Biology of Stratum Corneum: Tape Stripping and Protein Quantification

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40 Biology of Stratum Corneum: Tape Stripping and Protein Quantification

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Introduction

Stratum corneum (SC) adhesive tape stripping has been utilized in the measurement of stratum corneum mass, barrier function, drug reservoir, and percutaneous penetration of topical substances. The process involves a methodical, relatively noninvasive layer-by-layer removal of the SC, which comprises the outermost epidermal cell layers. Complete SC removal may require over 70 tape strips [1, 2]. The quantity of SC harvested diminishes with each sequential strip, possibly due to increased SC cohesiveness in deeper layers. Thus, the mass of any single strip depends on the mass removed by the prior strip [3]. SC removal may rely on the interaction between the adhesive stripping force and the cohesive intercellular force [3].

Tape Stripping Studies

Tape stripping was first devised in the 1940s, and examined by Pinkus in 1951. Pinkus demonstrated a remarkable burst of mitotic epidermal activity post-stripping, concluding that the lost horny layer is replaced by basal mitotic division [4]. The degree of hyperplasia correlates with the level and duration of barrier disruption [5]. Nevertheless, mitotic rate may remain five times greater than baseline six days after stripping [6]. Keratinocyte hyperproliferation may be a response to water barrier disruption or cytokine release secondary to epidermal injury [5, 6]. Adhesive stripping increases: epidermal lipid synthesis, lamellar body production/secretion in the stratum granulosum, epidermal DNA synthesis, epidermal cytokine production, dermal inflammation, and presence of TNF and IL-1α in skin [6]. Conversely, occlusion of stripped human skin via adhesive application suppresses mitotic activity; adhesive occlusion may provide artificial restoration of the lost barrier [6]. Similar experiments in mice do not support these findings [6].

The SC is essential to life, protecting the human body from desiccation and external penetration of deleterious agents. The SC is composed of a nucleated, keratin-rich corneocytes embedded in an extracellular multilamellar lipid matrix organized into membrane-like bilayers; intercorneocyte communication occurs through desmosomes [7]. Many other SC models exist, but none of them fully integrate all aspects of the skin barrier function. The SC is thin, less than 20 μm thick, and composed of about 10–15 tightly stacked layers, depending on the location [8]. Ceramides, cholesterol, and free fatty acids comprise the lipid matrix of the SC, providing invaluable roles in the barrier structure and function [7]. Their synthesis is required for barrier homeostasis; as with DNA, a burst of lipid synthesis (due to synthesis of their rate-limiting enzymes) occurs following barrier perturbation [9]. Lipid levels decrease in aged human skin, possibly due to SC pH increases and subsequent lipid processing impairment; this is described further, below [7].

The SC provides the rate determining step for the passage of most molecules across skin [10]. Therefore, topical agent concentration within the SC is directly related to that in the epidermis and dermis, the typical target sites. Additionally, corneocytes and intercellular lipids are responsible for preventing insensible water loss [11]. The transepidermal water loss can be measured with an evaporimeter, and frequently used to assess skin barrier integrity [11]. Anatomically, regional SC variations in percutaneous drug absorption, lipid composition, TEWL measurements, mean thickness, and number of cell layers have been described. Despite its structural heterogeneity, each layer of SC equally contributes in preventing water loss [11]. In doing so, the SC behaves as a membrane compatible with Fick's laws of passive diffusion [11].

TEWL increase as a function of tape strip number depends on the factors including: anatomical site, pressure, pressure duration, and tape removal rate [12]. Loffler et al. demonstrated that TEWL increased fastest on the forehead, followed by the back, and finally, the forearm [12]. These findings may be explained by the differences in SC thickness, differences in spontaneous desquamation (SC cohesion), and pressure resistance because of inherent viscoelasticity and type of tissue underlying the skin [12]. Rapid removal (vs. slow),

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shorter pressure duration (2 vs. 10 s), and higher pressure (330 g cm^{-2} vs. 165 cm^{-2}) all produced earlier TEWL increases [12].

A similar study by Breternitz et al. revealed the highest rise of TEWL on the cheek, compared to the back, upper arm, and forearm [13]. Interestingly, the cheek also demonstrated the greatest increase in SC hydration after stripping [13]. Breternitz et al. further established greater, earlier TEWL increase with higher pressure (7 N stamp vs. 2 N) and longer application (10 vs. 2 s) [13]. Moreover, using the thumb, stretching the skin, and utilizing a roller or stamp all result in varying quantities of harvested SC [13]. The use of thumb removed most of SC and produced the highest TEWL, even when compared with usage of a roller or skin stretching [13]. Occlusion of the test site prior to the stripping procedure resulted in higher TEWL values [13]. Occlusion results in water retention and degradation of the intercellular proteins [13]. In conclusion, reliable, reproducible results depend on standardization of the aforementioned variables.

Kalia et al. found that initial tape strips removed thicker layers of the SC, relating this to decreased number of desmosomes closer to the skin surface [10]. Kalia et al. demonstrated decreased impedance with increasing depth achieved, theorizing that removal of the upper corneocyte layers and lipid matrix diminishes structural opposition to ion flow, facilitating ion transport [10]. In addition, TEWL increased disproportionally with later tape strips; removing only the upper SC layers was insufficient to significantly enhance the water loss [10]. Removal of 6–8 μm of SC (deeper layers) typically resulted in significant TEWL increases [10]. Removal of the outermost layers affected impedance more than TEWL, with a 40% decrease in impedance after removal of only 4 μm of SC. Nonetheless, a correlation between TEWL increase and impedance decrease was observed. Upon completion of the tape stripping experiment, full return to the basal values of impedance occurred after 3 days, while TEWL recovery time was 5–6 days [10]. External layers, more crucial in impedance, are formed prior to deep compact layers [10].

The aforementioned findings suggest a gradation in water-regulating ability within the SC, with the deepest layers most responsible for controlling water flux [10]. However, via simple mathematical deduction, these results, in fact, support a Fickian model [10]. Though structurally heterogeneous and complex, the SC behaves as a homogenous barrier to water in vivo [10]. The water transport route may be homogeneous throughout SC, with each layer contributing equally to the barrier [10]. The best fit curve plotting experimental values of TEWL as a function of tape stripping frequency closely resembled a theoretical curve based on Fick's first law of diffusion [14]. The first half of the theoretical curve fit the actual curve; in the second half, experimental data show slightly higher TEWL values than Fick's theoretical values [14]. The authors of the study offer plausible explanations for this discrepancy [14].

In contrast to most studies, Schwindt et al. demonstrated that quantity of harvested SC was constant with each strip in a given anatomical site and volunteer [11]. Schwindt et al. found a linear relationship (in all anatomical sites) between 1/TEWL and the total mass of removed SC, further establishing that the SC acts as a Fickian membrane for steady state water diffusion [11]. It also appears that intercellular lipids, not corneocytes, are the determining factor for SC water diffusion [11]. This linear relationship was also described by another group, plotting 1/TEWL as a function of SC thickness (13 subjects examined) [15]. *Table 40.1* summarizes the results from three studies quantifying SC thickness.

Tape construction influences outcome [13]. Three brands of adhesive tapes, utilized in vivo, displayed statistically equivalent mean water diffusion coefficients, SC permeability, and SC mass/thickness removal [3]. After 40 strips, however, a proprietary adhesive stripped the most, while a rayon adhesive stripped the least [3]. TEWL increased significantly as deeper SC layers were reached with proprietary and polyethylene adhesives, but not with rayon tape [3]. Tape properties, subject properties, or a combination may account for variation

Table 40.1

Calculations of SC thickness in vivo in man

Authors	No. of subjects	Anatomical site	No. of strips	Mean total SC thickness (μm)
Kalia et al. [10]	3	Forearm	22–28	12.7 ± 3.3
Schwindt et al. [11]	6	Lower back	Up to 35	11.2
		Abdomen	Up to 35	7.7
		Thigh	Up to 35	13.1
		Forearm (ventral)	Up to 35	12.3 ± 3.5
Pirot et al. [15]	13	Forearm (ventral)	15	12.6 ± 5.3

Thickness appears to be a function of anatomical site

in barrier disruptive properties. Variation may also be accounted for by unique adhesive systems; adhesives of different tape brands may bind similarly to cellular SC, but differently to extracellular components of the SC barrier. These extracellular components (e.g., free fatty acids, ceramides, and lipids) are essential to barrier function. Furthermore, apparently 5–7 μm of SC removal resulted in significant TEWL elevations, a depth unobtainable by the rayon tape (*Table 40.2*) [3]. This implies that structural elements of the water barrier may not be homogeneously distributed. In some subjects, neither the proprietary adhesive nor the polyethylene adhesive disrupted the water barrier; these individuals experienced no barrier disruption at any of six tested sites, suggesting variation of water barrier disruption to be a function of the individual.

Demonstrating that removal of the same amount of SC from different individuals does not result in similar increases in TEWL, Kalia et al. asked whether this variation was secondary to inter-individual differences in intact membrane thickness [16]. Kalia et al. demonstrated that once inter-individual differences in the thickness of the intact SC are corrected for (by normalizing the SC thickness removed with respect to calculated total SC thickness), the same degree of barrier disruption induces the same increase in TEWL in each individual [16]. Stated differently, removal of the same percentage of SC in two individuals results in equivalent barrier disruption. TEWL rises considerably only after about 75% of the SC has been removed, presenting a very consistent barrier to water loss in the healthy human population [16].

Table 40.2

Relationship between protein removal and TEWL, from Bashir et al. [3]

Tape type	Location (forearm)	No. of strips	Mean thickness removed (μg)	TEWL (g m^{-1} h^{-1})
Proprietary	Dorsal	40	8.10	30.33
	Ventral	40	5.83	30.80
Polyethylene	Dorsal	40	7.25	31.98
	Ventral	40	4.96	30.83
Rayon	Dorsal	40	4.99	13.4
	Ventral	40	2.99	11.95

Note, there are significant differences in TEWL and mean thickness removed depending on tape construction. The dorsal forearm, in all cases, had greater SC thickness removed than the ventral forearm

Tape Stripping and Aging

Aged skin demonstrates increased susceptibility to the xerosis, exogenous, and environmental insults, and diminished ability to recover from these insults, indicating a suboptimal epidermal barrier. It is believed that no definitive studies have compared aged vs. normal SC thickness; nonetheless, some authors believe aged SC to be thicker, with decreased lipid content and deficient water-binding capacity [8]. TEWL is decreased in the aged, as is topical absorption [8]. The aging barrier was elegantly examined by Ghadially et al.; results are summarized below.

Aged humans (>80 years) have prolonged barrier recovery rates after tape stripping or acetone application compared to control subjects (20–30 years) [17]. 24 h after acetone treatment, 50% recovery occurred in control subjects compared to 15% in aged subjects [17]. Photoaging, in combination with this chronologic aging, may further delay recovery [9]. Furthermore, delays in SC lipid reappearance after barrier disruption have been described in aged murine epidermis [17].

Additionally, tape stripping studies have revealed decreased cohesiveness in aged skin [9]. In fact, barrier perturbation (TEWL $\geq$20 g m^{-2} h^{-1}) occurred after 18 $\pm$ 2 strippings in aged skin versus 31 $\pm$ 5 strippings in control skin [17]. Fortunately, topical lipid formulations, containing predominantly cholesterol, may accelerate barrier recovery in aged human skin [18].

The above findings may be explained by reduced delivery of secreted lipids to the epidermal surface in the elderly. There is a global diminution ($\approx$30%) of ceramide, cholesterol, and free fatty acid contents in the aged murine skin [17]. This reduction could be due to the decreased production and/or increased destruction; cytokines (e.g., IL-1α) and growth factors may play a role [9]. Additionally, decreased secretion of lamellar body contents (at stratum granulosum-stratum corneum interface) with fewer extracellular lamellar bilayers (at stratum corneum interstices) contributes to a more porous extracellular SC matrix [17].

Ghadially et al. further examined the effect of lipids on SC barrier function [19]. As described previously, SC of aged mice displays decreased lipid content and extracellular bilayers. This may result in impaired barrier recovery after a tape stripping insult (18.7 vs. 60.8% recovery by 24 h in aged vs. young mice). Upon further examination, Ghadially et al. determined that cholesterol synthesis is decreased significantly under basal conditions. Furthermore, sterologenesis fails to reach absolute levels obtained in young epidermis following tape stripping perturbation.

A 40% decrease in activity of HMG-CoA reductase, the rate-limiting enzyme in sterologenesis, was observed under basal conditions in aged mice. Despite a greater than 100% increase in HMG-CoA reductase activity after barrier perturbation in aged mice, absolute levels did not attain those reached in treated, young epidermis.

Ghadially et al. also supplemented aged murine SC with an equimolar mixture of SC physiological lipids (cholesterol:ceramide:linoleic acid:palmitic acid) or cholesterol alone [19]. Either mixture applied once enhanced the recovery after barrier disruption. Additionally, after four applications of either mixture, electron microscopy demonstrated repletion of extracellular spaces with normal lamellar bilayer structures.

Further work examining the role of aging on the SC remains to be done. Tape stripping and TEWL studies of aged skin are currently underway.

Protein Quantification

After harvesting of SC onto adhesives is complete, protein can be measured via several methods. For decades, weighing (gravimetry) was the preferred method, despite its inherent inconvenience (weighing before and after stripping under constant hydration conditions). Additionally, results were subjected to inflation secondary to absorption of exogenous (topically applied) or endogenous (sebum, sweat, and interstitial fluid) substances within the SC. Initial strips were most affected by this absorption.

One decade ago, a novel colorimetric method was developed and validated by Dreher et al. [20]. This colorimetric method relies on a protein assay similar to one developed by Lowry et al. over half a century ago. Lowry's method involved measurement of protein with a folin phenol reagent after alkaline copper treatment [21]. It was demonstrated to be simple, sensitive, specific, and easily adaptable to small scale analyses, making it suitable for measurement of miniscule absolute protein amounts [21]. Dreher's method relies on spectrophotometry and colorimetry, based on the calibration of stained SC proteins to the corneocyte mass [22]. Drawbacks include time-consuming preparation of tape strips with necessary destruction of the original strips.

The Bradford dye reaction, which relies on Coomassie Brilliant Blue G-250 dye, is similar to Dreher's method. The dye binds protein, resulting in ionic and hydrophobic reactions, with a spectral shift from reddish-brown to blue. Maximal absorption for the bound form of the dye is 595 nm, the optimal wavelength for colorimetric measurement once the reaction has occurred. Despite disadvantages (e.g., serial dilutions), it is a fast and generally reliable method for protein quantification.

Dreher's colorimetric method has been successfully adapted to 96-well microplates, effectively shortening analysis time [23]. Note that limited areas of adhesive tape are not predictive of SC removal on the entire tape [23]. Alternatively stated, SC distribution on tape is not homogeneous [23].

A pivotal study examined direct spectroscopic SC protein quantification via absorption in the visible range (595 and 600 nm), with and without staining of corneocyte aggregates, and the UV range (278 nm) [24]. Correlation coefficients R^2 were 0.71 and 0.74, respectively. The results demonstrated weak SC protein absorption with immense light scattering [24]. The Coomassie brilliant blue protein coloring did not increase light absorption by SC proteins, and thus, could not decrease the interference secondary to light scattering [24]. The absorption techniques utilized in this study cannot accurately predict corneocyte aggregate quantity.

Latter studies utilizing wavelengths of 430 nm have established optical spectroscopy in the visible range as a sensitive and reproducible method of protein quantification [2]. Absorbance in this range depends exclusively on quantity of corneocyte aggregates, and adequately reflects SC mass [2]. Corneocyte aggregates, adhering to tape strips, decrease transmission of visible light by scattering, reflection, and diffraction. The resulting pseudo-absorption has been successfully correlated with mass of removed SC particles [2]. Absorbance measurement allows facile determination of absolute mass from corneocyte aggregates harvested via tape stripping. Topically applied substances do not interfere with the spectroscopic measurements as they do with gravimetric measurements, explaining mass differences in the most superficial strips (when compared with gravimetry) [25].

Practically comparing spectrally measured quantity (absorbance) with corneocyte aggregate weight requires correction for: topical applications in upper SC layers, interstitial fluid in deeper SC layers, the "stack effect" which decreases absorbance, and the tape stripping procedure itself (e.g., nonhomogeneous removal of tape or incomplete tape contact with skin) [2]. Once these factors are corrected for (primarily by excluding analysis of the most superficial and deep strips), $R^2 = 0.93$, demonstrating proportionality between quantification methods [2].

A multicenter study involving 24 subjects found a correlation coefficient of $R^2 = 0.94$ when comparing UV/VIS spectroscopy (430 nm) with conventional weight determination [25]. Superficial (first five) and deep (19–23) strips were excluded on the basis of weight-enhancement;

application on an oil-water emulsion (part of the study) inflated superficial strip weight, and intrinsic interstitial fluid increased deep strip weight [25]. Nonhomogeneous strips and those subjected to handling errors were excluded [25]. Only 66% of total strips were utilized to determine the correlation coefficient [25]. Weigmann et al. explain that pseudo-absorption/weight correlation can be extrapolated to the deepest layers of the SC [25].

A recent study demonstrated strong correlation (R^2 = 0.92 and R^2 = 0.95) between pseudo-absorption at 430 nm and both protein absorption at 278 nm and absorption of Trypan blue-stained proteins at 652 nm [22]. However, protein absorption at 278 nm was characterized by a weak band, implying application limited to tape strips with high amounts of corneocytes [22]. Mass determination based on the UV absorption is further limited by the potential superpositioning of strong absorption bands from exogenous substances and/or tape components in the same spectral range. Unlike the previous study, correlation was described using all the tape strips (superficial and deep), regardless of adherent exogenous or endogenous components [22].

Lademann et al. tested an inexpensive, easily reproducible optical device ("corneocyte density analyzer"), based on a slide projector, which also measures corneocyte pseudo-absorption at 430 nm [1]. When compared with standard UV-visible spectrometric measurements, a correlation factor of R^2 = 0.95 was demonstrated [1]. The device may simplify calculation of removed SC, without messy chemistry or an expensive spectrometer; it includes a mechanical autofeed system, well suited for the handling of tape strips [1].

Colorimetric Bioassay of Keratolytic Efficacy

The desquamating effects of three keratolytics are presented in table-format (*Table 40.3*) using the data obtained from colorimetric protein assays described by Dreher et al. (mentioned above) [20]. The process begins with cutaneous application of the agent; the agent is placed on a patch and taped onto the subject's skin for a predetermined number of hours. After this period, placement and removal of tape strips (number varies by study) onto the site of topical treatment are performed. The assay involves immersion and shaking of SC adhering tapes in sodium hydroxide solution resulting in extraction of the soluble SC protein fraction. The solution, now containing SC protein, is neutralized with hydrogen chloride, as the assay is ineffective under strongly alkaline conditions. The protein assay is performed using the Bio-Rad Detergent Compatible Protein Assay Kit and following the prescribed microassay procedure. This assay is similar to the Lowry assay, and is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. Finally, absorbance at 750 nm is measured using a Hitachi U-2001 UV-vis Spectrophotometer. This method allows for quantification of microgram amounts of SC, diminishing confounding factors, namely vehicle and water uptake by the SC.

The protein measured using the assay described can be compared amongst groups, with statistical analysis allowing determination of strong and weak keratolytics. SC removal via tape stripping in treatment and control groups is attributable to keratolytic mechanisms, which loosen SC cohesion. The disintegrated SC is subsequently collected by the adhesive.

In the first keratolytic bioassay using this technique, salicylic acid was examined [26]. Keratolytic efficacy of salicylic acid was determined as a function of pH. The test preparations were: aqueous vehicle control of pH 7.4, 2% SA aqueous solution of pH 3.3, 2% SA aqueous solution of pH 3.3 with menthol, and 2% SA aqueous solution of pH 6.95 [26]. A statistically significant mass of SC was removed after 6 h and 20 tape strips in all three

Table 40.3

Studies using colorimetric protein assay to measure keratolytic potential

Authors	Drug	Result
Bashir et al. (2005)	Aqueous solution 2% Salicylic Acid – 3 formulations	Statistically significant mass of SC removed after 6 h and 20 tape strips in all three experimental groups (salicylic acid pH 3.3, salicylic acid pH 3.3 w/ menthol, salicylic acid pH 6.95) compared to vehicle, untreated, and untreated but occluded groups.
Waller et al. (2006)	Aqueous solutions of 0.05 % all-trans RA, 2% BPO, and 2% SA	Statistically significant mass of SC removed after 6 h and 25 tape strips in all three experimental groups compare to vehicle, untreated, and occluded groups. The first 10 tape strips from SA group removed more protein than the other groups; at 10–15 strips, treatments were comparable; at 16–25 strips, protein removed from BPO sites was greatest.

All agents tested demonstrated significant efficacy in SC removal. SA had superior superficial removal, while BP had superior deep removal

experimental groups compared to vehicle, untreated, and untreated but occluded groups [26]. However, after 10 strips, the SA pH 3.3 solution with menthol and the SA pH 6.95 solution removed significantly more SC than any other group, including the SA pH 3.3 solution [26]. These data suggest that a neutral preparation of SA results in a pronounced keratolytic effect. Moreover, the neutral preparation was associated with the least skin irritation among treatment groups [26]. This finding differs from that of a previous study, which demonstrated superior SA skin penetration in an acidic solution compared to neutral solution [27].

In the second bioassay using the aforementioned technique, salicylic acid, benzoyl peroxide (BPO), and retinoic acid were examined [28]. The test preparations were: 0.05% all-trans retinoic acid, 2% salicylic acid at pH 6.95, 2% BPO, vehicle, untreated skin, and occluded but untreated skin [28]. After 3 h of treatment, only BPO treatment removed significantly more SC on 25 strips than untreated skin, while the other treatments did not achieve statistical significance [28]. At 3 h, SA had greater SC amounts removed in the first 10 (superficial) strips, while deeper strips (11–25) demonstrated BPO to have the greatest SC removal [28].

Statistically significant masses of SC were removed after 6h and 25 tape strips in all three experimental groups when compared to vehicle, untreated, and occluded groups [28]. At 6 h, the first 10 tape strips from the SA group removed more protein than the other groups; at 10–15 strips, all treatments were comparable; at 16–25 strips, BPO removed the most protein [28].

These in vivo human results indicate that all treatments tested are effective keratolytics, which may account for their effectiveness against acne vulgaris. Furthermore, it appears that salicylic acid may be a more suitable treatment for mild, superficial acne while BPO may be optimal for deeper, inflammatory acne. BPO's ability to loosen SC at deeper levels complements its antimicrobial/anti-inflammatory properties, resulting in an effective anti-inflammatory agent for papulo-pustular acne. Additionally, BPO appears to be effective even with short-term administration. RA had inferior SC disruption at 3 h but significant disruption at 6 h, indicating time-dependent keratolytic effects, consistent with its complex nuclear receptor interactions and alteration of gene transcription.

Conclusion

Taken together, the SC is beginning to reveal some of its secrets. Much remains to be done.

Cross-references

- Corneocyte Size and Cell Renewal: Effects of Aging and Sex Harmones
- Stratum Corneum Cell Layers
- The Stratum Corneum and Aging

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