Visualization of the Lipid Barrier and Measurement of Lipid Pathlength in Human Stratum Corneum

Submitted: August 31, 2000; Accepted: April 23, 2001; Published: May 15, 2001.

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ABSTRACT Detailed models of solute transport through the stratum corneum (SC) require an interpretation of apparent bulk diffusion coefficients in terms of microscopic transport properties. Modern microscopy techniques provide a tool for evaluating one key property—lipid pathway tortuosity—in more detail than previously possible. Microscopic lipid pathway measurements on alkali expanded human SC stained with the lipid-soluble dyes methylene blue, Nile red, and oil red O are described. Brightfield, differential interference contrast, fluorescence, and laser scanning confocal optics were employed to obtain 2-dimensional (2-D) and 3-dimensional (3-D) images. The 2-D techniques clearly outlined the corneocytes. Confocal microscopy using Nile red yielded a well-delineated 3-D structure of expanded SC. Quantitative assessment of the 2-D images from a small number of expanded SC samples led to an average value of 3.7 for the ratio of the shortest lipid-continuous pathway to the width of the membrane. This was corrected for the effect of alkaline expansion to arrive at an average value of 12.7 for the same ratio prior to swelling.

KEYWORDS: Stratum Corneum, Alkaline Expansion, Microscopy, Lipid Pathlength, Tortuosity

INTRODUCTION

Since the elucidation of the biphasic structure of the stratum corneum (SC) (ie, flattened, proteinaceous corneocytes embedded within an ordered lipid matrix), much has been written about the implications of this structure for solute transport. It has long been known that the SC provides the skin's primary diffusion barrier (1). Correlations of skin permeability coefficients, k_p , versus physical

properties of a wide variety of permeants have shown that skin can be effectively modeled as a simple lipid barrier to compounds having at least moderate water and oil solubilities (2-6). In combination with the structural detail and evidence from electron microscopy (7) and other physical characterization techniques (8,9), this observation has led many researchers to conclude that the primary transport pathway for most materials traversing the SC is intercellular (6-8, 10). If this is true, it follows that the arrangement of the corneocytes within the lipid matrix is a key determinant of the skin's permeability, as it would influence the effective pathlength for diffusion. Related analyses of the impact of corneocyte shape on skin permeability have shown that shape and, by implication, stacking arrangement are important for compounds whose lipid/protein permeability ratio is greater than 1000 (2,11,12).

Stacking and layering of corneocytes have been studied by 2-dimensional (2-D) light microscopy, using the alkaline expansion technique to swell the tissues prior to analysis (13-20). Methylene blue, an absorbent dye, has most commonly been used as a lipid stain (13,18,19); however, phase contrast (15,18) and fluorescent staining (16) techniques have also been described. Alkaline expansion clarifies the corneocytes (13,14) and improves the visualization of the lipid layers, which are otherwise too closely spaced to be resolved. Swelling occurs largely in the apical-to-basal direction (21) and is likely to be confined to the corneocytes, as discussed in the Appendix. Corneocyte arrangement is maintained during swelling (13,14), as the corneocytes are

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fastened together by desmosomes, which restrict their movement (22,23). The same result is expected for mild tissue-handling procedures that do not disrupt desmosomes. Qualitative analysis of these earlier studies has established that corneocytes in rodent skin stack in highly aligned columns, except for the footpad area (15-19). However, the stacking of corneocytes in human SC appears to be less ordered (19,22). A quantitative description of this difference and its implications for diffusive transport through the lipids has not heretofore been presented.

In the current study, we present alternative methods for staining and examining alkaline-expanded SC in both 2 and 3 dimensions. The 2-D methods include brightfield microscopy using an oil red O stain (24) and differential interference contrast. Fluorescent staining with Nile red allows the extension to 3-dimensional (3-D) visualization.

We analyzed the 2-D micrographs obtained in this study by making quantitative estimates of the lipid pathlength across the SC. These data provide the basis for designing a 3-D microstructural model of human SC (T.F.W. and J.M. Nitsche, unpublished data, 2001). Pathlengths were compared with the values predicted from 2-D "brick-and-mortar" models of the SC (2,6) to provide a quantitative measure of corneocyte stacking in these tissue samples. The results show that a considerable degree of disorder can exist in human SC.

MATERIALS AND METHODS

Chemicals

Methylene blue was purchased from Fisher Scientific (Pittsburgh, PA). Nile red was purchased from Sigma Chemicals (St Louis, MO). Oil red O was obtained from Polyscientific (Gaithersburg, MD). OCT embedding media was purchased from Electron Microscopy Sciences (Washington, PA). Sorensen-Walbaum buffer (0.1 M glycine, 0.1 M NaCl, and 0.1 M NaOH), pH 12.5, was used for alkaline expansion of SC. Water was deionized and distilled, and all chemicals were reagent grade.

Human skin

Cryoprotected, cadaveric, split-thickness skin specimens (stored in 10% glycerol) were obtained from Ohio Valley Tissue and Skin Center

(Cincinnati, OH) and stored at -70°C until use. Donor age group (60-75 years) and sampling sites—unspecified (back, abdomen, or thigh) for brightfield microscopy and dorsum for fluorescence and confocal work—were recorded.

Preparation of skin specimens for cryosectioning

A piece of the frozen split-thickness skin approximately 6 cm² was cut and thawed in water at room temperature. A hand-held scalpel was used to cut 4 mm X 7 mm pieces, which were placed dermal side down on aluminum foil-wrapped glass microscope slides. The specimens were covered with OCT embedding medium and frozen on dry ice in an orientation that allowed sectioning perpendicular to the epithelial surface. The molds were placed on dry ice, and the tops of the specimens were covered with the media to facilitate thorough freezing. The tissues were sectioned on a cryotome (Cryostat MHR; Slee Technik, Mainz, Germany) at varying thicknesses, ranging from 8 µm for light microscopy to 40 µm for confocal imaging. They were then placed on poly-L-lysine coated glass slides, which were stored at 4°C until examination.

Alkaline expansion and staining of stratum corneum *Methylene blue staining*

The tissue section slide was immersed in 0.5% (wt/vol) methylene blue in 95:5 (vol/vol) ethanol:water for 2 minutes and rinsed gently in water. Excess water was wicked off with absorbent tissue. The SC was then expanded using half-strength Sorensen-Walbaum buffer for 10 to 20 minutes, and the preparation was covered with a glass coverslip.

Oil red O staining

The tissue section slide was placed in 100% propylene glycol at room temperature for 5 minutes. The slide was then placed in 0.7% (wt/vol) oil red O in propylene glycol at 60°C for 7 minutes and transferred to 85:15 (vol/vol) propylene glycol:water at room temperature for 3 minutes, followed by rinsing with water (25). Alkaline expansion of the SC was performed as described above.

Nile red staining

A stock solution containing 0.05% (wt/vol) Nile red in acetone was stored at 4°C, protected from light. Prior to each staining, the stock was diluted to 2.5

$\mu\text{g/mL}$ with 75:25 (vol/vol) glycerol:water, followed by brisk vortexing (26). The tissue sections were expanded in alkaline buffer as described above and gently rinsed with water. A drop of the glycerol-dye solution was applied to each tissue section and immediately covered with a coverslip.

Brightfield microscopy

Photomicrographs of expanded SC stained with methylene blue were obtained with a 35-mm Zeiss MC63 (Oberkochen, Germany) camera mounted on a Zeiss microscope using a 40X objective lens and Kodak (Rochester, NY) TMAX-100 black and white print film. The oil red O image was captured by a Nikon (Tokyo, Japan) Microphot-FXA microscope with a Spot2 digital camera (Diagnostic Instruments, Inc, Sterling Heights, MI) using a 40X objective lens.

Fluorescence microscopy

Nile red fluorescence was captured with a Leitz (Wetzlar, Germany) microscope equipped with a fluorescein filter cube (450- to 490-nm excitation filter, 510-nm dichroic mirror, and 515-nm long pass emission filter) and a Leitz-Vario-Orthomat camera, using a 40X objective lens and Kodak Elite Chrome-400 color film. This fluorescein filter cube enabled the differentiation of polar and neutral lipids. A fluorescent image of Nile red-stained SC was obtained with a Nikon Microphot-FXA microscope and a Spot2 digital camera, but using a 60X oil-immersion objective lens and a slightly different fluorescein filter cube (460- to 500-nm excitation filter, 505-nm dichroic mirror, and 510- to 560-nm emission filter). The image was captured in the black-and-white mode to decrease the exposure time and photobleaching of the dye. Subsequently, this Nile red image was pseudocolored using Metamorph Imaging System software (Universal Imaging Corp, West Chester, PA).

Nomarski Differential Interference Contrast (DIC) microscopy

A DIC image of an oil red O-stained specimen was obtained with a Spot2 digital camera mounted on a Nikon Microphot-FXA microscope with a 63X oil immersion objective. The DIC optical components were adjusted to obtain bright/dark effects for an apparent 3-D representation.

Confocal microscopy

An inverted Zeiss Axiovert 100M Laser Scanning Confocal Microscope 510 (LSM510) with a 63X water immersion objective lens was used to capture the confocal images. Nile red was excited with the 488-nm argon laser line. A set of optical sections through the specimen (called a "Z series") was obtained by coordinating the movement of the fine focus of the objective with image collection to get a stack of 2-D images (27). Sections were acquired in consecutive planes perpendicular to the apical surface. The images were 512 X 512 pixels, with a pixel size of 0.14 μm X 0.14 μm . The optical section thickness was 0.9 μm . The distance between optical sections was 0.8 μm for the 2-D image and 0.45 μm for the 3-D image. A 3-D view was modeled by making maximum projections of the Z series at several different angles about the apical to basal axis (LSM510 software). Audio Video Interlaced (AVI) files were generated using Animation Shop (JASC Software, Minneapolis, MN).

Lipid pathlength measurement and tortuosity calculation

Prints of the light micrographs were prepared and the corneocyte boundaries identified. Paths were constructed on the print from arbitrary points on the SC surface to the viable epidermal surface using, as a rule, the principle that the path should follow the shortest route across the membrane that does not traverse the interior of a corneocyte. These paths were determined using the analyst's judgment. The pathlength was evaluated by adding segment lengths measured with a ruler, then normalizing by the average width of the membrane in the vicinity of the chosen path. Four to five such paths per micrograph were constructed, and the results were averaged to give a value, τ_{ge} , related to membrane tortuosity in 2 dimensions. This value is equal to the "geometrical tortuosity" of the expanded membrane, as defined in the Appendix. Subsequent reanalysis of several of these paths using the Metamorph software led to very similar pathlength estimates.

The value of τ_{ge} determined in this manner reflects the length of the lipid pathway in the expanded SC specimen, relative to the membrane thickness. Because the SC swells primarily in the transverse dimension (21), the corresponding value for the

membrane prior to expansion, τ_g , must be larger than τ_{ge} . Values of τ_g were calculated as follows.: First, an estimate of the SC thickness for each sample prior to expansion was made by counting the number of corneocyte layers, N , and multiplying the result by $0.875 \mu\text{m}$. This procedure makes use of commonly accepted values for corneocyte ($0.8 \mu\text{m}$) and lipid lamellae ($0.075 \mu\text{m}$) thickness in air-dried skin (6,12). The transverse expansion factor, E_t , was calculated as ratio of the measured width of the SC for the alkali-expanded sample to that calculated from $0.875 \mu\text{m} \times N$. The lateral expansion factor, E_l , was taken to be 1.11, in accordance with measurements of lateral swelling of human epidermis in water (21). Values of τ_g were then calculated using Equation 1, which is derived in the Appendix.

$$\tau_g - 1 = \frac{E_t}{E_l} (\tau_{ge} - 1) \quad (1)$$

Pathlength calculation via this procedure is insensitive to small angular variations in sample alignment. Only rotations along the axis of rotation parallel to the plane of sectioning and to the plane of the corneocytes have an effect on τ_{ge} . Using Equation A-7 listed in the Appendix, it is possible to show that a misalignment of θ degrees along this axis lowers the measured value of τ_{ge} by a factor slightly less than $\cos \theta$. The same deviation *increases* the calculated value of E_t by a factor of $1/\cos \theta$. Since τ_g is related to the product of E_t and τ_{ge} (Equation 1), the alignment error cancels to first order in the calculation of τ_g . Numerical estimates of this effect made using Equations 1 and A-7 show that a 10° misalignment yields an error in τ_g of less than 1%, while a 20° misalignment yields an error of only 2%. These effects are much smaller than the other sources of uncertainty in this analysis, ie, the small number of samples analyzed, subjective judgment as to the shortest path, and local variations in SC thickness.

RESULTS AND DISCUSSION

Figure 1 shows corneocyte stacking in cross sections of human epidermis following alkaline expansion using a variety of histochemical stains and methods. Brightfield optics with methylene blue (Figure 1A) and oil red O (Figure 1B) clearly outline the lipid boundaries of the corneocytes, while fluorescence and DIC methods reveal additional features. Nile red

Figure 1A. Brightfield microscopy of alkali expanded human SC stained with methylene blue. The stacking arrangement of corneocyte layers is clearly discernable; body site is unspecified.

Figure 1B. Brightfield microscopy of alkali expanded human SC stained with oil red O, showing localization of dye in the intercellular spaces; dorsum skin.

Figure 1C. Fluorescence image of Nile red-stained human SC illustrating the change in lipid environment from viable epidermis to the SC as shown by the red to yellow-gold shift in wavelength; body site is unspecified.

Figure 1D. Fluorescence image of Nile red-stained human SC observed with alternative filter settings.

Figure 1E. Oil red O-stained human SC viewed with DIC optics; dorsum skin.

Figure 2. Example of lipid pathlength determination. The winding path on the left traces the shortest, uninterrupted lipid pathway, h_{lp} , from an arbitrary point on the SC surface to the other side of the membrane. The straight lines show the width of the membrane, h_e . The resulting value for geometrical tortuosity, $\tau_{ge} = h_{lp}/h_e$, describes the increased pathlength of the wandering lines relative to the straight-line distance.

fluoresces yellow-gold in the presence of neutral lipids and red in the presence of polar lipids (26,28,29). The red to yellow shift in fluorescence wavelength seen in Figure 1C illustrates the transition from polar lipids in the granular and spinous layers to neutral intercellular lipids in the SC (brightly stained boundaries). Nile red fluorescence in Figure 1D was captured with a different fluorescein filter cube, then pseudocolored to appear green. The oil red O-stained DIC photomicrograph in Figure 1E presents an apparent 3-D image due to the

Table 1. Two-dimensional geometrical tortuosity, τ_{ge} , of lipid pathway in expanded human stratum corneum (SC) estimated by hand analysis of the images in Figure 1.

Figure	Donor, sex, age, site	τ_{ge}
		Mean $\pm$ SD (no. of determinations/image)
1A	Donor #1, unspecified	3.3 $\pm$ 0.1 (4)
1B	Donor #2, male, 72, back	4.0 $\pm$ 0.7 (4)
1C	Donor #1, unspecified	3.9 $\pm$ 0.6 (4)
1D	Donor #2, male, 72, back	3.0 $\pm$ 0.7 (5)
1E	Donor #2, male, 72, back	4.4 $\pm$ 0.7 (5)
Mean $\pm$ SD		3.7 $\pm$ 0.7

Table 2. Geometrical tortuosity, τ_g , of lipid pathway in unexpanded human stratum corneum (SC) for the images in Figure 1, calculated according to Equation 1 using $E_l = 1.11$.

Figure	τ_{ge}	$h_e, \mu m^a$	$h, \mu m^b$	E	τ_g
1A	3.3	68	11.4	5.9	13.2
1B	4.0	41	11.4	3.6	10.7
1C	3.9	52	8.8	5.9	16.4
1D	3.0	43	7	6.1	12.0
1E	4.4	43	13	3.3	11.1
Mean $\pm$ SD					12.7 $\pm$ 2.3

^aMeasured from Figure 1 images.

^bCalculated from Figure 1 image by multiplying the number of corneocyte layers by 0.875 μm (6).

shadowing effect caused by variations in refractive index within the specimen. This image shows the corneocyte boundaries in sharp contrast to their interior and seems to be an attractive method to use in combination with digital image analysis techniques.

The images in Figure 1 show varying degrees of order for stacking of corneocytes within the SC. Figure 1A shows the highest degree of alignment; however, the arrangement is less ordered than has been reported for rodent epithelia (17-19) and some human specimens (19,22). The stacking arrangement appears to be random in the other images (Figure 1B-1E). It would be of interest to test for correlations between these arrangements—and the resulting differences in effective lipid pathlengths—with regional differences in skin permeability. If the diffusion pathways are truly intercellular and if they are significantly different from site to site, then one would anticipate a direct relationship between the precision of the stacking arrangement and skin permeability. This difference in stacking arrangement could be a factor in the higher permeability of rodent skin as compared to human skin.

Figure 3A. Confocal fluorescence images after staining with Nile red. The specimen was mechanically and optically cross-sectioned in the same apical-to-basal plane; dorsum skin. The apical surface is toward the top of each image. Video available in HTML version. To download video and required Microsoft Media Player, please visit: http://www.pharmsci.org/scientificjournals/pharmsci/journal/01_13.html.

Figure 3B. Confocal fluorescent images of Nile red-stained SC representing a 3-dimensional view of the tissue. The apical surface is toward the top of each image. Video available in HTML version. To download video and required Microsoft Media Player, please visit: http://www.pharmsci.org/scientificjournals/pharmsci/journal/01_13.html.

Figure 2 shows an example of a lipid pathlength determination performed on the Nile red-stained image in Figure 1C. Algorithms for determining such shortest paths have been known since the time of Euler (30); however, the paths in Figure 2 reflect human judgment only. Table 1 shows the results of applying this method of analysis to the remainder of the images in Figure 1. The calculated values of τ_{ge} ranged from 3.0 to 4.4 (mean, 3.7). The values were corrected for the effect of alkaline expansion according to Equation 1 to arrive at a 2-D value for the unperturbed membrane, τ_g . Results of this calculation are shown in Table 2. The mean value of τ_g was 12.7, more than 3 times the value of τ_{ge} . Relating this value to the mean pathlength and effective diffusivity of the full 3-D structure is nontrivial and beyond the scope of the present manuscript. It is, however, one of our long-range objectives. As Johnson et al (6) point out, in order to do this one must account also for the excluded volume of the corneocytes in addition to pathlength. The latter leads to a much greater reduction in permeability than suggested by τ_g alone.

It is worth noting that the alkaline expansion technique produces swelling of the SC comparable to that obtained with full hydration. Thus, the expanded SC value, τ_{ge} , of 3.7, calculated in our study may be applicable for in vivo exposure under occlusion or other hydrating conditions.

It is interesting to compare the results in Table 2 with those obtained from brick-and-mortar models of the SC. Michaels et al (2) obtained an expression for the lipid pathlength in a 2-D model consisting of a fully offset array of corneocytes embedded in a lipid matrix—the "brick mason's" model of corneocyte arrangement. We show in the Appendix, using current best estimates for corneocyte and lipid layer dimensions, that this model leads to an estimated value for τ_g of 22.5. Johnson et al (6) adopted nearly the opposite arrangement, with slightly overlapped corneocyte stacks traceable to micrographs of mouse ear epithelia (17,18). Their model leads to a value for τ_g of 5.8 (see Appendix). The average value for τ_g of 12.7 determined in this report indicates that the human SC samples we examined had lipid pathlengths intermediate between that calculated for fully offset corneocytes and that calculated for highly aligned columns.

Figure 3 shows images of expanded SC obtained by laser scanning confocal microscopy. The 2-D optical sections stained with Nile red in Figure 3A are devoid of the "out of focus" flare that is seen with nonconfocal microscopes (eg, Figure 1D). To obtain a model of the 3-D arrangement of the SC, maximum projections were made through a stack of optical sections at several different angles about the apical to basal axis (Figure 3B). Although the apical-to-basal dimension of the tissue in Figure 3B has been greatly increased by the alkaline expansion, desmosomal linkages between the cells prevent their sliding over one another to any great extent. Thus, one may be able to directly calculate lipid pathlengths in 3 dimensions by tracing fluorescent boundaries using a suitable image analysis algorithm. Alternatively, appropriate analysis of the 2-D projections (Figures 1 and 3A) may allow one to reconstruct the 3-D pathlengths. Analysis of these images is an ongoing effort in our laboratories.

CONCLUSIONS

Fluorescence, brightfield, and DIC microscopy, in combination with alkaline expansion and a geometrical model to account for the effect of swelling, can be used to estimate intercellular lipid pathlengths across human SC. Confocal optics show potential for extending this work from 2 to 3 dimensions. Application of these techniques to a small number of human SC samples led to calculated

Figure 4. Schematic diagram for brick-and-mortar model of SC, redrawn from Johnson et al (6). Key model parameters are the corneocyte aspect ratio, $\alpha = d/t$, the lipid/corneocyte thickness ratio, $\beta = g/t$ and the offset ratio, $\omega = d/d_s$.

average lipid pathlengths, relative to the SC width, of about 3.7 after expansion and 12.7 in unexpanded membranes.

ACKNOWLEDGEMENTS

Financial support was provided by the Procter & Gamble Company's International Program for Animal Alternatives and by the National Science Foundation GOALI program. We thank Drs Raymond Boissy and Ravi Kothari for helpful discussions.

APPENDIX

Geometrical tortuosity estimates for 2-D brick-and-mortar models of the stratum corneum—Consider the arrangement of corneocytes embedded in a lipid matrix shown in Figure 4. This model, originally presented by Johnson et al (6), is entirely analogous to the earlier model by Michaels et al (2). The only difference is the variable offset ratio, $\omega = d/d_s$. Johnson and coworkers chose a columnar arrangement of corneocytes corresponding to $\omega = 8$ based on references presenting micrographs of mouse ear epithelium (17,18). Michaels et al considered the fully offset arrangement, corresponding to $\omega \approx 1$. Both groups restricted the analysis to the case in which $s = g$, equivalent to a constant distance between corneocytes.

We wish to construct an expression for the length, h_{lip} , of the shortest path through the lipid matrix relative to the membrane width, h . The ratio $\tau_g = h_{lip}/h$ will heretofore be defined as the "geometrical tortuosity" of the lipid pathway. It should not be confused with the effective tortuosity, τ^* , defined by Johnson et al (6) as the ratio by which the

transmembrane flux is reduced by impermeable corneocyte impediments, nor with the mean pathlength for diffusion, $\tau = a\tau^*$, where a is the area fraction of lipid in a corneocyte layer. The reason for defining τ_g in this manner is that this quantity can be directly estimated from SC micrographs, regardless of the manner in which the corneocytes are stacked. Comparison of the measured values of τ_g with those calculated from precisely ordered structures such as shown in Figure 4 gives an estimate of the average corneocyte offset in the membrane.

Referring again to Figure 4, we construct expressions for h and h_{lip} as follows:

$$h = Nt + (N - 1)g \quad (\text{A-1})$$

$$h_{lip} = h + (N - 1)d_s \quad (\text{A-2})$$

Here N is the number of corneocyte layers and $N-1$ is the number of lipid layers. The value of τ_g is given by

$$\begin{aligned} \tau_g &= h_{lip} / h \\ &= 1 + \frac{d_s}{[N/(N - 1)]t + g} \end{aligned} \quad (\text{A-3})$$

Note that the corneocytes are fully aligned when $d_s = 0$, in which case $\tau_g = 1$.

We define, as did Michaels et al (2), the corneocyte aspect ratio as $\alpha = d/t$ and the lipid/corneocyte thickness ratio as $\beta = g/t$. For simplicity we consider the case $g = s$. Since $\alpha = (d_s + d_l)/t = (1 + \omega)d_s/t$, Equation A-3 can be rewritten as

$$\tau_g = 1 + \frac{\alpha/(1 + \omega)}{[N/(N - 1)] + \beta} \quad (\text{A-4})$$

For the fully aligned case, $\omega = \infty$, and Equation A-4 (like Equation A-3) yields $\tau_g = 1$. For the fully offset case, $\omega = (\alpha - \beta)/(\alpha + \beta)$. This may be seen by noting that the values of d_s and d_l for this case are $\frac{1}{2}(d + s)$ and $\frac{1}{2}(d - s)$, respectively. In this limit, with the further approximation $N/(N - 1) \cong 1$, Equation A-4 yields $\tau_g = 1 + \frac{\alpha + \beta}{2(1 + \beta)}$, which is equal to the value estimated by Michaels et al (2) for this structure.

Johnson et al (6) chose the values $N = 15$, $d = 40 \mu\text{m}$, $t = 0.8 \mu\text{m}$, $s = g = 0.075 \mu\text{m}$, and $\omega = 8$ for their geometrical model of the SC. Using these values and Equation A-4, one obtains $\alpha = 50$, $\beta = 0.094$, and τ_g

$= 5.8$. Using the same values of N , α , and β , but choosing $\omega = 0.996$ to represent the fully offset case, one obtains $\tau_g = 22.5$. Partially aligned arrays of corneocytes sharing these dimensions would thus be expected to yield values for τ_g within the range of 5.8 to 22.5.

Correction for alkaline expansion— When the SC swells in water, the transverse (apical-to-basal) dimension of the tissue increases dramatically and the lateral dimension increases slightly (21). Based on water sorption (31,32) and lateral expansion (21) measurements, the transverse expansion factor for fully hydrated SC relative to its dehydrated state is $E_t = 4-6$ and the lateral expansion factor is $E_l \cong 1.11$. Lipid lamella spacing does not increase as a result of hydration (33); hence, the swelling appears to be confined to the corneocytes.

Less is known quantitatively about SC swelling in alkali. Qualitatively, it appears to be similar to swelling in water (14). Our estimates for the transverse expansion factor range from $E_t = 3.3-6.1$ (Table 2). We assume, for the present analysis, that the lateral expansion factor is $E_l = 1.11$, as found for swelling in water. The impact of these factors on SC tortuosity can then be found by an analysis similar to that leading to Equation A-4. Pathlengths in expanded SC are calculated as follows:

$$h_e = E_t h \quad (\text{A-5})$$

$$h_{lip/e} = h_e + (N - 1)E_l d_s \quad (\text{A-6})$$

The expanded tortuosity factor is equal to the ratio of these values, as in Equation A-3:

$$\tau_{ge} = \frac{h_{lip/e}}{h_e} = 1 + \frac{(N - 1)E_l d_s}{E_t h} \quad (\text{A-7})$$

Rearrangement of Equation A-7 and substitution of the previously defined value for h from Equation A-1 yields

$$\tau_{ge} - 1 = \frac{(N - 1)E_l d_s}{E_t [Nt + (N - 1)g]} \quad (\text{A-8})$$

Comparison of Equations A-8 and A-3 reveals that

$$\tau_{ge} - 1 = \frac{E_l}{E_t} (\tau_g - 1) \quad (\text{A-9})$$

Rearrangement of Equation A-9 yields Equation 1 in the text.

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