{sc}}$ for the DIW treated tissue measured using the substrate curvature technique as a function of drying time in 15% RH and 25°C air is shown in Figure 4.4. The $\sigma_{\text{sc}}$ of the initially wet tissue was measured to be $0 \pm 0.2$ MPa, and no change was detected for a period of ~ 2 hrs. This was followed by a rapid increase in $\sigma_{\text{sc}}$ which then began to stabilize after ~ 4 hrs to a final value of ~ 2.47 MPa at 8 hrs. The uniaxial drying stress measured using the micro-tension test under identical drying conditions is included in the figure for comparison. By contrast, the uniaxial $\sigma_{\text{sc}}$ values began to rise immediately after exposure to the drying environment and began to stabilize after ~ 4 hrs to a final value of ~ 1.48 MPa
after 8 hrs. However, the time after which the drying stress began to stabilize was similar in both cases following the onset of the first drying stresses.

![Graph](image1)

**Figure 4.3** (a) SC thickness and modulus as a function of drying time for DIW treated tissue exposed to 15% RH and 25°C air. (b) SC modulus as a function of RH for DIW treated tissue exposed to 15, 30, 45 and 100% RH air at 25°C for 8 hrs. Error bars: mean ± SD with n = 4 for each test condition and are smaller than the data symbols in (b).
We attributed the immediate development of drying stresses in the micro-tension test to the fact that both sides of the SC specimen were exposed to the drying environment, thus allowing faster water loss. In the substrate curvature test, water can only escape from the original outer surface of the SC.

**Figure 4.4** The biaxial and uniaxial SC drying stress as a function of drying time for DIW treated tissue exposed to 15% RH and 25°C air. The biaxial SC drying stress is calculated using both Stoney’s thin film equation (Eq. 4.2) and the thick film analysis (Eq. 4.3) where \(\nu_{SC} = 0.4\).

We note finally that although the final \(\sigma_{sc}\) value measured using substrate curvature was significantly higher than the micro-tension value, they are entirely consistent with simple elastic predictions for films that are constrained under biaxial and uniaxial conditions while undergoing a transformation (drying) strain. Using simple Hooke’s laws for linear elastic films, it is easy to show that the ratio of the biaxial \(\sigma_{sc}\) and the uniaxial \(\sigma_{sc}\) is given by:

\[
\frac{\sigma_{sc}^{\text{biaxial}}}{\sigma_{sc}^{\text{uniaxial}}} = \frac{1}{1 - \nu_{SC}}
\]

(Eq. 4.7)
Using $\nu_{SC} \sim 0.4$ for dried SC, the expected ratio of 1.8 from Eq. 4.7 is close to the measured stress ratio of 1.7. This means that the biaxial constraint of the SC in the substrate curvature method results in stresses that are consistent with the uniaxial micro-tension results.

SC stresses were calculated using both the Stoney’s equation (Eq. 4.2) and the more complex thick-film equation (Eq. 4.3) and both approaches yielded almost identical results [Figure 4.4]. The thick-film analysis requires $E_{SC}$ and $\nu_{SC}$ values during the drying period and both approaches require $h_{SC}$ values. The $E_{SC}$ and $h_{SC}$ values were measured for the same SC dried under identical conditions along with the assumed $\nu_{SC}$ values as noted in the experimental section. Values were allowed to vary linearly from their initial to final values over the ~4 hr drying period over which the drying stress was observed to increase and stabilize. Initial and final values of the SC thickness were confirmed with separate measurements of the substrate curvature specimen using a digital micrometer. With these values, both Stoney’s analysis and the thick-film analysis yielded equivalent results, and there was no sensitivity of $\sigma_{sc}$ to $E_{SC}$ and $\nu_{SC}$ as anticipated by the thin-film assumption. On the other hand, both approaches are sensitive to $h_{SC}$. Our assumption of linear varying SC thickness during the drying period introduces some uncertainty for the intermediate $\sigma_{sc}$ values, but the maximum error did not exceed 15% based on the maximum possible variation of the actual SC thickness (comparing the measured SC thickness in Figure 4.3a with the assumed linear variation). The initial and final $\sigma_{sc}$ values are not affected by this assumption. The added complexity of $in situ$ measurements of $h_{SC}$ to reduce the error of the intermediate stress values was not considered compelling for this study.

The substrate curvature instrumental error in stress measurements was calculated to be 0.003 MPa based on the measurement system’s specified RMS noise level of 0.0001 m$^{-1}$ for the curvature values. Given the magnitude of the stresses measured, the instrumental error was negligible. To check for SC adherence to the glass substrate and to ensure that no sliding of the initially wet tissue occurred at the interface with the glass substrate, we monitored
distinguishing features on the SC for displacements during drying with an optical microscope as shown in Figure 4.5a.

Figure 4.5 (a) Optical microscopy image showing four distinguishing features on the surface of SC monitored to measure the strain associated with slippage of the wet SC on the glass substrate while drying. (b) The corresponding in-plane strains are shown as a function of drying time. The magnitudes of the strains are < 0.3%.
In the substrate curvature test, the SC should exhibit no in-plane displacements or strains if fully adhered to the glass substrate. By monitoring the positions of the four features apparent on the SC, the corresponding in-plane strains were calculated and are shown as a function of drying time in Figure 4.5b. The magnitude of the strains measured was small and within the error of our measurements (< 0.3%) confirming tissue adherence to the substrate and no detectable sliding throughout the experiment.

![Figure 4.6 SC drying stress as a function of drying time for DIW treated tissue exposed to 15, 30, 45 and 100% RH air at 25°C for up to 8 hrs.](image)

**Figure 4.6** SC drying stress as a function of drying time for DIW treated tissue exposed to 15, 30, 45 and 100% RH air at 25°C for up to 8 hrs.

### 4.4.3 Moisture and Temperature Effects

The $\sigma_{sc}$ values for DIW treated tissue as a function of drying time in 15, 30, 45 and 100% RH and 25°C air are shown in Figure 4.6. At the beginning of all experiments, the SC was wet and the $\sigma_{sc}$ was ~ 0 MPa as expected. After ~ 2 hrs, the $\sigma_{sc}$ values were observed to increase and they stabilized after ~ 4 hrs at different plateau $\sigma_{sc}$ values depending on the drying environment. The rate of
increase of $\sigma_{sc}$ with time was higher for specimens exposed to dry compared to moist air. The plateau $\sigma_{sc}$ values together with additional values measured in a number of other moist air environments with selected RH and temperature are summarized in Table 4.1. No drying stresses were observed in the SC exposed to the 100% RH air suggesting that the SC remained fully hydrated throughout the experiment.

<table>
<thead>
<tr>
<th>Drying Temperature, T (°C)</th>
<th>Relative Humidity, RH (%)</th>
<th>Chemical Potential of Water, $\mu_w$ (kJ/mol)</th>
<th>SC Final Drying Stress, $\sigma_{sc}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.0</td>
<td>15.0</td>
<td>-241.72</td>
<td>2.47 ± 0.06</td>
</tr>
<tr>
<td>26.5</td>
<td>22.5</td>
<td>-240.49</td>
<td>1.88 ± 0.04</td>
</tr>
<tr>
<td>25.5</td>
<td>30.0</td>
<td>-239.92</td>
<td>1.59 ± 0.04</td>
</tr>
<tr>
<td>32.5</td>
<td>27.1</td>
<td>-239.11</td>
<td>1.20 ± 0.02</td>
</tr>
<tr>
<td>32.1</td>
<td>27.3</td>
<td>-239.15</td>
<td>1.26 ± 0.01</td>
</tr>
<tr>
<td>25.5</td>
<td>45.0</td>
<td>-238.92</td>
<td>1.02 ± 0.04</td>
</tr>
<tr>
<td>25.0</td>
<td>100.0</td>
<td>-237.02</td>
<td>0.068 ±0.002</td>
</tr>
<tr>
<td>30.0</td>
<td>100.0</td>
<td>-236.20</td>
<td>0.021 ± 0.002</td>
</tr>
<tr>
<td>32.1</td>
<td>100.0</td>
<td>-235.86</td>
<td>0.015 ± 0.004</td>
</tr>
<tr>
<td>34.1</td>
<td>100.0</td>
<td>-235.54</td>
<td>0.019 ± 0.002</td>
</tr>
</tbody>
</table>

Table 4.1 Drying conditions and final drying stress values for human SC included in Figure 4.7.

The final SC moisture content after drying is expected to determine the plateau $\sigma_{sc}$ levels measured. SC moisture content is determined by water loss and this should be related to the chemical potential of water, $\mu_w$, in the drying environment. To check this hypothesis, the plateau $\sigma_{sc}$ values were plotted as a function of $\mu_w$ in Figure 4.7. A strong linear relationship is apparent between $\sigma_{sc}$ and $\mu_w$ values ($R^2 = 0.9984$) as the $\sigma_{sc}$ decreases with increasing $\mu_w$. Note that $\mu_w$ is a function of both temperature and RH and the plot includes all the conditions listed in Table 4.1.
Figure 4.7 SC final drying stress as a function of chemical potential of water in the drying environment. The linear fit shows a strong correlation between the drying stress and the chemical potential of water (R² = -0.998). Error bars: mean ± 1.96 x SEM with n = 32 for each test condition.

We finally assessed the substrate curvature technique’s ability to measure changes in $\sigma_{sc}$ with changing moisture content and temperature of the drying environment. The moisture content of the air was cycled between 15% and 100% RH over 2 hr periods after an initial drying period in 15% RH air and the resulting $\sigma_{sc}$ levels are shown in Figure 4.8a. As expected, the drying stress varied inversely with humidity and the $\sigma_{sc}$ values alternated between ~ 2.1 - 2.8 MPa and ~ 0 MPa as the RH was cycled between 15 and 100%. The effect of temperature of the air drying environment was assessed by cycling the temperature between 35 and 45°C following an initial 10 hr drying period in 27% RH and 32°C air. The resulting $\sigma_{sc}$ values as a function of time are shown in Figure 4.8b and are clearly observed to cycle with changing temperature. After
the first cycle, the change in $\sigma_{sc}$ measured with each temperature cycle was $\sim 0.4$ MPa.

Figure 4.8 SC drying stress as a function of drying time for specimen exposed to (a) 15% RH and 25°C drying environment for $\sim 8$ hrs and then cycled between 100% RH and 15% RH at 2 hr intervals, and (b) 27% RH and 32°C drying environment for $\sim 8$ hrs and then cycled between 35 and 45°C at 27% RH.
Over the four temperature cycles examined, there was some evidence of a reduction of the peak stress achieved at the higher temperature part of the cycle. The temperature range of the cycle includes the onset of melting of the crystalline lipid component in the SC at around 40°C [36,37]. Following lipid melting, the rate of water desorption has been reported to increase and cause a sharp increase in the storage modulus [36]. This together with the possibility of mechanical creep of the SC at the higher temperatures and stress levels may account for the variation of peak stress, but this is clearly an area for future attention.

We note that in experiments where the temperature is changed, we must take into account the stresses that result from thermal expansion mismatch between the SC and glass substrate. These stresses are in addition to the stresses that result from SC shrinkage due to drying. Using thin-film mechanics, the change in the SC stress, $\Delta \sigma_{sc}$, resulting from thermal expansion mismatch between the substrate and the SC layer when the temperature is changed from $T_o$ to $T$ is given by:

$$\Delta \sigma_{sc} = \frac{E_{sc}}{1 - \nu_{sc}} \left( \alpha_G - \alpha_{sc} \right) (T - T_o)$$

(Eq. 4.8)

where $\alpha_G$ is the thermal expansion coefficient of the glass substrate, and $\alpha_{sc}$ is the thermal expansion coefficient of the SC. $\alpha_G$ is $\sim 3 \cdot 10^{-6} / ^\circ C$, whereas $\alpha_{sc}$ is higher and on the order of $10^{-4} / ^\circ C$ [38] similar to other biological tissues [39] and polymeric materials. Using our measured value of $E_{sc} \sim 272$ MPa and assuming $\nu_{sc} \sim 0.4$, $\Delta \sigma_{sc}$ is $\sim -0.4$ MPa when the tissue is heated from 35 to 45°C and +0.4 MPa when cooled over the same range. Since the SC has a higher expansion coefficient, it expands more than the glass substrate when heated and since it is constrained by the substrate, it develops compressive (negative) stresses. On the other hand, when heated the $\mu_w$ increases at constant RH. The results shown in Figure 4.8b suggest that the resulting drying stresses in the SC should be more tensile (positive) compared to those at the lower temperature. For these experiments, the drying and thermal expansion strains result in opposite changes in SC stress with changing temperature and this must be appreciated in interpreting the resulting trends. If the SC were on a substrate with the same
thermal expansion coefficient, then the stresses achieved during heating from 35 to 45°C would be larger by ~ -0.4 MPa. The thermal expansion mismatch does not affect the experiments conducted at constant temperature.

### 4.4.4 Chemical Treatment Effects

The plateau $\sigma_{sc}$ levels of SC initially treated with GLY, SDS and DIW (control) and then dried for 8 hrs in 15% RH and 25°C or 27% RH and 32°C air is plotted as a function of the chemical potential of water, $\mu_w$, in the drying environment in Figure 4.9.

![Figure 4.9](image)

**Figure 4.9** SC final drying stress as a function of chemical potential of water for specimens treated with GLY, SDS and DIW (control) and exposed to either 15% RH and 25°C or 27% RH and 32°C air. GLY was found to significantly decrease the drying stress and SDS increased stress values. Error bars: mean ± 1.96 x SEM with $n = 26$ for SDS treated tissue, $n = 30$ for GLY treated tissue and $n = 32$ for DIW (control) treated tissue.
The plateau $\sigma_{sc}$ values of the SDS, GLY and DIW treated SC were 3.51, 1.06 and 2.47 MPa for specimens dried at 15% RH and 25°C, and 1.42, 1.01 and 1.26 MPa for specimens dried at 27% RH and 32°C, respectively. For both conditions, the $\sigma_{sc}$ of the damaging SDS treated SC was significantly higher than that of the control, while the $\sigma_{sc}$ of the moisturizing GLY treated tissue was significantly lower with respect to the control. For all conditions, the $\sigma_{sc}$ levels were higher for specimens dried in an environment with a lower chemical potential of water, consistent with the previous results.

4.5 DISCUSSION

4.5.1 Substrate Curvature

It is well known that isolated SC exhibits significant shrinkage when dried from a hydrated condition. If the SC is constrained from shrinking, then stresses develop as it dries and attempts to contract. In vivo, the constraint results from the underlying skin layers, which resist SC shrinking in the plane of the SC. The out-of-plane SC dimension is not constrained and free to contract without any through-thickness stress buildup. The substrate curvature technique provides the same type of constraint with an artificial elastic substrate. In this study, we showed that the technique provides an accurate method to measure the resulting SC drying stress. The measured biaxial stresses more closely approximate the biaxial in vivo stress state of SC compared to uniaxial stresses measured in a uniaxial tension test. Furthermore, water loss occurs only through the outer SC surface, again more closely approximating in vivo drying conditions. Drying stresses that result from water loss following treatment can be systematically studied in selected drying environments.

The technique also has experimental advantages compared to tensile testing. SC can be more easily transferred to the substrate, particularly when it is mechanically fragile after treatment. The SC mechanical properties and thickness compared to those of the substrate employed in this study mean that the mechanics “thin-film” assumption is satisfied, making the SC stress analysis particularly easy in terms of the measured elastic curvature of the substrate using
Stoney’s equation. SC mechanical properties are therefore not required to
determine the SC stresses, which simplifies the analysis compared to other
methods, such as tensile testing.

An important requirement for the accuracy of the method is that sliding at
the interface between the SC and substrate does not occur. We initially employed
a cyanoacrylate based epoxy to adhere the SC to the substrate. However, with
clean glass surfaces, the SC was found to adhere well enough to the glass surface
so an adhesive was not required. We demonstrated this by showing that no net
displacements of strains could be detected in the SC during the drying
experiment [Figure 4.5]. This further simplifies the method, since the added
epoxy bonding step is not required together with the possibility of epoxy
impregnation into the SC layer. We note, however, that sliding of the SC on the
substrate should be considered in the case of tissue treatments that may lubricate
or otherwise decrease the SC adhesion to substrate.

4.5.2 Water Effects
The dependency of $\sigma_{sc}$ on RH [Figure 4.6], the linear relationship between
$\sigma_{sc}$ and $\mu_W$ [Figure 4.7] and the buildup and relaxation of $\sigma_{sc}$ as the tissue was
cycled between dry and moist air [Figure 4.8a] all demonstrate that the residual
stress state of the tissue strongly depends on the water content of the SC, which is
determined by the drying environment. The hydration characteristics of the
tissue have been extensively investigated using methods such as gravimetry
[40,41,42] and spectroscopic techniques including infrared [43], Raman [44] and
nuclear magnetic resonance spectroscopy [45,46]. These studies suggest that
water in SC exists in two different states, namely, bound water and unbound
(free) water. The bound water has a different average intermolecular hydrogen
bonding energy, a lower molecular reorientation rate than unbound water, and
does not freeze at temperatures as low as $-50^\circ$C [40]. It consists of tightly and
loosely bound water [40,45,47]. At less than 10% (w/w) water content in the
tissue, water is tightly bound to primary hydration sites such as the strong polar
groups of keratin chains in corneocytes, covalently bound lipids of the cellular
protein envelope and intercellular lipids. At 10-40% (w/w) water content, additional water is loosely bound to secondary hydration binding sites, namely, tightly bound water and dipolar sites on the protein. Above 40% (w/w) water content, unbound water is present in the tissue in addition to bound water. This water is located primarily in the interior of the corneocytes and in the intercellular regions [40,48].

With only tightly bound water present in dry environments (< 20% RH), SC has a dense, semi-crystalline structure. There is a strong interaction between the keratin chains due to hydrogen bonding between the polar-side groups of the keratin chains [49,50]. The intercellular lipids are closely packed and rigid. As the tissue hydrates, first the primary hydration sites become saturated and then additional water binds to the secondary hydration binding sites [51]. This reduces the interaction between the keratin chains [52]. In the case of the lipids, increasing water presence reduces the intermolecular forces between the intercellular lipids and loosens their packing [53]. As the tissue continues to hydrate, secondary hydration binding sites become saturated and water can no longer be sorbed locally. Consequently, water condenses as unbound water [51]. The unbound water disrupts the hydrogen bonding between the keratin chains allowing them to be less constrained and move more easily relative to each other while being strained [35,49]. Meanwhile, the intercellular lipids of the hydrated tissue are less tightly packed and more fluid and permeable.

SC can be further hydrated if conditioned at very moist environments (> 90% RH) or soaked in water. Increasing unbound water in the tissue can have damaging effects on SC depending on the length of exposure to hydration. Recent studies have shown that short exposures to water (< 1 hr), even with repetitive applications during a day or over many days, have no damaging effect on SC intercellular lipids [54,55]. However, when SC is exposed to water for long periods of time (> 1 hr), significant swelling in the corneocytes followed by formation of amorphous appearing material and pooling of water in the intercellular lipids have been observed [55,56]. The dilation of the lipids due to pooling of water has been shown to disrupt SC lamellar lipid ultrastructure and produce corneocyte separations [55]. Furthermore, degradation of
corneodesmosomes between corneocytes has been associated with increased hydration content [23,48]. The studies above suggest that the limited 25-min water treatment employed in our work will not give rise to water driven damage in the tissue.

The hydration state of SC has direct implications on its mechanical properties and propensity for cracking. With respect to elastic behavior, the in-plane tensile tests exhibited a decreasing modulus from ~265 to 14 MPa with increasing humidity [Figure 4.3b]. This trend was consistent with literature reported values for human SC dried under similar conditions and is associated with the effect of water on molecular mobility in the SC. With increasing water content, the interaction between keratin chains is progressively weakened as discussed above. This involves substitution of existing protein-protein hydrogen bonds with water-mediated bonding to facilitate greater chain mobility [36,49,57]. Similar trends have been observed with nano-indentation measurements to obtain the compression modulus of porcine SC, and the SC modulus has been measured to decrease from ~120 to 26 MPa with increasing humidity [58]. Interestingly, the effect of hydration on the SC in-plane and out-of-plane fracture properties is different. The in-plane fracture (tearing) energy of the tissue has been shown to increase from 580 to 1390 J/m² with increasing RH from 35 to 85% [59]. This trend was not observed for out-of-plane fracture properties due to constrained plasticity [17]. The intercellular delamination energy of the tissue has been shown to decrease from ~4 to ~1 J/m² with increasing RH from 40 to 100%. This decrease has been associated with the separation of interfaces in the presence of water in the intercellular space [18]. Peripheral corneodesmosomes that connect the cells in-plane may also contribute to high toughness values [26]. It must also be noted that the in-plane fracture energies reported above were calculated using linear elastic behavior. Viscoplastic behavior of the SC, particularly at higher hydrations, and the unconstrained nature of the tearing configuration suggest that the values above need to be treated with caution [17].
4.5.3 Modeling Water Effects

Despite the complex interaction of water on the components and structure of SC discussed above, we now demonstrate the surprising result that the principal effect of drying on the stresses developed may be simply explained by the shrinkage of the SC that is associated with the volume of water lost during drying. We develop a thin-film mechanics model from which we are able to predict the water loss from measured values of the SC drying stress, elastic modulus and SC thickness. We then validate the model by comparing these predictions to the measured water loss using mass measurements of the SC during drying.

![Diagram](image)

**Figure 4.10** A schematic illustration showing (a) wet SC with a thickness of $h_0$ before being attached to the substrate and exposed to a drying environment, and (b) the dry SC with a thickness of $h_f$ on the glass substrate after 8 hrs long exposure to the drying environment.
We begin by considering the unconstrained (stress-free) dilatation of the SC, which we express in terms of the volume of water lost during drying:

\[ \Delta^{\text{dry}} = \frac{\Delta V}{V_{\text{SC}}} = \varepsilon_1^{\text{dry}} + \varepsilon_2^{\text{dry}} + \varepsilon_3^{\text{dry}} \]  

(Eq. 4.9)

where \( \Delta V \) is the water volume lost, \( V_{\text{SC}} \) is the volume of the initially wet SC, and \( \varepsilon_1^{\text{dry}}, \varepsilon_2^{\text{dry}} \) and \( \varepsilon_3^{\text{dry}} \) are the stress-free drying strains in the orientations shown in Figure 4.10. Note that \( \Delta^{\text{dry}} \) will be negative corresponding to shrinkage of the SC with drying. We assume equal biaxial in-plane drying strains, \( \varepsilon_1^{\text{dry}} = \varepsilon_2^{\text{dry}} \), in the plane of the SC due to moisture loss, while the out-of-plane drying strain, \( \varepsilon_3^{\text{dry}} \), can be different\(^5\) as defined below. When the SC is adhered to the glass substrate, the in-plane drying strains are fully constrained and must be accommodated by equal and opposite in-plane elastic strains that can be calculated using Hooke’s law so that:

\[ \varepsilon_1^{\text{dry}} = \varepsilon_2^{\text{dry}} = -\left(1 - \frac{V_{\text{SC}}}{E_{\text{SC}}} \right) \sigma_{\text{SC}} \]  

(Eq. 4.10)

where \( \left( \frac{E_{\text{SC}}}{1 - V_{\text{SC}}} \right) \) is the biaxial SC modulus. The total out-of-plane strain, \( \varepsilon_3^{\text{total}} \), during the substrate curvature experiment includes an elastic Poisson’s contraction of the SC thickness due to the in-plane drying stress, \( \sigma_{\text{SC}} \), in addition to the shrinkage due to drying. To determine \( \varepsilon_3^{\text{dry}} \) we must therefore subtract the Poisson contraction strain, \( \varepsilon_3^{\text{el}} \), from \( \varepsilon_3^{\text{total}} \) which can then be expressed in terms of the initial, \( h_0 \), and final, \( h_f \), SC thickness yielding:

\[ \varepsilon_3^{\text{dry}} = \varepsilon_3^{\text{total}} - \varepsilon_3^{\text{el}} = \frac{h_f - h_0}{h_0} + \left(\frac{2V_{\text{SC}}}{E_{\text{SC}}^z} \right) \sigma_{\text{SC}} \]  

(Eq. 4.11)

\(^5\) The mechanics model considered above assumes that the SC is an anisotropic material where both the elastic properties and shrinkage of the tissue normal to the plane of the SC film may be different than the in-plane elastic properties and shrinkage. This model can easily be described using hexagonal elastic constants and isotropic in-plane properties.
where \( -\frac{2\nu_{SC}}{E_{SC}^\perp} \sigma_{SC} \) is the out-of-plane Poisson contraction for the bi-axially stressed SC and \( E_{SC}^\perp \) is the out-of-plane modulus. The dilatation of the SC during drying can now be written in terms of measured quantities:

\[
\Delta^{\text{dry}} = \frac{\Delta V}{V_{\text{wet}}} = -2\left(\frac{1-\nu_{SC}}{E_{SC}}\right)\sigma_{SC} + \left(\frac{h_f - h_0}{h_0} + \frac{2\nu_{SC}}{E_{SC}^\perp} \sigma_{SC}\right)
\]

(Eq. 4.12)

Finally, the mass of water lost during drying of the SC that accounts for the drying stress can be obtained from:

\[
m_w = \rho_w \Delta V = \rho_w V_{\text{wet}} \Delta^{\text{dry}}
\]

(Eq. 4.13)

where \( \rho_w \) is the density of water.

From the measured drying stress, modulus and SC thickness measurements, the predicted water loss is compared to the measured water loss in Figure 4.11a. The calculated water loss slightly exceeds the measured values by up to \( \sim 16\% \) for the SC dried in 45% RH air. This may be related to the value of \( E_{SC}^\perp \) we used in the model as discussed below. The strong linear relationship (\( R^2 \sim 0.9985 \)) between the predicted and measured moisture loss suggests that the SC drying stress is mainly related to the reduction of the volume occupied by water, regardless of the bound states of water that were discussed. This surprising result suggests that that the reorientation of proteins and lipids which have commonly been thought to provide a significant contribution to the drying strains and stresses may in fact be quite small.

We note finally that for the calculations above we assumed isotropic elastic behavior with \( E_{SC} \approx E_{SC}^\perp \) and similarly for the Poisson’s ratios. With this assumption we find from Eqs. 4.9 and 4.10 that the SC out-of-plane shrinkage during drying is much larger than the in-plane shrinkage as shown in Figure 4.11b. This result is generally accepted for drying of SC although the magnitude of the difference together with the predicted water loss are sensitive to the value of \( E_{SC}^\perp \). While there is a lack of published data for \( E_{SC}^\perp \) given the structure of SC, it is generally accepted to be lower than the in-plane modulus \( E_{SC} \).
Figure 4.11 (a) The predicted moisture loss as a function of the measured moisture loss calculated assuming $E_{sc}^{\perp} \approx E_{sc}$ (solid symbols) and $E_{sc}^{\perp} \approx \frac{1}{10} E_{sc}$ (open symbols). The data fits show a linear relationship between the two ($R^2 = 0.998$ assuming $E_{sc}^{\perp} \approx E_{sc}$ and $R^2 = 0.985$ assuming $E_{sc}^{\perp} \approx \frac{1}{10} E_{sc}$). (b) The in-plane strain during drying of SC as a function of the out-of-plane strain.

One study using nano-indentation suggests that $E_{sc}^{\perp} \sim 120$ MPa for porcine SC [58], which is significantly lower than the in-plane modulus $E_{sc} \sim 750$ MPa.
conditioned under similar conditions [49]. If we make the assumption that $E_{sc}^{\perp} = \frac{1}{10} E_{SC}$ the values of $\varepsilon_{3}^{dry}$ predicted by the model would be reduced by $\sim 30\%$. The predicted water loss would be reduced by $\sim 22\%$ for SC exposed to the 15% RH air. These reduced values of water loss and $\varepsilon_{3}^{dry}$ are shown in Figure 4.11a and b. The two predictions of water loss fall close to, but on either side, of the ideal behavior and therefore likely represent reasonable bounds for the actual behavior depending on the specific value of $E_{sc}^{\perp}$.

4.5.4 Chemical Treatment Effects

For both drying conditions, the $\sigma_{sc}$ of the tissue treated with the harsh cleanser surfactant, SDS, was significantly higher than that of the control treated tissue as shown in Figure 4.9. SDS treatment is known to have damaging effects on all SC components and is widely used to study surfactant-induced dry scaly skin [24,25]. It disrupts the barrier properties of the intercellular lipids increasing their fluidity and permeability [60]. As a result, more water can penetrate into the tissue during treatment. SDS also binds to SC proteins resulting in conformational changes in their structure leading to transient swelling and hyper-hydration of corneocytes [2,7,61,62]. Thus, the water content of the tissue during exposure to SDS is much higher compared to that of the tissue exposed to DIW. Additionally, following exposure to SDS, SC has been reported to return to a lower hydration state compared to that of the tissue exposed to DIW because surfactant binding reduces the ability of SC proteins to bind and hold water [2]. Finally, the in-plane modulus of SDS treated tissue has been observed to be slightly lower than that of DIW treated tissue and similar trends have been reported for the out-of-plane modulus of SC [58].

The studies above suggest that the increased $\sigma_{sc}$ values of SDS treated tissue compared to DIW treated tissue in our work are related to the increased volume of water lost during drying which is anticipated from our model above. The other possible causes of high SC drying stress values may be conformational changes in the keratin chains following SDS binding and contraction of lipids due
to extraction of a fraction of the intercellular lipids or changes in the lipid composition and content. It is difficult to say if the SDS concentration (and exposure time) used in our study will induce extraction of the intercellular lipids. At present, there are contradictory results in the literature regarding the extraction of intercellular lipids from SC due to surfactant treatment. The $\sigma_{sc}$ of the SDS treated tissue was significantly reduced when the availability of water in the drying environment increased suggesting that the damage caused by SDS may be suppressed when the tissue is conditioned at moist environments.

In contrast to the harsh SDS treatment, the $\sigma_{sc}$ of the tissue treated with GLY was significantly lower than that of the control treated tissue for both drying conditions. This trend was expected considering that the GLY is a well known moisturizing treatment which increases water holding capacity of SC and accelerates the recovery of its barrier function after exposure to damaging treatments [63,64]. Following exposure to GLY, corneocytes and SC intercellular spaces have been observed to expand and the tissue has been reported to be at a higher hydration state compared to that of the tissue exposed to DIW [65]. Additionally, after exposure to GLY, SC in-plane modulus significantly decreases with respect to that of the DIW treated tissue and the extensibility of SC significantly increases [23].

The reported effects of GLY above on SC hydration and mechanical properties suggest that the decreased $\sigma_{sc}$ values of GLY treated tissue compared to DIW treated tissue in our work may be due to the decreased volume of water lost during drying and the decreased SC modulus. The other possible cause of low drying stress may be expansion of lipids due to GLY increasing their fluidity. The effects of corneodesmosome degradation are not considered, since it is reported to occur when the tissue is conditioned at moist environments (> 80% RH) [23]. We note that the $\sigma_{sc}$ of the GLY treated tissue remained the same at different drying conditions. This means that the tissue maintains a certain level of hydration independent of the availability of water in the drying environment after being treated with GLY. Altogether, the data in Figure 4.9 suggests that the
substrate curvature technique can be used to easily detect and distinguish the $\sigma_{sc}$ of SC treated with different chemicals.

**4.5.5 Implications for Damage**

We now consider the biomechanics model described in the introduction to infer the propensity for dry skin damage by computing the strain energy release rate, $G$, from the measured values of $\sigma_{sc}$ and $E_{sc}$ and comparing them to the SC resistance to cracking, $G_c$. $G$ values for the cracking configurations shown in Figure 4.1 for DIW treated SC as functions of RH of the drying environment are shown in Figure 4.12a. It is clear from the figure that $G$ values increase with decreasing RH of the drying environment. We note that the mechanical driving force for skin damage is particularly sensitive to the SC stress, since $G$ scales with the square of $\sigma_{sc}$. On the other hand, the increasing stiffness of the SC which is related to the value of $E_{sc}$ has the opposite effect on the magnitude of $G$ since it appears on the denominator of Eq. 4.1. As shown in Figure 4.3, the value of $E_{sc}$ increases with decreasing RH of the drying environment which would act to decrease $G$ values. However, the SC stress dominates and the values of $G$ increase markedly with decreasing RH suggesting that the driving force for dry skin cracking and chapping damage increases as the skin is exposed to increasingly dry environments.

The $G$ values determined must exceed the SC resistance to cracking, $G_c$, in order for such skin damage processes to occur. $G_c$ values reported in the literature range from $\sim 0.5 - 8 \text{ J/m}^2$ for human SC treated in different drying environments [17,18,19]. The $G$ values estimated clearly equal or exceed $G_c$ values in the lower part of the range that we have previously reported (they are likely to be higher due to the epidermal/dermal viscoelastic relaxation processes mentioned in the introduction). Taken together, the results for the value of $G$ and the associated $G_c$ values for dry skin strongly suggest that the drying stress in SC provides the principal mechanical driving force for the formation and propagation of dry skin damage.
Figure 4.12  a) Crack driving force, G, values that result from the SC drying stresses for surface cracking, channel cracking and delamination as a function of the RH of the drying environment for DIW treated tissue exposed to air at 15, 30, 45 and 100% RH and 25 °C for 8 hrs. b) SC drying stress, $\sigma_{SC}$, intercellular delamination energy, Gc, and the normalized crack driving force, G/Gc, shown as a function of chemical treatment (SDS, DIW and GLY) for SC exposed to 15% RH air at 25 °C. The cracking potential is significantly increased after the SC is damaged by the SDS treatment, and the cracking potential is markedly reduced after moisturizing with GLY.

The results shown in Figure 4.12a also suggest that the driving force for surface cracking which involves the propagation of a through thickness SC crack
into the underlying epidermal layers has the largest value of G making this the most prevalent form of skin damage. These results are suggested from the mechanical models for the different possible SC cracking configurations but are clearly consistent with clinical observations of severe dry skin damage. The surface cracking configuration provides a direct path for environmental species to penetrate the SC barrier and enter the underlying skin layers.

Using the biomechanics model it is also possible to predict the effect of chemical treatments on skin damage processes. By observing the ratio of $G / G_c$ and using the values for DIW treated tissue exposed to a drying environment as a control, the propensity for SC cracking for SDS and GLY treated tissue exposed to the same 15% RH and 25°C drying environment can immediately be observed [Figure 4.12b]. The normalized cracking ratio for SDS treated tissue increased significantly with the higher drying stresses compared to the DIW treated control, suggesting that the harsh SDS treatment enhances the propensity for cracking. In contrast, the normalized cracking ratio for the GLY treated tissue with the lower drying stresses was significantly reduced compared to the control, indicating the beneficial effects of moisturizing treatments on reducing dry skin damage.

### 4.6 ACKNOWLEDGEMENTS

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### 4.7 REFERENCES


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Chapter 5 Application of Substrate Curvature Method to Differentiate Drying Stresses in Topical Coatings and Human SC

5.1 Abstract

Despite the extensive use of topical coatings in cosmetics, their effect on the mechanical properties of human skin and the perception of skin tightness in the form of drying stresses is not well understood. We describe the application of a recently developed substrate curvature technique to characterize stresses in drying and non-drying occlusive topical coatings. We then extend the technique to measure the combined effects of the coating applied to human stratum corneum (SC) where the overall drying stresses may have contributions from the coating, the SC and the interaction of the coating with the SC. We show how these separate contributions in the coating and SC layers can be differentiated. (This section is currently in press: K. Levi and R.H. Dauskardt. Application of Substrate Curvature Method to Differentiate Drying Stresses in Topical Coatings and Human SC. International Journal of Cosmetic Science, 2009)

5.2 Introduction

Topical coatings are extensively used in medical and cosmetic formulations [1]. Despite their extensive use, little is understood of their effects on the perception of skin “dryness” and “tightness”. It is also unclear whether these perceptions are related to the drying of the coating itself or the interaction of the coating with the underlying SC. For example, the coating may affect the water content of the SC or may contain molecules that diffuse into the SC and change its mechanical properties and stress state. In addition, the coating may
itself develop drying stresses that may be perceived by the consumer as skin tightness. In a recent paper, we described the substrate curvature technique to measure drying stresses in SC [2]. In the present study, we demonstrate how the method can be used to measure the stresses that develop in topical coatings with different drying characteristics. We then extend the technique to measure the combined effects of the coating applied to SC where the overall drying stresses may have contributions from the coating, the SC and the interaction of the coating with the SC. We show how these separate contributions in the coating and SC layers can be differentiated.

The selected topical coatings included a non-drying occlusive, NDO, coating and a drying occlusive, DO, coating. The DO coating developed stresses as high as 0.54 MPa on its own whereas the NDO coating did not develop any stresses. Both NDO and DO coatings were observed to be equally effective in reducing the drying stresses in SC and maintaining higher moisture content when exposed to the drying environment. However, the overall stresses from the SC and the DO layers were larger and would lead to the perception of greater skin tightness. The drying stress of the tissue following application of the coatings is discussed in terms of the effect of the coatings on the hydration and chemical state of the SC components.

5.3 MATERIALS AND METHODS

5.3.1 Tissue Preparation

Human SC was acquired from the abdomen of a 75 year old deceased Caucasian female donor. Methods for SC isolation were nominally similar to those previously described [2,3]. The study was approved by the Stanford University Institutional Review Board and conducted according to Declaration of Helsinki Principles. All comparative testing was performed on specimens taken from adjacent regions of the tissue from this single donor to reduce variations. The epidermal layer was separated from the dermis by immersion in a 35°C water bath to remove adipose tissue and afterward 60°C water bath for 1 min followed by careful mechanical separation from the dermis using a flat-tipped spatula. The
SC was then detached from the epidermal layers by soaking in a trypsin enzymatic digest solution (0.1% (w/w) in 0.05M, pH 7.9 Tris Buffer) at 35°C for 135 minutes. During separation, the orientation of the outer SC surface was recorded. The isolated SC was rinsed with distilled water (DIW) at room temperature and allowed to dry under atmosphere on filter paper (Grade 595 Filter Paper, Schleicher & Schuell MicroScience GmbH, Dassel, Germany) and then removed and stored under ambient conditions of ~ 45% RH and ~ 18°C. Unlike most other soft tissues in the human body, SC is not vascularized and does not undergo rapid apoptosis and structural damage after such harvesting. The trypsin digest only causes removal of “living” cells (uncornified cells) of the full thickness epidermis. It does not alter the structure of the cornified SC layer. SC isolated in this manner can be appropriately stored with minimal change for extended periods [2,3]. The thickness of the untreated tissue was measured with a digital micrometer (Digimatic Micrometer, Mitutoyo Corp., Japan) to be 18 ± 1 μm thick and, as expected, was fairly homogenous for localized regions in the human body [4].

5.3.2 Substrate Curvature Technique for Multiple Layers

The substrate curvature method developed to measure the drying stress of SC tissue as a function of hydration, temperature and chemical treatment has been previously described [1]. Briefly, in-plane tensile or compressive stresses that develop in a film adhered to an elastic substrate result in concave or convex elastic curvature of the substrate, respectively. The relationship between the SC film stress, $\sigma_{sc}$, and the substrate curvature, $K$, was previously shown to be accurately given by the Stoney’s equation [1]:

$$\sigma_{sc} = \left( \frac{E_{sub}}{1 - \nu_{sub}} \right) \frac{h_{sub}^2 K}{6h_{sc}}$$  \hspace{1cm} (Eq. 5.1)

where $E_{sub}$, $\nu_{sub}$ and $h_{sub}$ and $h_{sc}$ are the Young's modulus, Poisson's ratio and thickness of the substrate and SC specimen thickness, respectively.

In the present study we extend the substrate curvature technique to measure the effects of topical coatings on SC stresses. This requires taking the
contribution of the coating to the overall curvature of the specimen into account. If the treatment involves a coating applied to the SC that develops stresses after application, the total substrate curvature, $K_T$, will be given by:

$$K_T = K_{sc} + K_{coat}$$

(Eq. 5.2)

where $K_{coat}$ is the curvature associated with the stress developed in the treatment coating, and $K_{sc}$ is the curvature associated with the drying stress of the underlying SC. If the coating does not develop any stresses, then $K_{coat} = 0$ and the curvature measured is related to the stress, $\sigma_{sc}$, in the SC only. On the other hand, if the treatment coating hardens and develops stresses of its own, $\sigma_{coat}$, then these will add to the curvature measured. If the multiple films together satisfy the thin-film assumption, then Stoney’s equation can be applied to relate the contribution of individual film stresses to the substrate curvature. Any significant changes in the SC and coating thickness, $h_{coat}$, during the experiment must be updated and included in Stoney’s equation (Eq. 5.1). Note finally that the overall substrate curvature is measured and the individual SC and coating stresses cannot be deconvoluted without additional information. For example, if the coating stresses are separately measured, then their contribution to the substrate curvature can be determined and subtracted from the total curvature using Eq. 5.2 to obtain the curvature related to the SC stresses provided that the presence of the SC under the coating does not change the coating stresses.

### 5.3.3 Specimen Preparation and Treatments for Substrate Curvature

Square (25 x 25 mm) specimens were excised from the isolated SC and subsequently submersed in 50 mL of DIW (control) for 25 minutes prior to testing. As previously described, a 177 $\mu$m thick glass cover slip (Fisher Scientific, 12-541-B) with a Young’s modulus of 69 GPa and a Poisson’s ratio of 0.2 was submersed and positioned under the treated SC prior to the end of 25 min soak in DIW. As water was removed with pipettes, the SC settled onto the cover slip, fully coating the side of the cover slip opposite to the Cr/Au films without forming any visible wrinkles in the SC. Excess SC at the edges of the cover slip was removed with a razor blade (VWR, 55411-050). Curvature measurements reported
represent the curvature change from the initial value to negate the effects of initial curvature value. The temperature and RH of the air in the instrument was controlled and measured with a hygrometer (TM325, The Dickson Company, Addison, IL) at 5 min intervals.

![Figure 5.1](image)

**Figure 5.1** A schematic illustration showing (a) NDO or DO coating on SC, and b) NDO or DO coating on the glass substrate.

All specimens were exposed to a 26% RH and 26°C drying environment for 8 hours to characterize the drying stress without any coating applied as a control. Initial and final \( h_{sc} \) values were measured using a digital micrometer (Digimatic Micrometer, Mitutoyo Corp., Japan) and assumed to vary linearly from their initial to final values over the ~2 hr drying period over which the drying stress was observed to increase and stabilize. The specimens were then placed in a 100% RH environment for 2 hrs to return the SC drying stress to zero. The SC was treated with either a NDO (Ultra Color Rich Lipstick, Avon Products Inc., New York, NY) or a DO (CoverGirl Outlast # 531, Procter & Gamble Company, Cincinnati, OH) coating using a casting knife applicator (3580/1, Elcometer, Rochester Hills, MI). The thickness of both coatings was 27 \( \mu m \). The
coated SC specimens shown in Figure 5.1a were then exposed to the same 26% RH and 26°C drying environment for another 8 hrs. In separate experiments, the stresses developed in the coatings during drying was measured by applying 27 μm thick NDO and DO coatings directly on the glass substrate [Figure 5.1b] using the casting knife applicator and then exposing them to the same drying environment.

5.3.4 Statistical Analysis

SC drying stresses are presented as mean ± 1.96 x the standard error of the mean (SEM) in which the mean values reported are expected to fall within these bounds with 95% confidence. Three to four specimens were tested for each condition yielding 8 – 13 final drying stress values per specimen.

5.4 Results

The coating stress and associated substrate curvature of the DO and NDO coatings applied directly to the glass substrate and exposed to the 26% RH and 26°C drying environment are shown as a function of time in Figure 5.2a. As expected, the DO coating hardened in the drying environment, and a positive curvature developed in the substrate as a result of the presence of tensile drying stresses in the coating. The maximum coating stress of ~ 0.54 MPa determined using Stoney’s equation (Eq. 5.1) was achieved after ~ 1 hr and exhibited only a marginal decrease of ~ 6.3% over the following 7 hr period. The NDO coating did not harden and no substrate curvature was detected over the full 8 hr drying period indicating zero coating stresses.

The substrate curvature and SC stresses measured for the SC specimens prior to application of the coatings and exposed to the 26% RH and 26°C drying environment for 8 hrs is shown in Figure 5.2b and c, respectively. The σ_sc values stabilized at ~ 2.0 MPa after ~ 2 hrs in the drying environment and remained constant.
Figure 5.2 (a) The curvature and stress values as a function of drying time for DO and NDO coatings exposed to 26% RH and 26°C air. (b) The total curvature values as a function of drying time for SC treated with DO and NDO coatings and exposed to 26% RH and 26°C air. (c) SC drying stress as a function of drying time for SC treated with DO and NDO coatings and exposed to 26% RH and 26°C air.
After 8 hrs in the drying environment, $\sigma_{sc}$ values reduced to $\approx$ 0 MPa when the specimens were exposed to 100% RH air. The coatings were then applied and the substrate curvature was measured as a function of time in the same drying environment as shown in Figure 5.2b. The curvature of the specimen with the NDO coating increased and reached a maximum value after $\approx$ 2 hrs that was significantly lower than the same specimen without the coating. It then exhibited only a minor decrease over the subsequent 6 hr period. The curvature of the specimen with the DO coating increased more rapidly and exhibited a distinct peak after $\approx$ 1.5 hrs followed by a rapid decrease over $\approx$ 0.5 hrs, where it stabilized at a value similar to the specimen without the coating and significantly higher than the specimen with the NDO coating. The reason for the peak is considered in the discussion section on the effect of topical coatings on SC drying stress.

The resulting stresses in the SC for the coated specimens were determined in the following manner. The NDO coating did not harden, and the results shown in Figure 5.2a confirm that no coating stresses developed. The curvature measured was, therefore, associated with stresses in the SC only which were calculated using Stoney’s equation (Eq. 5.1) and shown in Figure 5.2c. In the presence of the NDO coating, the $\sigma_{sc}$ achieve a maximum value of $\approx$ 1.25 MPa, significantly lower than the specimen without the coating. For the specimen with the DO coating, we assume that the coating hardens and develops the same stresses as it did when applied directly to the substrate. Using Eq. 5.2, the curvature associated with the ND coating stresses [Figure 5.2a] can be subtracted from the total curvature [Figure 5.2b] to obtain just the curvature associated with stresses in the SC. The $\sigma_{sc}$ value can then be determined using Stoney’s equation (Eq. 5.1) as a function of time in the drying environment as shown in Figure 5.2c.

Interestingly, the $\sigma_{sc}$ values stabilize for the DO coated SC at $\approx$ 1.30 MPa which were almost identical to those of the SC with the NDO coating. The principal effect of the occlusive coatings on the SC is to maintain higher moisture content in the SC when exposed to the drying environment. Therefore, the reduced and similar $\sigma_{sc}$ values with the coatings present suggests that both
coatings are equally effective at maintaining higher moisture content in the SC. On the other hand, it should be noted that the overall curvature of the specimen with the SC and DO coating was much higher than that for the NDO coating indicating that the combined stresses of the SC and DO coating was higher. This is consistent with the perception of greater skin tightness reported for drying compared to non-drying occlusive coatings.

5.5 Discussion

In the present study, we described that the substrate curvature technique can be extended to measure the drying stresses of occlusive topical coatings applied on skin together with their effects on the drying stresses of the tissue. When applied on glass, the NDO coating was observed to be free of residual drying stresses for hours after exposure to air suggesting that there was no moisture diffusing out of the coating. In addition, significant (~ 0.5 MPa) and rapidly increasing drying stresses were observed for the DO coating [Figure 5.2a]. These stresses may be associated with the loss of water bound to the humectant molecules in the DO coating or contractions due to cross-linking of emollient molecules in the coating when exposed to air.

For the NDO and DO coated tissue, the final drying stresses were observed to be the same despite the initial difference in the stress profiles following application of the coatings [Figure 5.2c]. This demonstrates that both coatings are equally effective at maintaining higher moisture content in SC. To better understand the effect of occlusive barriers in maintaining higher moisture content in SC, we utilized a previously found relationship (shown in Chapter 4) between the SC drying stresses and the chemical potential of water in the drying environment. We found that the decreased SC stresses observed in the presence of the occlusive barriers were equivalent to SC stresses that would be observed in a 49% RH, 26°C drying environment without any occlusive barrier as shown in Figure 5.3. This suggests that the SC retains some water in the presence of the occlusive barriers. In the next chapter, we will calculate the percentage of water retained by the SC in the presence of moisturizing treatments.
For the DO treated SC, the stress relaxation following initial stress increase which gives rise to the observed peak may be due to the diffusion of moisturizing molecules such as emollients in the coating into SC and softening the tissue. This behavior is similar to behavior observed in other studies where diffusion of emollient molecules in the occlusive coating into the intercellular boundaries of SC causes significant softening of the tissue within minutes of application [5].

**Figure 5.3** (a) SC final drying stress as a function of chemical potential of water in the drying environment. The decreased SC stresses observed in the presence of the occlusive barriers are equivalent to SC stresses that would be observed in a 49% RH, 26°C drying environment without any occlusive barrier.
5.6 Conclusion

Using the substrate curvature method, we have evaluated the effect of DO and NDO coatings on SC drying stresses. Interestingly, both coatings were observed to be equally effective in reducing SC drying stresses and maintaining higher moisture content in the tissue after the stresses in the coating and the SC were differentiated. The methodology presented in this study provides direct opportunities for studying the effects of moisturizing treatments and occlusive barrier films used to control water loss on SC and alleviate the propensity for dry skin damage.

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5.8 References


Chapter 6 Effect of Moisturizing Treatments on the Drying Stresses in Human SC

6.1 ABSTRACT

Moisturizers are widely used to alleviate dry skin and to improve skin “feel” although little is known about their effects on dry skin stresses that develop on human skin. We characterize the effect of a range of moisturizing molecules on the drying stresses developed in human stratum corneum (SC). Following moisturizing treatment, the SC was observed to have distinctive stress profiles with drying time depending on the effectiveness of the treatment. The stress values of specimens treated with the humectant moisturizers were observed to increase and stabilize after a few hours in the drying environment, whereas the stress values of specimens treated with the emollient and occlusive moisturizers were observed to rise rapidly to a peak stress value and then slowly relax to a final stress value. Attenuated total reflectance Fourier transform infrared spectroscopy was employed to investigate the effects of the treatments on SC lipid extraction and conformation. The role of moisturizing molecules on the drying stresses in SC is discussed in relation to their effects on the underlying SC structure. We discuss how these stresses may contribute to the reported perception of the treatments.

6.2 INTRODUCTION

Moisturizers are formulated based on their biochemical and moisture occlusive properties and widely used to improve skin “feel” and alleviate dry skin damage [1,2,3,4]. Control of transepidermal water loss (TEWL), effects on skin components and dielectric properties, irritation potential, optical imaging, together with consumer product perceptions are used to assess efficacy [5]. However, these do not assess the effects of moisturizing treatments on skin
biomechanical function, which affect not only the perception of dry skin stiffness and tightness, but also underlie the biomechanical component for dry skin damage [6,7].

In the present study, we directly measure the effect of selected humectant and emollient moisturizing treatments together with an occlusive barrier on the drying stresses in human SC as a function of drying time using a recently developed application of the substrate curvature method [6,7]. We demonstrate that the moisturizers act to significantly reduce SC drying stresses and alleviate the propensity for dry skin damage. The selected treatments include various concentrations and different formulations of the nonvolatile trihydroxylated humectant, glycerol (GLY), widely used as a hydrating agent in personal care products; a range of ester based emollients with partially occlusive behavior including alkyl lactate (AL), diisopropyl adipate (DA), ethylhexyl palmitate (EP), isostearyl neopentanoate (IN), isocetyl stearoyl stearate (ISS) and octyldodecyl stearoyl stearate (OSS); and the purified mixture of hydrocarbons taken from petroleum, petrolatum (PET), long considered as the “gold standard” occlusive treatment with emollient characteristics in cosmetic science.

Following moisturizing treatment, the SC was observed to have distinctive drying stress profiles depending on the effectiveness of the treatment. The stress values of specimens treated with the humectant moisturizers were observed to increase and stabilize after a few hours in the drying environment and then remain relatively constant until the end of exposure to the drying environment. In contrast, the stress values of specimens treated with the emollient and occlusive moisturizers were observed to rise rapidly to a peak stress value and then slowly relax to a final stress value. The final stress value of all moisturizer treated specimens was lower compared to that of the DIW (control) treated tissue. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was employed to investigate the effects of the treatments on SC lipid extraction and conformation. The effect of moisturization on the SC drying stresses is linked to hydration and chemical state of the SC components, providing a new understanding of the role of moisturizing treatments on skin
biomechanics. Finally, we discuss how these stresses may contribute to the reported perception of the treatments.

6.3 MATERIALS AND METHODS

6.3.1 Tissue Preparation

Human SC was acquired from the abdomen of a 60 years old deceased Caucasian female donor. The study was approved by the Stanford University Institutional Review Board and conducted according to Declaration of Helsinki Principles. All comparative testing was performed on specimens taken from adjacent regions of the tissue from this single donor to reduce variations. The epidermal layer was separated from the dermis by immersion in a 35°C water bath to remove adipose tissue and afterward 60°C water bath for 1 min followed by careful mechanical separation from the dermis using a flat-tipped spatula. The SC was then detached from the epidermal layers by soaking in a trypsin enzymatic digest solution (0.1% (w/w) in 0.05M, pH 7.9 Tris Buffer) at 35°C for 135 minutes. During separation, the orientation of the outer SC surface was recorded. The isolated SC was rinsed with distilled water (DIW) at room temperature and allowed to dry under atmosphere on filter paper (Grade 595 Filter Paper, Schleicher & Schuell MicroScience GmbH, Dassel, Germany) and then removed and stored under ambient conditions of ~45% RH and ~18°C. Unlike most other soft tissues in the human body, SC is not vascularized and does not undergo rapid apoptosis and structural damage after such harvesting. The trypsin digest only causes removal of “living” cells (uncornified cells) of the full thickness epidermis. It does not alter the structure of the cornified SC layer. SC isolated in this manner can be appropriately stored with minimal change for extended periods [6,7,8].

The thickness of the untreated tissue was measured with a digital micrometer (Digimatic Micrometer, Mitutoyo Corp., Japan) to be $17 \pm 1 \mu m$ thick and, as expected, was fairly homogenous for localized regions in the human body [9]. Square (25 x 25 mm) specimens were excised from the isolated SC for the substrate curvature.
Table 6.1 The treatments used in this study, their compositions, physical states and their mechanism of action to moisturize skin are listed above. For the emollients, the main component of the composition is listed first. The remaining ingredients of the composition, which were used to construct the emollient molecule are in parenthesis.

6.3.2 Moisturizing Treatments

The moisturizing treatments used in this study are listed in Table 6.1 and classified according to their mechanism of action to moisturize skin. The liquid moisturizing treatments consisted of 10%, 30% and 100% GLY (v/v) in DIW

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Composition</th>
<th>Physical State</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLY</td>
<td>10%, 30% and 100% (v/v) in DIW</td>
<td>Liquid</td>
<td>Humectant</td>
</tr>
<tr>
<td>GLY-A</td>
<td>40-50% (v/v) Glycerin, 40-50% (v/v) Water, 1-5% (v/v) Glyceryl Polyacrylate</td>
<td>Solid (Gel)</td>
<td>Humectant</td>
</tr>
<tr>
<td>GLY-B</td>
<td>40% Glycerin (v/v), Cetearyl Alcohol, Stearic Acid, Sodium Cetearyl Sulfate, Methylparaben, Propylparaben, Dilauryl Thiodipropionate, Sodium Sulfate</td>
<td>Solid (Cream)</td>
<td>Oil / Lamellar Gel / Water emulsion</td>
</tr>
<tr>
<td>AL</td>
<td>C12-C15 Alkyl esters, (Lactic acid, Lauryl and myristyl alcohol)</td>
<td>Liquid</td>
<td>Emollient</td>
</tr>
<tr>
<td>DA</td>
<td>Diisopropyl adipate, (Adipic acid, Isopropyl alcohol)</td>
<td>Liquid</td>
<td>Emollient</td>
</tr>
<tr>
<td>EP</td>
<td>Ethylhexyl palmitate, (Palmitic Acid, 2-ethylhexanol)</td>
<td>Liquid</td>
<td>Emollient</td>
</tr>
<tr>
<td>IN</td>
<td>Isostearyl neopentanoate, (Neopentanoic acid, Isostearyl alcohol)</td>
<td>Liquid</td>
<td>Emollient</td>
</tr>
<tr>
<td>ISS</td>
<td>Isocetyl steaeryl stearate, (Stearic acid, Isocetyl alcohol)</td>
<td>Liquid</td>
<td>Emollient</td>
</tr>
<tr>
<td>OSS</td>
<td>Octyldodecyl stearoyl stearate, (Stearic acid, Octyldodecanol)</td>
<td>Liquid</td>
<td>Emollient</td>
</tr>
<tr>
<td>PET</td>
<td>100% White Petrolatum USP</td>
<td>Solid (Gel)</td>
<td>Occlusive</td>
</tr>
</tbody>
</table>
(Invitrogen Corp., Carlsbad, CA) and ester based emollients with different molecular weights (MWs), viscosities and spreading characteristics, such as: “light” AL (Ceraphyl 41) and DA (Ceraphyl 230), “medium” EP (Ceraphyl 368) and IN (Ceraphyl 375), “heavy” ISS (Ceraphyl 791) and OSS (Ceraphyl 847, ISP Corp., Wayne, NJ). The solid moisturizing treatments consisted of GLY-A Gel (Lubrajel XD, International Specialty Products (ISP) Corp., Wayne, NJ) and GLY-B (Norwegian Formula Hand Cream, Neutrogena Co., Los Angeles, CA) and PET (White Petrolatum USP, Walgreen Co., Wilmette, IL).

6.3.3 Substrate Curvature Technique

The substrate curvature method developed to measure the drying stress of SC tissue as a function of hydration, temperature and chemical treatment has been previously described [6]. Briefly, in-plane tensile or compressive stresses that develop in a film adhered to an elastic substrate result in concave or convex elastic curvature of the substrate, respectively. The resulting curvature was measured in scanning laser substrate curvature instrument (FLX-2320, Tencor Instruments, Mountain View, CA). 22 x 22 mm borosilicate glass cover slips 179 μm thick (Fisher Scientific, 12-541-B) with a Young’s modulus of 69 GPa and a Poisson’s ratio of 0.2 were used as the substrate. Cr/Au films were deposited by evaporation onto one side of the glass substrate to improve reflectivity.

The relationship between the SC film stress, \( \sigma_{sc} \), and the elastic curvature, \( K \), was expressed with the Stoney’s equation as in our previous studies [6,10]:

\[
\sigma_{sc} = \left( \frac{E_{sub}}{1 - \nu_{sub}} \right) \frac{h_{sub}^2}{6h_{sc}} K
\]

(Eq. 6.1)

where \( E_{sub} \), \( \nu_{sub} \), \( h_{sub} \) and \( h_{sc} \) are the Young’s modulus, Poisson’s ratio, thickness of the substrate and SC specimen thickness, respectively. The relationship above is based on the “thin-film” assumption that generally requires the product of the film biaxial modulus and thickness to be \( \leq 1/80 \)th of the equivalent product for the substrate to ensure an error less than 5% in the resulting film stress. In the present study, the ratio of the film and substrate products easily satisfied the thin-film assumption.
The extension of the substrate curvature technique to measure the effects of topical coatings on SC stresses requires taking the contribution of the coating to the overall curvature of the specimen into account. If the treatment involves a coating applied to the SC that develops stresses after application, the total substrate curvature, $K_T$, measured is [7]:

$$K_T = K_{sc} + K_{coat}$$  \hspace{1cm} (Eq. 6.2)

where $K_{coat}$ is the curvature associated with the stress developed in the treatment coating, and $K_{sc}$ is the curvature associated with the drying stress of the underlying SC. If the coating does not develop any stresses, then $K_{coat} = 0$ and the curvature measured is related to the stress, $\sigma_{sc}$, in the SC only. On the other hand, if the treatment coating hardens and develops stresses of its own, $\sigma_{coat}$, then these will add to the curvature measured. In the present study, none of the moisturizing coatings developed any stresses; therefore the measured curvature was solely related to the stress, $\sigma_{sc}$, in the SC.

To prepare the specimens, a glass cover slip on filter paper was submersed and positioned under the treated SC prior to the end of 25 min soak in DIW. As water was removed with pipettes, the SC settled onto the cover slip, fully coating the side of the cover slip opposite to the Cr/Au films without forming any visible wrinkles in the SC. Excess SC at the edges of the cover slip was removed with a razor blade (VWR, 55411-050). The specimen was then positioned Au-face towards the laser in the substrate curvature instrument in the same orientation as the initial curvature measurement. Subsequent curvature measurements represent the curvature change from the initial value. The temperature and RH of the air in the instrument was controlled to achieve the desired drying conditions and measured with a hygrometer (TM325, The Dickson Company, Addison, IL) at 5 min intervals. The flow of dry air into the instrument was 6 L / min.

All specimens were dried at 7% RH and 22°C for 8 hrs to characterize the drying stress without any moisturizing treatment applied as a control. Initial and final $h_{sc}$ values were measured using a digital micrometer (Digimatic Micrometer, Mitutoyo Corp., Japan) and assumed to vary linearly from their initial to final values over the ~4 hr drying period over which the drying stress
was observed to increase and stabilize. The specimens were then placed in a 100% RH environment for 2 hrs to return the SC drying stress to zero. The SC was subsequently coated with either a liquid or solid moisturizing treatment. The liquid moisturizers were dipcoated on the specimens following exposure to these chemicals for 5 min. The draw speed used during the dipcoating process was 200 μm / s. The solid moisturizers were applied as coatings on the specimens using a precise casting knife applicator with a micrometer controller (3580/1, Elcometer, Rochester Hills, MI). The initial thickness of all solid coatings was 27 μm.

The coated SC specimens were then exposed to the same 7% RH and 22°C drying environment for another 8 hrs. The initial and final thicknesses of the liquid and solid coatings and the underlying SC tissue were measured by looking at the sides of the specimens using an optical microscope. For cases where the moisturizing treatment did not form an oily film on the tissue, the final thickness value of the tissue was confirmed with the digital micrometer. The tissue thickness, hSC, was measured using a digital micrometer or optical microscopy and was assumed to vary linearly from their initial to final values over the ~1 hr drying period over which the drying stress was observed to increase and stabilize. In separate experiments, the stresses developed in the coatings during drying were measured by applying the solid or liquid moisturizing treatments directly on the glass substrate, and then exposing them to the same drying environment.

6.3.4 Infrared Spectroscopy

The Fourier Transform Infrared (FTIR) studies were performed using a Vertex 70 FTIR spectrometer (Bruker Optics Inc., Billerica, MA) equipped with a deuterated triglycine sulfate detector and a micro ATR (A529-P MIRacle) accessory supporting a ZnSe-crystal. Data collection and spectral calculations were performed using OPUS (version 5.5) software. All spectra (2 cm⁻¹ resolution) were obtained in the frequency range 4000-750 cm⁻¹ and normalized by the Amide I peak at ~ 1650 cm⁻¹ largely due to carbon-oxygen (C = O) stretching with a small contribution from nitrogen-hydrogen (N-H) bend. 2nd derivative of the spectra was used to identify peak positions.
Figure 6.1 ATR-FTIR spectra of SC showing asymmetric and symmetric C-H bond stretching absorbances after DIW treatment.

Following moisturizer treatment and exposure to the drying environment for 8 hrs, SC specimens on glass were blotted with filter paper (Grade 595 Filter Paper, Schleicher & Schuell MicroScience GmbH, Dassel, Germany) to remove excess treatment and then analyzed with ATR-FTIR. The moisturizing coatings used in this study were also analyzed to distinguish the peaks associated with the SC from those associated with the treatments themselves. Finally, the effect of the moisturizing coatings on SC was analyzed with ATR-FTIR as a function of tissue depth using a delamination technique that has been previously used to characterize the intercellular delamination energy of SC layers [11,12]. The advantages of this technique compared to the tape stripping technique in terms of producing quantitative cohesion energies and well defined delaminated surfaces have also been described previously [11].
The SC tissue was adhered to an elastic polycarbonate substrate (Hyzod® GP, Sheffield Plastics Inc., Sheffield, MA, ~ 40 x 10 x 2.88 mm) using a cyanoacrylate adhesive (Instant Krazy Glue® BRUSH, Elmer’s Products Inc., Columbus, OH). The SC orientation was noted to ensure that the outer SC surface was facing out. Prior to treatment, specimens were equilibrated at 100%RH and 22°C for 2 hrs. Specimens were then dip-coated with the moisturizing treatments, exposed to the drying environment for 8 hrs and blotted with filter paper to remove excess treatment as described above. A second polycarbonate beam was then adhered onto the SC, and the specimens were mounted at the SC-free end via loading tabs in an adhesion test system (Delaminator Test System, DTS company, Menlo Park, CA) with a computer controlled DC servoelectric actuator operated in displacement control. Specimens were loaded in a purely tensile, Mode I, fashion, and delaminated using a 2 μm/s displacement rate. Two successive specimens were prepared from completely separated specimens by adhering new substrates onto the inner side of the previously tested specimen.

In this study, we were particularly interested in SC peak near 2850 cm⁻¹ shown in Figure 6.1 for SC treated with DIW and exposed to 7% RH and 22°C drying environment. This peak has been associated with the hydrocarbon chains of the SC lipids and is due to symmetric carbon-hydrogen (C-H) stretching from CH₂ groups [13]. In the literature, changes in SC lipid fluidity (degree of disorder of the lipid acyl chains) have been associated with the location of the C-H stretching absorbance peaks. Increasing lipid fluidity shifts the peaks to a higher wavenumber [14]. The height of both symmetric and antisymmetric C-H stretching absorbance peaks (at ~ 2920 cm⁻¹) compared to that of DIW treated specimen and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak (at ~ 1540 cm⁻¹) were used to qualitatively evaluate if the treatments have caused lipid extraction or not. Note that the ester peak at ~ 1740 cm⁻¹ may have been due to the presence of sebaceous lipids on the skin sample and was observed to significantly decrease after a single delamination.

6.3.5 Statistical Analysis
The SC drying stresses measured as a function of drying time following selected treatments are presented as mean values ± 1.96 x the standard error of the mean (STDEM) in which the mean values reported are expected to fall within these bounds with 95% confidence. Three to four specimens were tested for each treatment yielding ~ 18 - 20 final drying stress values per specimen for the substrate curvature method. In the ATR-FTIR study, four scans were made at different regions of the specimens to confirm the trends. The C-H stretching absorbances and peak heights are reported as mean ± standard deviation (SD) of four samples.

6.4 Results

6.4.1 Glycerin Based Humectants

The SC drying stresses measured for the SC specimens prior to application of the glycerin based humectant coatings and exposed to the 7% RH and 22°C drying environment for 8 hrs are shown in Figure 6.2. The $\sigma_{SC}$ values stabilized at ~ 3.4 MPa after ~ 2 hrs in the drying environment where they remained constant for 8 hrs. The $\sigma_{SC}$ values then reduced to ~ 0 MPa when the specimens were exposed to 100% RH air. The humectant coatings (10, 30, 100% GLY (v/v) in DIW) were then applied, and the overall substrate curvature was measured as a function of time in the same drying environment. Since no stresses developed in the humectant coatings, the overall substrate curvature was solely due to the curvature associated with the drying stresses of the underlying SC tissue. The $\sigma_{SC}$ value could then be determined using Stoney’s equation (Eq. 6.1) as a function of time in the drying environment as shown in Figure 6.2.

The $\sigma_{SC}$ values of specimens treated with the humectant coatings were observed to increase and stabilize after ~ 2 hrs in the drying environment where they remained relatively constant. With increasing GLY concentration, the final $\sigma_{SC}$ values and the initial rate of increase in $\sigma_{SC}$ with time, $d\sigma_{SC}/dt$, to the maximum drying stress values were observed to decrease as shown in Figures
6.3a and 3b, respectively. The implications of the maximum stress and drying stress rate are discussed at the end of the section.

![Graph showing SC drying stress as a function of drying time for SC treated with the humectant coatings and exposed to 7% RH and 22°C air.](image)

**Figure 6.2** SC drying stress as a function of drying time for SC treated with the humectant coatings and exposed to 7% RH and 22°C air.

The ATR-FTIR data of the SC specimens treated with the humectant coatings and exposed to the 7% RH and 22°C drying environment for 8 hrs is shown in Figure 6.4a. The height of the symmetric and antisymmetric C-H stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak are listed in Table 6.2. For all concentrations of glycerol in DIW, there was no significant change in the ratio of the heights of the symmetric C-H stretching peak and the Amide II peak compared to DIW (control) treated tissue suggesting the humectant treatments did not cause lipid extraction.
Figure 6.3 (a) The initial $\sigma_{SC}$ values following treatment, the peak $\sigma_{SC}$ values and the final $\sigma_{SC}$ values after 8 hr long exposure to the drying environment are shown with respect to those of DIW (control) treated tissue for SC treated with the humectant coatings. (b) The drying stress rate is shown with respect to that of DIW (control) treated tissue for SC treated with the humectant coatings.
Figure 6.4 (a) ATR-FTIR spectra of SC showing asymmetric and symmetric C-H bond stretching absorbances after humectant treatment. (b) The location of the symmetric C-H stretching peak as a function of the delamination number for SC specimens treated with the humectant coatings and exposed to the 7% RH and 22°C drying environment for 8 hrs.
The location of the symmetric C-H stretching peak was measured on delaminated SC surfaces following treatment with the humectant coatings and exposure to the 7% RH and 22°C air for 8 hrs. The peak location is shown in Figure 6.4b for each delamination number, N. Data shown for N = 0 represents the top surface of the SC tissue. The location of the peak was observed to decrease with increasing delamination number similar to trends recently reported using tape-stripping [15]. This decrease has been associated with the conformational changes in SC lipids from a disordered to an ordered state with increasing depth into the tissue [15]. Peak positions clustered above the DIW control suggest increased lipid fluidity following the treatment as noted in the infrared spectroscopy section. For SC treated with 30% (after 3rd delamination) and 100% GLY, the location of the peak was observed to be slightly higher compared to that of the control or 10% GLY treated tissue suggesting increased lipid fluidity for the high glycerin concentrations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-H Stretching Peak Heights (A.U)</th>
<th>Symmetric C-H Stretching Peak / Amide II Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symmetric</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>DIW</td>
<td>0.93 ± 0.02</td>
<td>1.40 ± 0.01</td>
</tr>
<tr>
<td>10% GLY</td>
<td>0.94 ± 0.02</td>
<td>1.41 ± 0.03</td>
</tr>
<tr>
<td>30% GLY</td>
<td>0.92 ± 0.02</td>
<td>1.37 ± 0.02</td>
</tr>
<tr>
<td>100% GLY</td>
<td>0.91 ± 0.03</td>
<td>1.36 ± 0.03</td>
</tr>
</tbody>
</table>

**Table 6.2** The height of the symmetric and antisymmetric C-H stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak for SC treated with the humectant coatings.

Topically applied humectants function to attract water mainly from the viable skin layers to the SC and from the environment if the ambient RH exceeds 70% [16]. In our substrate curvature measurements, the humectant coatings could neither attract water from the underlying skin layers as it would *in vivo* nor
could it draw water from the external dry (7% RH) environment. For this reason, the resulting drying stresses should be explained solely in terms of the effect of humectant coatings on SC water loss and the SC components themselves.

With increasing GLY concentration, the final $\sigma_{SC}$ values were observed to decrease and the rate of the increase in $\sigma_{SC}$ over time to stable stress values was significantly lower compared to that of DIW suggesting that these formulations were able to slow down the SC water loss during exposure to the drying environment. The kinetics of water loss from SC is likely to slow down in the presence of increasing concentrations of glycerin since glycerin has a proclivity to bind with water. To gain more insight into the possible contribution of changes in SC water loss to the decrease in the final $\sigma_{SC}$ values, we utilized a previously found relationship between the SC drying stresses and the chemical potential of water in the drying environment and related these stresses to the modeled water loss during drying as shown in Figure 6.5a [6]. Using this relationship, we were able to determine the percentage of the water retained by the GLY treated SC to the water lost by the DIW treated tissue following exposure to 7% RH and 22°C air. The percentage of water retained by the SC was ~ 2, 4 and 8% for 10, 30 and 100% GLY treated SC, respectively [Figure 6.5b].

The observed retention of water in the SC following treatment with different concentrations of glycerin in DIW is consistent with previous findings in the literature. Following exposure to formulations with high concentrations of glycerin, corneocytes and SC intercellular spaces have been observed to expand and the tissue has been reported to be at a higher hydration state compared to that of the tissue exposed to DIW [17].

The other possible cause of lower SC stresses with increasing concentration of glycerin may be the increased lipid fluidity. When glycerin is added to SC lipids in vitro, it has been reported to interact with the intercellular lipids, enhance water absorption, and maintain the intercellular lipid matrix in a fluid, liquid crystalline state [18,19]. Considering that the SC lipids account for 5-30% of the total tissue volume, their expansion with increasing lipid fluidity is likely to lower the SC stresses. However, at this point, their exact contribution is
not known. In this study, the effect of glycerin on corneodesmosome degradation was not considered since it is reported to occur when the tissue is conditioned at moist environments (>80% RH) [20].

**Figure 6.5** (a) SC drying stress as a function of % water loss. The final $\sigma_{SC}$ values of GLY treated specimens and the corresponding % water loss values are plotted in blue. (b) % retained water in the tissue following treatment with varying concentrations of GLY.
Finally, we used the substrate curvature technique to measure the SC drying stresses following application of two different treatments containing similar concentrations of glycerin, GLY-A and GLY-B. SC drying stresses were measured prior to and after application of the treatments in the same drying environment as described above. Since when applied directly on the glass, no stresses developed in the treatments, the overall substrate curvature was solely due to the curvature associated with the drying stresses of the underlying SC tissue. The measured $\sigma_{SC}$ values are shown as function of time in the drying environment in Figure 6.2.

The $\sigma_{SC}$ values of specimens treated with GLY-A increased to a peak $\sigma_{SC}$ value and then slowly decreased to a final $\sigma_{SC}$ value higher than that of the control over the course of 8 hrs as shown in Figure 6.2. In contrast, the $\sigma_{SC}$ values of specimens treated with GLY-B increased and stabilized at $\sigma_{SC}$ values lower than that of the control after ~ 2 hrs in the drying environment where they remained relatively constant.

The significantly higher final $\sigma_{SC}$ and $d\sigma_{SC}/dt$ values for specimens treated with GLY-A (Figure 6.3a and b) suggest that this treatment enhanced the water loss from SC during exposure to the drying environment. This was a surprising result considering that the GLY-A is added into novel skin care formulations as an effective moisturizer. This non-drying gel with a simple chemical composition consisting of glycerin and glyceryl polyacrylate does not readily release the water contained within its molecular structure even under severe drying conditions due to its clathrate (group of molecules that form a cage-like matrix) structure. Water molecules are held within the glyceryl polyacrylate clathrate matrix and released when disrupted by the salt content, pH and surface temperature of skin. GLY-A is also very hygroscopic – it can absorb water from the atmosphere and through semi-permeable membranes (osmotic effect) and in vivo it is reported to facilitate the evacuation of water and toxins by the blood and the lymphatic vessels [21]. We believe that the increased $d\sigma_{SC}/dt$ and SC stresses following application of the GLY-A treatment may be associated with the poor diffusion of the gel ingredients into the SC and the strong affinity of both glycerin and
glyceryl polyacrylate in the gel to absorb water from the humid SC in the meantime. Finally, the relaxation in SC drying stresses may be attributed to the availability of the water held by the gel to the SC with increasing diffusion of its ingredients into the tissue. The other possible causes of this decrease may be the expansion of the SC lipids due to increased lipid fluidity and softening of the tissue in the presence of GLY-A treatment.

The final $\sigma_{SC}$ value of GLY-B treated tissue was the same as that of the tissue treated with 100% GLY although the formulation contained only 40% (v/v) glycerin. We believe that the lower stress values observed with GLY-B may be due to the synergistic effect of its ingredients and the increased penetration of glycerin in the presence of the emulsifiers used in the treatment. Also, based on the formula composition, GLY-B is an oil/lamellar gel/water type emulsion that forms a hydrophobic film when applied on SC, which can improve skin barrier function, thus lowering the SC stress value.

The results of the study above show that the efficacy of a treatment on lowering skin stresses depends on the effect of the whole formulation on skin rather than the effect of its individual ingredients. Therefore, the assumption of a treatment containing specific moisturizers improving skin “feel” and alleviating dry skin should be treated with caution.

### 6.4.2 Ester based emollients with partially occlusive behavior

The molecular structure of the main component of the emollient coatings and the calculated (ACD / ChemSketch, Version 12.01, Advanced Chemistry Development Inc.) partition coefficient value, logP, are shown in Table 6.3. The partition coefficient was used as a measure of the hydrophilic / lipophilic balance. From the calculated logP values, DA was the least lipophilic treatment and the OSS was the most lipophilic. Since the calculated logP value of DA was $\sim$ 2.8, we expected that it would diffuse rapidly into the SC and partition well between its hydrophilic and lipophilic domains [22]. With logP values higher than 5, the other emollient molecules were unlikely to partition into the hydrophilic domains.
of the SC. They were expected to partition only into the skin lipids and have extreme difficulty partitioning out of the SC [23].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Molecular Structure</th>
<th>logP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA</td>
<td><img src="image" alt="DA structure" /></td>
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</tr>
<tr>
<td>AL</td>
<td><img src="image" alt="AL structure" /></td>
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<tr>
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<td>11.15</td>
</tr>
<tr>
<td>ISS</td>
<td><img src="image" alt="ISS structure" /></td>
<td>20.02</td>
</tr>
<tr>
<td>OSS</td>
<td><img src="image" alt="OSS structure" /></td>
<td>26.92</td>
</tr>
</tbody>
</table>

**Table 6.3** The molecular structure of the main component of the emollient coatings and their corresponding calculated logP values are listed above.
The sensory profiles and physical characteristics of the emollients (MW, viscosity and spreading) are listed in Table 6.4. The emollients are divided into three types (light, medium and heavy) according to their MW. The spreading of the emollient was observed to increase as its MW and viscosity decreased.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type</th>
<th>MW (g)</th>
<th>Viscosity (Pa.s)</th>
<th>Spreading</th>
<th>Sensorial Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA</td>
<td>light</td>
<td>230.2</td>
<td>0.002</td>
<td>high</td>
<td>Dry initial feel, light after feel</td>
</tr>
<tr>
<td>AL</td>
<td>light</td>
<td>286.5 (average)</td>
<td>0.012</td>
<td>high</td>
<td>Dry initial feel, light after feel</td>
</tr>
<tr>
<td>IN</td>
<td>medium</td>
<td>354.4</td>
<td>0.015</td>
<td>medium</td>
<td>Medium initial, medium after feel</td>
</tr>
<tr>
<td>EP</td>
<td>medium</td>
<td>368.4</td>
<td>0.014</td>
<td>medium</td>
<td>Medium initial, medium after feel</td>
</tr>
<tr>
<td>ISS</td>
<td>heavy</td>
<td>791.4</td>
<td>0.064</td>
<td>low</td>
<td>Heavy initial feel, lubricious after feel</td>
</tr>
<tr>
<td>OSS</td>
<td>heavy</td>
<td>846.9</td>
<td>0.054</td>
<td>low</td>
<td>Heavy initial feel, lubricious after feel</td>
</tr>
</tbody>
</table>

Table 6.4 The physical characteristics of the emollient coatings (MW, viscosity and spreading characteristics) and their sensory profiles are listed above. Note: ISS and OSS feel drier compared to petrolatum (hydrocarbon class) which is very greasy, tacky and heavy. They feel heavier compared to DA, AL, IN, EP - rest of the esters investigated in this study.

The SC drying stresses measured for the SC specimens prior to application of the emollient treatments and exposed to the 7% RH and 22°C drying environment for 8 hrs are shown in Figure 6.6. The $\sigma_{SC}$ values stabilized at ~ 3.4 MPa after ~ 2 hrs in the drying environment where they remained constant. After
8 hrs in the drying environment, $\sigma_{SC}$ values reduced to $\sim 0$ MPa when the specimens were exposed to 100% RH air. The emollient treatments (AL, DA, EP, IN, ISS, OSS) were then applied and the overall substrate curvature was measured as a function of time in the same drying environment. Since, when applied directly on glass, no stresses developed in the emollient treatments, the overall substrate curvature was solely due to the curvature associated with the drying stresses of the underlying SC tissue. The $\sigma_{SC}$ value could then be determined using Stoney’s equation (Eq. 6.1) as a function of time in the drying environment as shown in Figure 6.6.

![Graph showing SC drying stress as a function of drying time for SC treated with ester based emollient coatings (DA, AL, IN, EP, ISS, OSS) and exposed to 7% RH and 22°C air.](image)

**Figure 6.6** SC drying stress as a function of drying time for SC treated with ester based emollient coatings (DA, AL, IN, EP, ISS, OSS) and exposed to 7% RH and 22°C air.

The $\sigma_{SC}$ values of specimens treated with the emollient treatments were observed to be non-zero just after application, rising rapidly to a peak value and then slowly relaxing over the course of 8 hrs in the drying environment as shown
in Figure 6.6. The initial $\sigma_{SC}$ value following application of the emollient, the peak $\sigma_{SC}$ value and the final $\sigma_{SC}$ value after 8 hrs of exposure to 7% RH and 22°C air are shown in Figure 6.7a. The drying stress rate values are shown in Figure 6.7b.

**Figure 6.7** (a) The initial $\sigma_{SC}$ values following treatment, the peak $\sigma_{SC}$ values and the final $\sigma_{SC}$ values after 8 hr long exposure to the drying environment are shown with respect to those of DIW (control) treated tissue for SC treated with the ester based emollients. (b) The drying stress rate is shown with respect to that of DIW (control) treated tissue for SC treated with the ester based emollients.
Following emollient treatment, the initial $\sigma_{SC}$ values varied between 0.15 MPa and 3.15 MPa and were observed to increase with decreasing logP and MW. SC stresses then rapidly increased to a peak value. The initial rate of increase in $\sigma_{SC}$ with time, $d\sigma_{sc}/dt$, varied between 2.9 MPa / hr and 7.1 MPa / hr and was observed to increase with increasing MW and logP and decreased spreading; DA was an exception to this trend. All $d\sigma_{sc}/dt$ values were higher than those of the DIW treated tissue. The possible explanations for the observed trends including exceptions are discussed in detail later in this section. The peak $\sigma_{SC}$ values of heavy emollients with poor spreading characteristics and DA were significantly higher than those of the other treatments. After rising to a peak $\sigma_{SC}$ value, the $\sigma_{SC}$ values of all specimens excluding AL and DA were observed to slowly decrease to a final $\sigma_{SC}$ value. In the case of DA treated SC, following rise to a peak $\sigma_{SC}$ value, stresses were observed to rapidly relax to a final $\sigma_{SC}$ value where they remained relatively constant for a period of 6 hrs. SC surface was observed to significantly dry after exposure to DA and the drying environment. In one sample, DA exposure was observed to induce significant cracking. In the case of AL treated tissue, after the sudden rapid decrease, $\sigma_{SC}$ values continued to decrease slowly and then increased to a secondary peak followed by a decrease to a final $\sigma_{SC}$ value.

The final $\sigma_{SC}$ values of emollient treated specimens varied between 0.61 and 3.14 MPa and were significantly lower than those of DIW treated tissue. Interestingly, the final $\sigma_{SC}$ values of specimens treated with ISS, the heaviest treatment with the highest logP value, and DA, the lightest treatment with the lowest logP value, were higher than those treated with the other emollients. As noted in the experimental section, at least 3 tests were conducted for each treatment and the drying stress curves were found to be highly reproducible. This included the small secondary peaks at 4.5 and 6 hrs of drying time post exposure noted for AL and OSS treatments, respectively.

The ATR-FTIR data of the SC specimens treated with the emollient coatings and exposed to the 7% RH and 22ºC drying environment for 8 hrs is
shown in Figure 6.8a and the height of the symmetric and antisymmetric C-H stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak are listed in Table 6.5.

![Graph and Table]

**Figure 6.8** (a) ATR-FTIR spectra of SC showing asymmetric and symmetric C-H bond stretching absorbances after ester based emollient treatment. (b) The location of the symmetric C-H stretching peak as a function of the delamination number for SC specimens treated with the emollient coatings and exposed to the 7% RH and 22°C drying environment for 8 hrs.
For AL, DA, EP and IN coated specimens, the height of the symmetric and antisymmetric carbon-hydrogen (C-H) stretching peaks and the ratio between the heights of symmetric C-H stretching peak and the Amide II peak were lower compared to DIW (control) treated tissue suggesting that all of these emollient coatings caused lipid extraction. The degree of lipid extraction (from high to low) was DA > AL > IN > EP > DIW. There was no lipid extraction for the ISS and OSS treated specimens.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-H Stretching Absorbances (A.U)</th>
<th>Symmetric C-H Stretching Peak / Amide II Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symmetric</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>DIW</td>
<td>0.93 ± 0.02</td>
<td>1.40 ± 0.01</td>
</tr>
<tr>
<td>DA</td>
<td>0.50 ± 0.05</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td>AL</td>
<td>0.80 ± 0.03</td>
<td>1.24 ± 0.03</td>
</tr>
<tr>
<td>IN</td>
<td>0.83 ± 0.03</td>
<td>1.30 ± 0.03</td>
</tr>
<tr>
<td>EP</td>
<td>0.86 ± 0.02</td>
<td>1.32 ± 0.02</td>
</tr>
<tr>
<td>ISS</td>
<td>1.08 ± 0.02</td>
<td>1.58 ± 0.04</td>
</tr>
<tr>
<td>OSS</td>
<td>1.03 ± 0.02</td>
<td>1.48 ± 0.01</td>
</tr>
</tbody>
</table>

**Table 6.5** The height of the symmetric and antisymmetric C-H stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak for SC treated with the emollient coatings.

The location of the symmetric C-H stretching peak measured on delaminated SC surfaces for SC specimens treated with the emollient coatings and exposed to the 7% RH and 22°C drying environment for 8 hrs is shown in Figure 6.8b as a function of the delamination number, N. In the same figure, the location of the symmetric C-H stretching peaks for the emollient coatings are shown. Following exposure to the drying environment (Del 0), the location of the
symmetric C-H stretching peak of SC treated with the moisturizing coatings excluding DA was higher compared to that of DIW treated tissue. This increase was treated with caution since all the emollients used in our study excluding DA had a C-H stretching peak very close to that of the SC. The increase in the peak location could have been due to the presence of the emollients on the SC surface despite the blotting of the specimens before testing. As the deeper layers of SC were probed, the effect of emollients on the tissue was observed to persist: the location of the symmetric C-H stretching peak was still higher (although not as drastically as before) compared to that of DIW treated tissue. To determine whether the increase in the peak location was due to the presence of the emollients or conformational changes in the SC lipids, we looked at several vibrational bands characteristic for the emollient treatments and checked for their presence in the spectra of SC treated with the emollients. For these vibrational bands, there was no noticeable difference between the spectra of SC treated with the emollients and SC treated with DIW after the 2nd and 3rd delaminations. We believe that the peak positions clustered above the DIW control suggest increased lipid fluidity following the treatment for these delaminations.

To accurately interpret the observed SC drying stress profiles (Figure 6.6) following application of the emollients and to correlate these stresses with the structural changes in SC components, we have to first review how emollients typically interact with skin. Emollients provide an oily partially occlusive film over the surface of the skin which fills in the interstices between the desquamating corneocytes abundant in dry skin conditions smoothing the rough SC surface [16,24]. The degree of their occlusivity depends on their molecular weight, viscosity and spreading characteristics on SC. If the emollient molecules penetrate into the SC, they may interact with the intercellular lipids or corneocytes depending on their hydrophilicity / lipophilicity and eventually be metabolized and modify lipid secretory mechanism of SC [25,26,27]. Considering that the composition of the emollient treatments used in our study (Table 6.1), it is likely for the emollient molecules to penetrate into the SC and interact with SC lipids. Fatty acid esters, fatty acids and alcohols have been extensively studied as
skin penetration enhancers for the developments of topical and transdermal drug delivery systems [28] where the successful penetration of these molecules were correlated with the lipid extraction in the skin [29,30].

![Graph showing absorbance vs. first SC stress](image)

**Figure 6.9** The first $\sigma_{SC}$ values following application of ester based emollient coatings as a function of the height of asymmetric and symmetric C-H bond stretching peak and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak. The linear fits show a strong correlation between the drying stress and the height of the asymmetric and symmetric C-H bond stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak.

The drying stress curves of specimens exposed to emollient treatments were different than those exposed to humectants or DIW. For all the emollient treatments, the initial $\sigma_{SC}$ value was non-zero. This value varied drastically for different treatments suggesting that this may be a treatment induced effect on SC. We observed that for treatments that resulted in lipid extraction, the initial $\sigma_{SC}$ values were correlated ($R^2 = -0.94$ for both) with the height of symmetric and
antisymmetric C-H stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak (Figure 6.9). Thus, we think that the initial stress value may be attributed to the contraction of SC lipids due to extraction of a fraction of the intercellular lipids following penetration of emollient molecules into the SC.

We have noted earlier that emollients are partially occlusive depending on their chemical and physical characteristics. Therefore, the rate and the amount of water loss from SC strongly depend on how well the emollient coating occludes the surface of the SC. In the case of our study, we believe that the rise in $\sigma_{SC}$ over a short period of time (~ 0.5 hrs) to a peak $\sigma_{SC}$ value is primarily related to the water loss from SC following extraction of a fraction of intercellular lipids due to emollient penetration. Heavy emollients with high lipophilicity and low spreading characteristics, ISS and OSS, were observed to have a significantly higher $d\sigma_{SC}/dt$ and peak $\sigma_{SC}$ value compared to light and medium emollients (excluding DA) suggesting that they may not be occluding the surface of the SC as well as other emollients.

The lightest emollient with the least lipophilicity and high spreading characteristics, DA, was observed to have similar $d\sigma_{SC}/dt$ and peak $\sigma_{SC}$ values with the heavy emollients. We attribute these high values to the possibility of DA to quickly penetrate both into the hydrophilic and lipophilic domains of the SC tissue (based on its calculated logP value) and compromise SC barrier properties by extracting SC lipids and increasing lipid rigidity as confirmed by spectroscopy results. Thus, despite the high spreading characteristics of DA, high $d\sigma_{SC}/dt$ and peak $\sigma_{SC}$ values develop due to its effect on SC components.

The light emollient AL with medium lipophilicity and high spreading characteristics and the medium emollients EP and IN with medium lipophilicity and medium spreading characteristics were observed to deliver lower $d\sigma_{SC}/dt$ and peak $\sigma_{SC}$ values with respect to other treatments. This trend was reasonable considering that these emollient treatments were likely to better occlude the SC due to their high or medium spreading characteristics. Among these emollients,
AL with high spreading characteristics had the lowest $d\sigma_{SC}/dt$ value, but the highest peak $\sigma_{SC}$ value. This may be associated with the higher lipid extraction observed after this treatment. Thus, despite the high spreading characteristics of AL, the changes in SC barrier properties due to extraction of the SC lipids may be increasing its peak $\sigma_{SC}$ values. While it is difficult to make conclusive statements on some observed trends, we believe that both spreading characteristics of the emollients and structural changes in the SC components determine SC barrier properties and the kinetics of water loss from SC consequently.

After rising to a peak $\sigma_{SC}$ value, SC stresses of all emollient treated specimens were observed to relax to a final $\sigma_{SC}$ value. For the light emollients, there was a sudden rapid decrease in SC stresses that may be indicative of damage (cracking) in the tissue. In the case of DA treated SC, our visual observations following exposure to this treatment and the drying environment strengthen this argument. For medium and heavy emollients, the stress relaxation to the final $\sigma_{SC}$ value is gradual. There are no sudden decreases in SC stresses. We attribute this stress relaxation to the expansion of the SC lipids due to increased fluidity and softening of the tissue due to increasing length of emollient exposure.

The final $\sigma_{SC}$ values for the emollient treatments didn’t exhibit a distinctive trend. The treatments that delivered lower $d\sigma_{SC}/dt$ and peak $\sigma_{SC}$ values were observed to also deliver lower final $\sigma_{SC}$ values with one exception, ISS.

Finally, we note that there may be a correlation between the stresses observed in our study and the sensory profile of moisturizing treatments listed in Table 6.4. Both light and heavy emollients are suggested to deliver a dry initial feel meaning following their application on skin the initial sensation perceived is dry, non-greasy whereas medium emollients are suggested to deliver light to medium initial feel. Interestingly, we observed that the peak $\sigma_{SC}$ values for light and heavy emollients were significantly higher than those of medium emollients that were lower than the threshold value (peak $\sigma_{SC}$ value for DIW treated tissue). All emollients are also suggested to deliver either light or more lubricious after
feel, meaning after a short time period the final sensation perceived is light for
the least lipophilic emollients (DA and AL) due to a faster penetration on SC, and
more lubricious and heavier feel for more lipophilic emollients (ISS and OSS)
with slower penetration into SC. This may be closely related to the results of our
study in which we observed that the final $\sigma_{\text{sc}}$ value for emollient treated SC was
significantly lower than that of DIW treated SC.

Figure 6.10 (a) SC drying stress as a function of drying time for SC treated with
PET and exposed to 7% RH and 22°C air. (b) The peak $\sigma_{\text{sc}}$, final $\sigma_{\text{sc}}$ and stress
rate values are shown with respect to those of DIW (control) treated tissue for SC
treated with PET.
6.4.3 Petrolatum

The SC stresses measured for the SC specimens prior to application of PET and exposed to the 7% RH and 22°C drying environment for 8 hrs is shown in Figure 6.10a. The $\sigma_{SC}$ values stabilized at ~ 3.4 MPa after ~ 2 hrs in the drying environment where they remained constant. After 8 hrs in the drying environment, $\sigma_{SC}$ values reduced to ~ 0 MPa when the specimens were exposed to 100% RH air. The PET coating was then applied and the overall substrate curvature was measured as a function of time in the same drying environment. Since when applied directly on glass, no stresses developed in the PET coating, the overall substrate curvature was solely due to the curvature associated with the drying stresses of the underlying SC tissue. The $\sigma_{SC}$ value could then be determined using Stoney’s equation (Eq. 6.1) as a function of time in the drying environment as shown in Figure 6.10a. Following application of the PET coating, the first $\sigma_{SC}$ value was non-zero. Then, the $\sigma_{SC}$ values of specimens were observed to increase to a peak $\sigma_{SC}$ value of ~ 2.80 MPa within ~ 2 hrs in the drying environment where they remained constant for an additional ~ 2 hrs. Then, SC stresses were observed to relax to a final value of ~ 2.25 MPa significantly lower than the final $\sigma_{SC}$ value of specimens treated with DIW. The average of the peak and final $\sigma_{SC}$ values are shown in Figure 6.10b.

The height of the symmetric and antisymmetric carbon-hydrogen (C-H) stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak were observed to be similar to those of DIW treated tissue (Table 6.6). The location of the symmetric C-H stretching peak for SC specimens coated with PET and exposed to the 7% RH and 22°C drying environment for 8 hrs is shown in Figure 6.11 as a function of the delamination number. In the same figure, the location of the symmetric C-H stretching peak for PET is shown. Following exposure to the drying environment (Del 0), the location of the symmetric C-H stretching peak of SC treated with the moisturizing coatings excluding DA was not significantly different than that of DIW treated tissue. As the deeper layers of SC were probed, the location of the symmetric C-H
stretching peak was observed to be higher with respect to that of DIW treated
tissue suggesting increased lipid fluidity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-H Stretching Absorbances (A.U)</th>
<th>Symmetric C-H Stretching Peak / Amide II Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symmetric</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>DIW</td>
<td>0.93 ± 0.02</td>
<td>1.40 ± 0.01</td>
</tr>
<tr>
<td>PET</td>
<td>0.98 ± 0.03</td>
<td>1.44 ± 0.04</td>
</tr>
</tbody>
</table>

Table 6.6 The height of the symmetric and antisymmetric C-H stretching peaks and the ratio of the height of symmetric C-H stretching peak to the height of the Amide II peak for SC treated with petrolatum.

The final SC drying stress following PET application was significantly lower with respect to that of DIW treated tissue. This result was somewhat expected considering that PET is regarded as one of the most effective occlusive emollients for skin care and its topical application on skin is used as a standard to demonstrate the reduction of TEWL following insults to the SC barrier [31,32,33]. It penetrates only to the very top layers of SC and partitions only into the skin lipids like the extremely hydrophobic (ISS and OSS) emollients tested in the previous section. Interestingly, for the PET coating, both the first $\sigma_{SC}$ value (~0.15 MPa) and the height of C-H stretching peaks were similar to those of the extremely hydrophobic (ISS and OSS) emollients tested in the previous section. However, unlike all the emollients tested in the previous section, both $d\sigma_{SC}/dt$ and the peak $\sigma_{SC}$ value were significantly lower than those of DIW suggesting that PET may be occluding the surface of the SC better than the other emollients. Despite the suggested better occlusive properties of PET, its application was not as successful in lowering SC drying stresses as some other emollients (AL, EP, IN and ISS) used in our study. Considering that the blue shift in C-H stretching absorbances was similar to that of AL, EP, IN coated specimens, it is not clear why the SC drying stresses for PET coated specimens did not relax as much after reaching the peak $\sigma_{SC}$ value.
Figure 6.11 The location of the symmetric C-H stretching peak as a function of the delamination number for SC specimens treated with the PET coating and exposed to the 7% RH and 22\(^\circ\)C drying environment for 8 hrs.

6.4.4 Implications for Alleviation of Dry Skin Damage

We now consider the biomechanics model described in the introduction to model the effectiveness of moisturizing treatments used in this study in alleviating dry skin damage. We infer the propensity for dry skin damage by computing the strain energy release rate, \(G\), from the measured values of \(\sigma_{sc}\) and \(E_{sc}\) following exposure to the moisturizing treatments and the drying environment for 8 hrs and comparing them to the measured SC resistance to intercellular delamination, \(G_c\). By observing the ratio of \(G / G_c\) and using the values for DIW treated tissue exposed to a drying environment as a control, we determine the efficacy of humectant and emollient moisturizers in alleviating dry skin damage as shown in Figure 12a and b, respectively.
**Figure 6.12** a) SC drying stress, $\sigma_{sc}$, intercellular delamination energy, $G_c$, and the normalized crack driving force, $G/G_c$, shown as a function of humectant moisturizing treatments. b) SC drying stress, $\sigma_{sc}$, intercellular delamination energy, $G_c$, and the normalized crack driving force, $G/G_c$, shown as a function of emollient moisturizing treatments.
The cracking potential was significantly reduced following application of the humectant and emollient treatments excluding DA and exposure to the 7% RH and 22°C drying environment. The increasing cracking potential following exposure to DA treatment and the drying environment suggests that this treatment may cause damage to the tissue.

6.5 Conclusion

Using the substrate curvature method presented in this study, we have evaluated the effect of a wide variety of moisturizing molecules on SC drying stresses. These stresses were observed to strongly depend on the physical and chemical characteristics of the moisturizing molecules and their influence on the underlying SC structure such as extraction of the SC lipids and changes in lipid fluidity. In general, occlusive and emollient molecules providing an exogenous hydrophobic barrier to water loss while enhancing the softness and flexibility of skin were observed to reduce drying stresses in SC more effectively than humectant molecules such as glycerol which promote water retention within the SC. For ester based emollients, a potential correlation was suggested between the sensory profiles of these molecules and their resulting drying stresses. This study may help in a better understanding of specific functions of moisturizers and enable the use of a biomechanics model to evaluate the efficacy of moisturizers in alleviating the potential energy for dry skin damage such as cracking and chapping of the tissue.

6.6 Acknowledgements

The authors would like to thank David Moore and Mihaela Gorcea from ISP Corp. for useful discussions and providing most of the moisturizing molecules used in this study. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Sciences Division of the U.S. Department of Energy, under Contract No. DE-FG02-07ER46391.
6.7 REFERENCES


Chapter 7 Effect of Corneodesmosome Degradation on the Intercellular Delamination of Human SC

7.1 ABSTRACT

The desquamation behavior of human stratum corneum (SC) has been associated with the progressive degradation of corneodesmosomes toward the outer skin surface. This process is facilitated by the action of specific enzymes in the SC such as the stratum corneum chymotryptic enzyme (SCCE) and the stratum corneum tryptic enzyme (SCTE). In order to better understand the effect of this degradation on SC mechanical properties, we have characterized the SC intercellular delamination energy in the presence and absence of an exogenous form of SCCE known as recombinant human tissue kallikrein 7 (rhK7). Transmission electron microscopy (TEM) of the intact and degraded corneodesmosomes was used to quantify the effect of enzymatic exposure. The enzymatic degradation process was demonstrated to decrease the density of intact corneodesmosomes leading to significant decreases in the SC intercellular delamination energy. (from K. Levi et al. Effect of Corneodesmosome Degradation on the Intercellular Delamination of Human Stratum Corneum. The Journal of investigative dermatology 2008; 128(9):2345.)

7.2 INTRODUCTION

Stratum corneum (SC) has a composite like structure consisting of heavily keratinized, disk-shaped corneocytes cells bound together by intercellular lipids and degraded desmosomal protein junctions or corneodesmosomes. To maintain a constant thickness, SC renews itself via the continual replacement of mature exterior cells with younger interior cells. Proper SC cell detachment and renewal,
or desquamation, has been associated with the progressive degradation of corneodesmosomes toward the outer skin surface, and this degradation is facilitated by the action of specific enzymes in the SC such as the stratum corneum chymotryptic enzyme (SCCE), also known as recombinant human tissue kallikrein 7 (rhK7), and the stratum corneum trypsin enzyme (SCTE) [1,2].

Surprisingly, current understanding of the effect of corneodesmosome degradation on the mechanical behavior of SC remains incomplete and largely limited to measurements of in-plane properties or qualitative tape stripping [3,4]. In particular, a quantitative relationship between human SC intercellular delamination energy and the density of intact corneodesmosomes has not been established. This limits our understanding of the contribution of corneodesmosome degradation on the normal physical process of corneocyte detachment as well as damage processes associated with abnormal desquamation conditions. Abnormal desquamation occurs when intracorneocyte (peripheral) and intercorneocyte (non-peripheral) corneodesmosomes remain intact and do not degrade thus preventing corneocytes from detaching from the SC layer as single cells [5,6]. Due to the absence of proper corneodesmosome degradation, corneocytes are thought to be much more cohesive in the outer SC and as a result of the defective proteolysis, they are shed in clusters forming visible scales that can vary from severe in disorders such as ichthyoses and psoriasis to mild-to-moderate in surfactant-induced dry skin [4,7].

We employ an in vitro fracture mechanics based double-cantilever-beam (DCB) method to quantitatively characterize the effect of enzyme-induced corneodesmosome degradation on the intercellular delamination energy of isolated human SC in a direction perpendicular to the skin surface. Using this approach, we have previously identified how selected treatments and exposures affect both the intercellular delamination energy and time dependent delamination of SC with respect to its cellular and intercellular structure [8,9,10,11]. We have also quantitatively characterized the increase in intercellular delamination energy with depth into the SC layer demonstrating the role of increasing corneodesmosome density on intercellular adhesion [11]. In the current study, rhK7 enzyme was used to facilitate the degradation of
corneodesmosomes thereby decreasing the density of intact corneodesmosomes. The effect of varying enzyme incubation period was also examined. To correlate SC intercellular delamination energy with the level of corneodesmosome degradation in the tissue, transmission electron microscopy (TEM) was employed.

The intercellular delamination energy of SC tissue was observed to decrease by ~ 67% from ~ 6 J/m² to ~ 2 J/m² after exposure to rhK7. TEM studies on the enzyme treated SC revealed a significant decrease in the intact corneodesmosome density compared to that of the tissue exposed to a control solution. These results suggest a strong link between the SC intercellular delamination energy and intact corneodesmosome density. The effect was mostly related to degradation of intercorneocyte corneodesmosomes since no apparent degradation was noted for intracorneocyte corneodesmosomes in the presence of the rhK7 enzyme. We believe that this represents the first direct quantitative demonstration of the effects of corneodesmosome degradation on the delamination properties of human SC.

7.3 MATERIALS AND METHODS

7.3.1 Tissue preparation

Human cadaver SC used in these experiments was obtained from a female Caucasian donor, 80 years of age, from the thigh. Epidermal tissue was separated from dermis by immersion of the donor tissue cleared of adipose tissue in a 35°C water bath followed by a 1 min soak at 60°C and subsequent mechanical separation from the dermis using a flat-tipped spatula. The stratum corneum was isolated from the underlying epidermis by soaking in a trypsin enzymatic digest solution (0.1% wt/wt in 0.05M, pH 7.9 Tris buffer) at 35°C for 135 min. During separation, the orientation of the outer SC surface was recorded. The isolated SC was rinsed with room temperature distilled water and allowed to dry on filter paper (Grade 595 General-Purpose Filter Paper, Schleicher & Schuell MicroScience GmbH, Dassel, Germany) then removed and stored in ambient
conditions of ~18°C - 23°C and ~35 – 55% relative humidity (RH). Comparative tests were performed on the single donor tissue specimens to reduce variability within test sequences. Unlike most other soft tissues in the body, SC is not vascularized and does not undergo rapid apoptosis and structural damage after such harvesting. The SC tissue isolated in this manner can be appropriately stored without major change for extended periods [12].

### 7.3.2 Enzyme Treatment

Recombinant human tissue kallikrein 7 (rhK7) enzyme (R & D Systems, Inc., Minnesota, MN) was activated with 25 mM Tris, 10 mM CaCl₂, 0.15 M NaCl, 50% glycerol, pH 7.5, thermolysin (R & D Systems, Inc., Minnesota, MN) formulation. After pre-incubating the rhK7 at 0.1 μg/μl with thermolysin at 0.01 μg/μl in TCNB buffer (50 mM Tris, 10 mM CaCl₂, 0.15 M NaCl, 0.05% Brij-35, pH 7.5) for 2 hours at 37°C, EDTA was added into the mixture to stop thermolysin activity. The enzyme was then stored in a 50 mM Tris, 150 mM NaCl, pH 8.5 buffer solution and this buffer was used for all the experiments.

SC was incubated in the buffer solution with the rhK7 enzyme (50 ng/ml) and without the enzyme for 1.5, 3 or 6 hours at 37°C. In order to measure enzyme activity, fluorogenic peptide substrate II (R & D Systems, Inc., Minnesota, MN) was used. Enzyme activity measurements were performed using a fluorescence plate reader with excitation at 320 nm and emission at 405 nm before and after soaking SC tissue. At the end of each incubation period, a small section of the treated tissue was fixated with Karnovsky’s fixative (2% glutaraldehyde, 2% paraformaldehyde in 0.15 M sodium cacodylate, 0.1 M sucrose, pH 7.4) for about 12 hours at 4°C. Fixated SC tissue was rinsed in a 50 mM Tris, 150 mM NaCl, pH 8.5 buffer solution three consecutive times for 15 minutes.

### 7.3.3 Transmission Electron Microscopy

Fixated SC specimens were transferred into 200 mM sodium cacodylate buffer for 1 hour. Then, half of the specimens were postfixed with 0.2% RuO₄ in
freshly prepared in Na cacodylate buffer for 10-15 minutes in the dark in a hood at room temperature. The other half of the specimens were postfixed with 2.0\% OsO$_4$ in freshly prepared sodium cacodylate buffer for 20 minutes in the dark in a hood at room temperature. At the end of the postfixation step, all specimens were washed twice in water for 10 minutes and then dehydrated with full power microwave in varying acetone solutions, 1x50\%, 1x70\%, 1x90\% and 2x100\% for 1 minute each step. Then, the specimens were infiltrated in microwave in 1x50\%, 2x100\% Spurr for 15 minutes for each step. All specimens were embedded in 100\% Spurr and cured at 60\°C for 2 days. Thin sections were examined with TEM1230 electron microscope (JEOL, Tokyo, Japan) equipped with a CCD camera (Gatan, Pleasanton, CA) at an accelerating voltage of 80 kV. An indication of the corneodesmosome degradation was obtained from the resulting TEM images (n = 4) by counting the number of intact and partially degraded corneodesmosomes that were clearly apparent in the images. The surface density of such intact and partially degraded corneodesmosomes was obtained after dividing by the total area of the micrographs examined. The area of the each micrograph was 102 $\mu$m$^2$.

### 7.3.4 Delamination energy measurements

The fracture mechanics technique developed to measure the delamination energy of SC tissue as a function of hydration, temperature and delipidization with varying tissue depth has been previously described [8,9,11]. Briefly, the thin SC tissue was adhered with a cyanoacrylate adhesive (Instant Krazy Glue © Gel, Elmer’s Products Inc., Columbus, Ohio) between two elastic substrates of polycarbonate (Hyzod® GP, Sheffield Plastics Inc., Columbus, Ohio) to form a double-cantilever beam (DCB) fracture mechanics specimen. The substrate dimensions of 40 mm x 10 mm x 2.88 mm were selected to ensure elastic deformation and the valid application of linear elastic fracture mechanics.

The specimens were mounted at the SC-free end via loading tabs in an adhesion test system (Delaminator Test System, DTS Company, Menlo Park, CA) with a computer controlled DC servoelectric actuator operated in displacement
control. The adhesion test apparatus and DCB specimens were placed in an environmental chamber (Model LH-6, Associated Environmental Systems, Ayer, Massachusetts or Model ZH-16-2-H/WC, Cincinatti Sub Zero [CSZ], Cincinatti, Ohio) to control the test environment. Specimens were loaded in a purely tensile, Mode I, fashion, and tested using 2 μm/s displacement rates during delamination extension with corresponding loads measured with a 222 N load cell. Periodic measurement of the critical load, $P_c$, and the delamination length, $a$, at incipient delamination extension were used to determine the delamination energy, $G_c$, from critical values of the strain energy release rate, $G$, as described elsewhere [13,14]. Four DCB specimens were tested for specimen exposed to the enzyme treatment or control treatment for 6 hours with each test yielding ~ 8 - 12 delamination energy values as the debond propagated along the length of the specimen. Reported delamination energy, $G_c$, values were obtained from averaging the individual data values for a given test or tissue condition. To measure delamination energy values through the SC thickness, single DCB specimens were tested as described below, and the 8 - 12 measured delamination energy values were averaged.

7.3.5 Graded delamination energy measurements

To measure delamination energies through the thickness of the SC, successive DCB specimens were prepared from completely separated specimens by adhering new polycarbonate substrates onto each side of the previously tested specimen as described elsewhere [11]. DCB specimens were fabricated and tested as described above. After testing the two new specimens, the substrates from the original delamination specimen would again be adhered to another set of new substrates to delaminate the remaining SC layer. In this manner the delamination properties of the SC were probed as a function of tissue depth.

7.3.6 Statistical analysis

Delamination energies measured as a function of conditioning are presented as mean values ± 1.96 x the standard error of the mean (STDEM) in
which the mean values reported are expected to fall within these bounds with 95% confidence. On average, \( n = 46 \) for each test condition. Delamination energies were compared using the Wilcoxon signed-ranks test for independent samples. The confidence interval was set at 95%. Delamination energies for graded properties measurements are presented as mean ± standard deviation (SD) with \( n = 16 \) for each test condition. The surface corneodesmosome density values are presented as mean ± standard deviation (SD) with \( n = 4 \) for each test condition.

![Graph](image)

**Figure 7.1** The activity of enzyme containing buffer solution with respect to the activity of buffer solution (control) is plotted as a function of incubation time. The enzyme activity is significantly higher than the buffer solution activity at the onset of tissue exposure to SCCE.

### 7.4 RESULTS

#### 7.4.1 Enzyme activity variation with incubation time

The activity of enzyme containing buffer solution with respect to the activity of buffer solution (control) is plotted as a function of incubation time in
Figure 7.1. The SC was present in the enzyme containing buffer solution. At the onset of exposure to the tissue, the activity of the enzyme containing buffer solution was significantly higher than the activity of the control solution demonstrating that enzyme was present in the buffer solution. After exposure to the tissue, the enzyme activity began to decrease, and the ratio of the activities approached one after ~ 3 hours of incubation.

![Graph showing delamination energy](image)

**Figure 7.2** The intercellular SC delamination energy, $G_c$, of the tissue exposed to the enzyme treatment. After exposure to rhk7 enzyme, the intercellular delamination energy is observed to decrease from $\sim 6 \text{ J/m}^2$ to $\sim 2 \text{ J/m}^2$. (For comparison to untreated SC: $p < 0.01$) Error bars: mean +/- 1.96 x STDEM.

### 7.4.2 Effect of enzyme induced corneodesmosome degradation on delamination

The intercellular SC delamination energy, $G_c$, of the tissue exposed to the enzyme treatment and the control treatment is shown in Figure 7.2. These data are from a single donor in directly adjacent locations from the SC tissue sample to mitigate tissue specimen variability. After being subjected to the rhk7 enzyme,
the delamination energy was observed to decrease markedly from \(~ 6 \text{ J/m}^2\) in the control to \(~ 2 \text{ J/m}^2\) after enzyme treatment.

![Diagram of SC layers and delaminations](image)

(a)

![Graph showing delamination energy](image)

(b)

**Figure 7.3** Intercellular delamination energy was measured after successive delaminations a) into the SC layer as indicated schematically, and b) showing the significant decrease in delamination energy values as a function of depth into the SC layer following enzyme treatment. Error bars: mean ± SD. Comparison between control and enzyme for the inner surface: \(p < 0.01\) for 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} delaminations.
7.4.3 Graded delamination energy values

Intercellular delamination energy values measured after repeated testing and specimen preparation using the original substrates and remaining SC layers are shown as a function of the delamination number in Figure 7.3. A schematic of the SC layer and approximate location of the repeated delaminations into the depth of the tissue are shown in Figure 7.3(a). Four delaminations were performed on each side of the SC following the initial delamination, although only 4 are shown as a function of depth into the SC tissue in Figure 7.3(b). Similar to results from the tissue sample shown in Figure 7.2, the first delamination energy measured was significantly lower in the case of the enzyme treated tissue compared to the control. For both enzyme treated and control treated SC, Gc values increased relatively slowly for the first two consecutive delaminations on the inner SC layer with the Gc values for the treated tissue consistently lower compared to the control tissue. Gc values began to increase significantly for the third and subsequent delaminations. On the other hand, after the initial delamination, Gc values increased significantly for the outer SC tissue indicating the presence of the cyanoacrylate adhesive. For the final fifth delaminations, both inner and outer Gc values began to converge on the adhesion energy value of the cyanoacrylate adhesive of ~ 325 J/m², indicating no remaining tissue present in the specimens.

7.4.4 Characterization of enzyme-induced corneodesmosome degradation

After exposure to the rhk7 enzyme for varying incubation times, the SC was characterized using TEM. Both intracorneocyte and intercorneocyte corneodesmosomes were clearly visible at corneocyte boundaries where they appeared as dark phases when fully intact as shown in Figure 7.4(a) and (d). Degradation of the corneodesmosomes following rhk7 enzyme exposure was also clearly apparent in the TEM images as has been previously reported [4,15, 16,17,18]. Degraded corneodesmosomes appeared to have light interiors associated with reduced electron scattering in the lower density degraded interior regions (Figure 7.4(b) and (c)). Such degradation was already apparent after 1.5
hours of exposure and confined to the intercorneocyte layer corneodesmosomes. No degradation was observed in intracorneocyte corneodesmosomes which remained intact in the interdigitated regions of neighboring corneocytes (Figure 7.4(d)). It was not possible in the present study to determine the exact extent of degradation. Rather, the resulting surface densities obtained from the micrographs of corneodesmosomes that appeared fully intact and those that were partially degraded was determined and is presented in Figure 7.5 for the enzyme and control treated tissues. Enzymatic treatment significantly increased the density of partially degraded corneodesmosomes from 0.056 corneodesmosomes/μm² to 0.147 corneodesmosomes/μm² while significantly decreasing the density of intact corneodesmosomes with respect to the control.

Figure 7.4 TEM micrographs of the SC tissue clearly showing a) elongated corneocytes and both intercellular lipids and intact corneodesmosomes for the tissue exposed to the control solution, partially degraded (PD) intercorneocyte corneodesmosomes following b) 3 hours, and c) 6 hours of enzyme exposure. Intracorneocyte corneodesmosomes were resistant to degradation for the rhk7 enzyme exposures employed and remained intact in the interdigitated regions of neighboring corneocytes within a layer as shown in d) following 3 hours.
Figure 7.5 Measured surface density of intact and partially degraded corneodesmosomes for SC exposed to rhk7 enzyme for 6 hours. After exposure, surface density of partially degraded corneodesmosomes increased significantly while the density of intact corneodesmosomes decreased. \( p < 0.01 \) for all conditions. Error bars: mean +/- SD

7.5 DISCUSSION

Our previous research clearly identified that delamination of SC is intercellular and the removal of mobile lipids in SC increases the intercellular delamination energy as covalently bound lipids are brought into closer proximity [8,9,10,11]. It was also suggested that the increase in intercellular delamination energy, \( G_o \), with depth into the SC layer is related to the increasing density and integrity of the corneodesmosomes, widely noted in the literature as central to SC cohesion [3,4,19,20,21,22,23]. Extending our prior studies on understanding the role of SC components on the intercellular delamination energy of SC, we sought to understand if the activation or deactivation of exogenous enzymes and their
subsequent effect on corneodesmosome degradation dominated the change observed in intercellular delamination energy or if the changes were related to other changes in the SC tissue.

After exposure to the exogenous rhk7 enzyme, we observed that the $G_c$ value significantly decreased with respect to that of the control treated SC tissue. This decrease was postulated to result from the degradation of corneodesmosomes in the presence of the exogenous enzyme. The enzymatic degradation of corneodesmosomes weakens the strong corneocyte-corneocyte interaction in the SC microstructure making the tissue more susceptible to delamination fracture as shown in Figure 7.2. The drying of the tissue following control or enzyme exposure was carried out at a relative humidity level of 45% RH at which the actual moisture content of SC as measured with respect to SC dry weight was ~ 5% wt/wt to reduce the effect of tissue hydration on the delamination energy. SC hydration above values of ~ 60% RH conditioned tissue has been shown to decrease intercellular delamination energies [9,10].

By performing multiple delaminations on the enzyme treated and control treated SC specimens, we were able to probe quantitatively the variation in intercellular delamination energy as a function of depth in the SC layer as described elsewhere [11]. For the first three delaminations towards the inner layers of SC where there are no effects of the cyanoacrylate adhesive used in the specimen preparation (Figure 7.3(a)), $G_c$ values exhibited a marginal increase as the intercellular delamination path moved inwards and sampled an increasing density of corneodesmosomes at the corneocyte boundaries. The enzyme treated SC exhibited consistently lower $G_c$ values with increasing depth into the tissue compared to the control treated tissue. This implies that the rhk7 enzyme treatment we employed and associated corneodesmosome degradation has a direct and measurable effect on lowering the intercellular delamination energy for multiple corneocyte layers into the SC. After the three delaminations, there was a significant increase in the $G_c$ of both enzyme treated and control specimens suggesting a transition from intercellular delamination to combined intercellular and cohesive delamination of the individual corneocytes and the first limited
presence of the cyanoacrylate adhesive in the delamination path as previously reported [11].

For the outer surface, the $G_c$ values for successive delaminations after the first delamination of the enzyme treated tissue was significantly higher with respect to that of the control. This suggests that there were fewer layers of corneocytes left on the outer surface of the enzyme treated tissue after the first delamination. As in previous studies [11], the significant increase in $G_c$ values for subsequent delaminations on the outer SC layers is associated with the effect of the cyanoacrylate adhesive that increasingly becomes apparent on the delamination path. Final delaminations on both sides exhibited $G_c$ values > 100 J/m² due to the cyanoacrylate adhesive on the delamination path (not shown in the figures). Delamination energies measured for DCB specimens containing only cyanoacrylate adhesive yielded $G_c$ values of ~ 325 J/m².

The TEM observations clearly revealed a significant increase in the level of partial degradation of corneodesmosomes for the enzyme treated tissue at varying incubation times (1.5 to 6 hours) with respect to that of the control treated tissue (Figure 7.4(a-d)). Whole corneodesmosomes are extensively cross-linked into the cornified envelope of corneocytes holding layers of corneocytes and the corneocytes within a single layer intact. As these linkages are emptied out by enzymatic degradation, the level of corneodesmosomal degradation dictates how easily the tissue will delaminate. This mechanism is very similar to the desquamation mechanism of SC where corneodesmosomal retention as a result of decreasing enzymatic activity can prevent both peripheral and non-peripheral corneocytes from detaching during desquamation. Thus, large clumps of corneocytes can accumulate on the surface of the SC [24]. The degraded corneodesmosomes were within neighboring corneocyte layers at all incubation times for enzyme treated tissue. Corneodesmosomes were still intact in interdigitated regions of neighboring corneocytes (Figure 7.4(e)) suggesting that the enzyme dosage employed in this study was not high enough to degrade the intracorneocyte layer corneodesmosomes.
7.6 CONCLUSION

We report on the effects of corneodesmosome degradation using a chymotryptic enzyme on the intercellular delamination of human stratum corneum. A direct and quantitative connection between intercellular delamination energy and corneodesmosome degradation was observed. The intercellular delamination energy of SC tissue was observed to decrease from \(~ 6 \text{ J/m}^2\) to \(~ 2 \text{ J/m}^2\) after exposure to rhK7 enzyme. TEM studies on enzyme treated SC revealed a significant increase in the partially degraded corneodesmosome density of the tissue with respect to that of the tissue exposed to a control solution. Both the results of mechanical testing and TEM studies on enzyme treated SC suggest a strong link between the intercellular delamination energy of SC and its corneodesmosome density. Accelerated degradation of corneodesmosomes in the presence of rhK7 enzyme lowered the density of intact corneodesmosomes making SC more susceptible to delamination. We believe that this represents the first direct quantitative demonstration of the effects of corneodesmosome degradation on the delamination properties of human SC.

7.7 ACKNOWLEDGEMENTS

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7.8 REFERENCES


Chapter 8 Conclusions

8.1 Introduction

The research described in this dissertation involves synergistic experimental and mechanics modeling efforts to provide a fundamental scientific and clinically relevant basis from which cracking and chapping associated with dry skin conditions in human stratum corneum (SC) can be quantitatively characterized and understood. We describe a mechanics approach to characterize both the driving force for dry skin damage, together with the resistance to the principal intercellular delamination processes. We describe environmental, biological and chemical treatments to systematically manipulate and influence components of the SC tissue including intercellular lipids, corneodesmosomes, and intracellular keratin. Taken together, this research represents a new and unique approach to characterize and model the fundamental biomechanics of human SC and skin damage processes. The major results from each experimental chapter are summarized below and possible areas of future work are discussed.

8.2 Summary of Results

Dry skin is one of the most ubiquitous and chronic skin conditions that generally result from the variable humidity and temperature conditions to which our skins are exposed. The inevitable results of these exposures are “tightness” of the skin and skin damage in the form of chapping and cracking. The skin tightness is directly related to contraction of the SC and the buildup of tensile residual stresses in the SC layer. These are referred to as SC drying stresses. In Chapter 4, we explained the use of the substrate curvature technique to characterize these stresses and reported extensively on the evolution of these stresses in SC in relation to the effects of hydration and damage caused by chemical treatments on the underlying SC structure. The SC drying stresses, ranging from 0 to 3.2 MPa, were found to be very sensitive to the relative humidity and temperature of the drying environment as well as harshness of the
chemical treatment. A strong correlation was found between the SC drying stress and the chemical potential of water in the drying environment. We demonstrated that the SC drying stress is mainly related to the reduction of the volume occupied by water regardless of the bound states of water in the tissue.

Then, we used a fracture mechanics approach to understand the implications of the drying stresses in SC as a mechanical driving force for damage propagation (e.g. cracking and chapping) in the tissue. The crack driving force $G$ was found for several cracking configurations (peeling, channel cracking, delamination, etc) and compared with the intercellular delamination energy, $G_c$, which is a property of the tissue provides a measure of the resistance to cracking. Finally, we developed a biomechanics framework in which skin stress and skin damage processes can be predicted and modeled in terms of environmental exposure, tissue conditioning, and treatment.

In Chapter 5, using the substrate curvature method, we have evaluated the effect of DO and NDO coatings on SC drying stresses. Interestingly, both coatings were observed to be equally effective in reducing SC drying stresses and maintaining higher moisture content in the tissue after the stresses in the coating and the SC were differentiated. The methodology presented in this study provided direct opportunities for studying the effects of moisturizing treatments and occlusive barrier films used to control water loss on SC and alleviate the propensity for dry skin damage.

In Chapter 6, we have evaluated the effect of a wide variety of moisturizing molecules on SC drying stresses. These stresses were observed to strongly depend on the physical and chemical characteristics of the moisturizing molecules and their influence on the underlying SC structure such as extraction of the SC lipids and changes in lipid fluidity. In general, occlusive and emollient molecules providing an exogenous hydrophobic barrier to water loss while enhancing the softness and flexibility of skin were observed to reduce drying stresses in SC more effectively than humectant molecules such as glycerol which promote water retention within the SC. For ester based emollients, a potential correlation was suggested between the sensory profiles of these molecules and their resulting drying stresses. Finally, we considered the biomechanics model described in
Chapter 4 to model the effectiveness of moisturizing treatments in alleviating dry skin damage.

Chapter 7 is included in the thesis to strengthen our understanding of how SC intercellular delamination is a property of the tissue that provides a measure of the resistance to shedding or peeling of the cell layers (desquamation). We used a chymotryptic enzyme, rhK7, known to have an active role in the desquamation of SC by degrading the linkages between corneocytes, corneodesmosomes, and reported the effects of corneodesmosome degradation on the intercellular delamination of human stratum corneum. A direct and quantitative connection between intercellular delamination energy and corneodesmosome degradation was observed. The intercellular delamination energy of SC tissue was observed to decrease from ~ 6 J/m² to ~ 2 J/m² after exposure to rhK7 enzyme. TEM studies on enzyme treated SC revealed a significant increase in the partially degraded corneodesmosome density of the tissue with respect to that of the tissue exposed to a control solution. Both the results of mechanical testing and TEM studies on enzyme treated SC suggest a strong link between the intercellular delamination energy of SC and its corneodesmosome density. Accelerated degradation of corneodesmosomes in the presence of rhK7 enzyme lowered the density of intact corneodesmosomes making SC more susceptible to delamination. This research presented the first direct quantitative demonstration of the effects of corneodesmosome degradation on the delamination properties of human SC.

8.3 Future Work

As already stated, the mechanical driving force for thin-film cracking associated with elastic stresses in the SC layer is described by a relationship of the form shown in Figure 1.2. We note that for the relatively low strains, hydration conditions and time scales important for SC cracking the tissue may be described as linear elastic. However, to be precise, the thin film mechanics framework for treating nonlinear aspects of the residual stress and fracture of viscoelastic films
and/or substrates should be adapted and applied depending on the extent of viscous relaxation present.

Residual drying stresses and their time dependency in the SC layer should also be measured using a range of thin-film constraint conditions. In the case of substrate curvature techniques, the substrate behaves in a linear elastic fashion and only thermal expansion mismatch between the substrate and SC layer and SC drying strains contribute to the drying stress achieved. By varying the thermal expansion of the substrate material, different levels of constraint may be achieved and characterized as a function of time. In the case of free-standing film techniques, the level and nature of the constraint can be independently prescribed.

In the future program, the level of stress relaxation that may occur under the different constraint conditions should also be considered. An indication of these has already been addressed in our prior SC research where the in-plane creep and stress relaxation processes were characterized under selected tissue hydration conditions. This will provide important information on the extent of relaxation that may occur during the course of the drying process and the time scales associated with the onset and nature of damage. Then, the detailed cracking configuration and possible “crack length” dependencies can be incorporated into the non-dimensional parameter Z (Figure 1.2).

With the inclusion of these details, critical conditions for the onset of damage and cracking may well include rate or time dependencies that determine when the cracking condition is met. Such dependencies relate not only to the magnitude of the crack driving force, G, but also to the resistance to cracking G_c. There are direct analogies in the literature to a range of rate dependent bridging mechanics models for cracking which contain a wide range of time-dependent variables including e.g. loading conditions, material properties and fluid viscosity.

Other central issues that will need to be resolved in a future program relate to the detailed nature of the parameters contained on the left hand side of the relationship in Figure 1.2 and their dependence on SC components. In this dissertation, we demonstrated that the SC drying stress is mainly related to the
reduction of the volume occupied by water regardless of the bound states of water in the tissue suggesting that the contribution of the reorientation of proteins which have commonly been thought provide a significant contribution to the drying strains and stresses may in fact be small. This will have to be further investigated in future work. Of the SC components, intracellular keratin and the cellular protein envelope are likely to have the largest effect on the driving force for cracking due to their expected effects on both the SC residual drying stress and elastic modulus. From a mechanics perspective, the total volume fraction of intercellular components (lipids and corneodesmosomes) is limited and cannot have a dominant effect on SC elastic and residual stress values. Our current SC research has reached similar conclusions with respect to our in-plane stress and elastic property measurements. However, future staff nevertheless be interested in all potential SC component contributions to elastic behavior and mechanical driving forces.
A1 Isolation of Stratum Corneum from Full Thickness Human Skin

A1.1 Isolation of Dermis from Full Thickness Skin

1. Line a large tray first with aluminum foil, then plastic wrap. Fill a plastic cylindrical container with ice to keep skin cool during fat removal.
2. Place ice in small pile on tray then place cylinder on it so that it does not roll around. Place skin over the cold cylinder and ice.
3. Trim the excess fatty tissue from the skin with durable scissors until the dermis is cleared of fat.
4. Rinse skin by dipping in a 400 mL beaker containing deionized (DI) water at room temperature. Repeat this procedure until water is clear of debris.
5. Transfer skin to a 1 L beaker containing DI water at 37°C and allow skin to soak 5 – 10 min.
6. Transfer the skin to a 2 L beaker containing DI water at 62°C. The weight of water should be approximately 6 times larger than the weight of the tissue. Within 5 seconds the temperature of the water should drop to 60°C. Stir the tissue with the observation thermometer for exactly one minute at 60°C.
7. To remove fat deposited over the stratum corneum, place skin over a tray lined with aluminum foil and plastic wrap then filter paper. Smooth the skin over the filter paper dermis side down. Place the tray at ~ 15° angle into a sink and pour water heated at 45°C, from top to bottom. Repeat rinsing procedure twice more.

A1.2 Isolation of Epidermis from Dermis

1. Place tissue, dermis side down, over a piece of rectangular filter paper which has been lined below with a piece of plastic wrap. During this step, it might be a good idea to take note of which side is the outer stratum corneum surface.
2. Holding the edge of the dermis down with a pair of flat tipped tweezers, the epidermis is pushed away with the edge of a round tipped spatula, moving a few millimeters back and forth, from side to side until all the epidermis has been separated.

3. The epidermis, with the stratum corneum side up, is then transferred to a glass tray about 9” x 14” x 2”, ¾ full with DI water. The floating epidermis is gently moved with the spatula until it stretches out with no wrinkles.

4. The surface of the floating epidermis is rinsed with DI water twice. Then, the floating epidermis is lifted out with a piece of filter paper and the moist filter paper / epidermis is placed in a glass tray until use.

5. If the epidermis is not going to be used immediately, the glass tray is sealed tightly with plastic wrap and stored at 4°C.

A1.3 ISOLATION OF STRATUM CORNEUM FROM EPIDERMIS

1. The filter paper holding the epidermis is placed in a glass tray over a double layer of filter paper soaked with a solution of 0.1% trypsin (weight percent) in 0.05 M Tris Buffer at pH 7.9. The tray is covered with plastic wrap and the epidermis allowed to incubate for 2 hours at 35°C in an oven. It is important to take note of the orientation of the stratum corneum to know which side is the outer surface.

2. The top filter paper and the treated epidermis are then transferred to a tray filled with DI water and the floating stratum corneum is moved gently with Q-tips to lose any remaining soft tissue. If necessary, the incubation is repeated until a clear sheet of stratum corneum is obtained.

3. The stratum corneum is then thoroughly rinsed in 3 – 4 trays of DI water and then lifted out with filter paper and placed in a clean glass tray for drying.

4. Once the stratum corneum is sufficiently dry (not completely dry), make sure to remove it from the filter paper so that it is not stuck to the filter paper. Placing one edge of the stratum corneum along the length of a glass stir bar is an effective way to controllably remove the stratum corneum from the filter paper. Take care
to not allow the (still damp) stratum corneum to contact itself as it adheres quite well to itself. If it does stick to itself, the tissue can be rewetted and allowed to dry again.

5. After drying place the tissue on a clean tray and store it at room temperature in a dry environment.

Note: The procedure used to isolate the SC is widely used in the literature and is based on the protocol suggested by Kligmann and Christophers in 1963. Following the trypsin digest at 35°C for 135 min, isolated SC was rinsed with room temperature distilled water five times and then allowed to dry on filter paper. Thus, the isolated SC should be clear of trypsin after the rinse. Also note that the trypsin causes removal of the “living” cells (uncornified cells) of the full thickness epidermis. It does not alter the structure of the cornified layer (SC). Different methods can be used to isolate the SC from the underlying tissues. For example, there is a mechanical method which involves repetitive stretching of the skin. This procedure is not widely used because it causes significant alteration of the SC.