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A Predictive Self-Organizing Multicellular Computational Model of Infant Skin Permeability to Topically Applied Substances

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Computational models of skin permeability are typically based on assumptions of fixed geometry and homogeneity of the whole epidermis or of epidermal strata and are often limited to adult skin. Infant skin differs quantitatively from that of the adult in its structure and its functional properties, including its barrier function to permeation. To address this problem, we developed a self-organizing multicellular epidermis model of barrier formation with realistic cell morphology. By modulating the parameters relating to cell turnover reflecting those in adult or infant epidermis, we were able to generate accordingly two distinct models. Emerging properties of these models reflect the corresponding experimentally measured values of epidermal and stratum corneum thickness. Diffusion of an externally applied substance (e.g., caffeine) was simulated by a molecular exchange between the model agents, defined by the individual cells and their surrounding extracellular space. By adjusting the surface concentration and the intercellular exchange rate, the model can recapitulate experimental permeability data after topical exposure. By applying these parameters to an infant model, we were able to predict the caffeine concentration profile in infant skin, closely matching experimental results. This work paves the way for a better understanding of skin physiology and function during the first years of life.

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AUTHOR SUMMARY

The processes characterizing infant skin permeability to chemical irritants and allergens are fundamental in understanding the triggers of the skin irritations and allergic reactions commonly found in infants. Even though noninvasive methods have been developed and used in understanding the unique nuances of infant skin structure and function on human subjects, measuring the effect of topical products on skin barrier to external permeability directly on infants is challenging both technically and ethically. For this reason, we developed a computational model of infant skin epidermis and epidermal permeation. The model can be supplied with data from clinical studies on adult subjects and can predict the permeability of infant skin under similar conditions, given the structural and functional differences between infant and adult epidermis. Furthermore, we validated the capability of the model to predict the concentration profiles of an external agent in the infant epidermis. The model has the potential to show the damaging effects of conditions, such as exposure to harsh surfactants or hard water, on the infant skin barrier as well as the barrier-enhancing effects of emollients and to help with improving disease management protocols for both irritant and allergic dermatitis.

INTRODUCTION

It has long been recognized that the fundamental function of the skin is to provide a barrier to water loss as well as to protect against external insults such as irritants and allergens (Blank, 1965). The study of the permeability dynamics of topically applied substances can provide useful quantitative information about the skin barrier function.

The epidermis is a self-organizing and self-renewing stratified epithelial tissue (Elias and Feingold, 2005). Throughout our lives, keratinocytes are produced continuously at the basal layer of the epidermis and undergo a highly controlled sequential differentiation process in the subsequent layers until they undergo nonapoptotic programmed cell death (cornification). This process results in the generation of corneocytes, the building blocks of the epidermal barrier that consist of the outermost layer, known as the stratum corneum (SC). Intercellular cross-links (corneodesmosomes) hold the corneocytes together (Nemes and Steinert, 1999). Parallel to the cornification process, carefully orchestrated secretion of organized lipid lamellae provides the extracellular hydrophobic mortar of the epidermal barrier (Harding, 2004; Madison, 2003). The path that an external substance needs to transverse to reach the viable part of the epidermis below the

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Abbreviation: SC, stratum corneum

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SC depends on the total thickness of the SC (Barbero and Frasch, 2017; Hadgraft and Lane, 2009; Kasting et al., 2019).

Historically, infant skin beyond the first few weeks after birth has been regarded as structurally and functionally equivalent to adult. Experimental data over the last two decades have challenged this dogma and have revealed that skin undergoes a maturation process that can last for several years after birth (Chiou and Blume-Peytavi, 2004; Stamatas et al., 2011; Visscher et al., 2017). This process involves higher keratinocyte proliferation and desquamation rates than in adults (Liu et al., 2018; Stamatas et al., 2010). Importantly, such higher turnover rates result in smaller corneocytes and thinner SC (Stamatas et al., 2010), creating shorter permeation paths for external agents. Subsequently, SC in babies is expected to be more permeable than that in adults, which is further supported by the higher transepidermal water loss rates (Nikolovski et al., 2008). This higher permeability is an important factor in the development of irritant and allergic dermatitis in the infant's skin and at least partially explains the higher incidence of atopic dermatitis early in life.

In a clinical setting, the quality of skin barrier function to external substance penetration is often measured indirectly through measuring the transepidermal water loss rate as a surrogate, although the validity of this approach has been challenged (Chilcott et al., 2002). More accurately, the quality of the outside in skin barrier can be measured by following a marker with a detectable signal through a confocal arrangement. Caffeine has been proposed as such a marker owing to its safety profile (Dias et al., 1999; Dobson and Hunt, 2018; Kreutzer and Bassler, 2014), its relatively fast skin penetration kinetics, and its ability to be detected by Raman spectroscopy (Stamatas and Boireau-Adamezyk, 2012). However, this method is time consuming and can be uncomfortable for the person undergoing the process of measurement, and for this reason, it is easier to apply the method on adults than on infants. Therefore, there is a need to develop a predictive computational model of infant skin that can translate experimental permeability data from adults.

Although computational modeling of cutaneous permeability is an attractive noninvasive approach, proposed models to date are static and involve the assumptions of tissue homogeneity at the skin compartment (epidermis, dermis, subcutis) or the epidermal layer level (Barbero and Frasch, 2006; Chen et al., 2013; Heisig et al., 1996; Naegel et al., 2008) and importantly are designed to the specifications of adult skin.

The aim of this work was to address these gaps through the development and validation of a dynamic self-organizing multicellular epidermis model with realistic cell morphology.

RESULTS

The first step to simulate external substance penetration in infant skin was to modify the agent-based structural model of the adult epidermis. Figure 1 shows a snapshot of the time of the rendered output of the simulation for adult and infant epidermis at steady state (>5,000 steps from model initiation). Visual comparison of the two models reveals that the infant epidermis is overall thinner. This simulated thinning of the strata and particularly of the SC (10 μm for infant SC compared with 18 μm for the adult SC) is a differentiating characteristic of this model accounting for the increased cell-turnover rate in the epidermis and approximates the experimentally measured values (7.3 μm for infant SC compared with 10.5 μm for adult SC) (Stamatas et al., 2010).

Besides the thickness of the epidermal layers, another emerging property of the computational model is the water concentration profile as a function of depth in the SC. The water concentration profiles corresponded to the two models and are shown in Figure 2a. For comparison, we show the experimentally measured average water profiles for adult and infant epidermis (Figure 2b) from previously published data (Nikolovski et al., 2008). Accounting for the differences between infant and adult SC thickness, the water concentration profiles are displayed as a function of depth normalized to the corresponding SC thickness. The difference between the water concentration profiles in infant epidermis and that in adult epidermis is a significantly higher water content in the top half of the SC for the infant epidermis. This observation is qualitatively recapitulated by the computational models (Figure 2c). Therefore, the models show that infant skin surface is more hydrophilic than adult skin, which is in agreement with the experimental observation.

The permeability of caffeine for infant and adult skin was measured clinically. To arrive at the model-simulated infant concentration profile, we followed the process shown in Figure 3a.

The average caffeine concentration profiles can be seen in Figure 3b and c (black-filled symbols), and the corresponding parameters are shown in Table 1. Although the gradients of the two profiles are not statistically different, the caffeine concentration measured at the SC surface, which relates to the partition coefficient, is slightly higher (15%) for the case of infant skin. This is not surprising given the more hydrophilic nature of the infant SC than the adult SC as evidenced by the higher water content close to the SC surface (Figure 2). Importantly, the total amount of caffeine that permeated the infant SC is 18% higher than the caffeine that permeated the adult SC (3.28 ± 1.00 and 2.78 ± 0.72 arbitrary units correspondingly).

A comparison of the computationally simulated caffeine concentration profiles with the experimental curves is shown in Figure 3b and c (gray-filled symbols). The two simulated profiles show very good fits with the experimental data. Note that although the adult profile was adjusted to fit the data, the infant caffeine concentration profile was not, and it is rather an emerging property of the model. The average concentration of caffeine that penetrated the infant epidermis as predicted by the computational model is statistically the same as the experimentally determined value.

DISCUSSION

To address the limitations of previous skin permeability models and the lack of models relating to infant epidermis, we developed and validated a dynamic agent-based computational model of topical substance permeation into infant epidermis. The model takes into consideration the self-organizing multicellular nature of the epidermis. The model's emerging properties, such as the thickness of epidermal layers and the water concentration profiles in the SC, agree

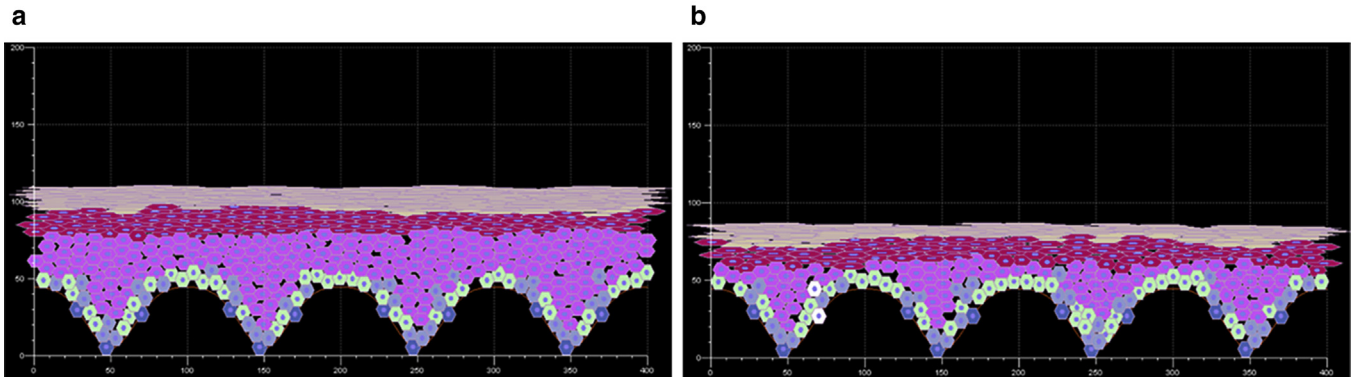

Figure 1. The computational model of the infant epidermis results in thinner layers than that of the adult model. Graphical output of (**a**) the adult and (**b**) the infant epidermal models. The colors of individual keratinocytes correspond to their differentiation state: dark blue for stem cells, light blue for transient amplifying cells, light green for basal cells, light pink for spinous cells, dark pink for granular cells, and tan for corneocytes. The dividing cells are colored white.

with published experimental observations. Validation of the model consisted of predicting the concentration profile of a topically applied substance (caffeine) in the infant epidermis.

The model presented in this paper derives from the two-dimensional version of a published three-dimensional model (Sütterlin et al., 2017) with the following modifications: (i) parametric adjustment to arrive at a model representing infant epidermal structure and (ii) addition of modules regarding the penetration of a topically applied substance. The adult and infant skin penetration models are used to show that one can use adult clinical data and through the modeling process arrive at conclusions regarding penetration in infant skin.

Cutaneous permeation of external substances that come in contact with the skin surface has medical implications (transdermal drug delivery, contact dermatitis, eczema). The exogenous permeation depends on the state of the skin barrier—whether it is a maturing infant skin or an aging adult skin. The study of topical agent delivery typically involves clinical, ex vivo, or in vitro experiments. On the basis of the results from such experiments, generalized computational models have been developed aiming to predict skin permeation kinetics of substances, starting from their physicochemical properties. These include quantitative structure–permeability relationships and mechanistic models (Chen et al., 2013; Tsakovska et al., 2017), some of which take into account the differences in skin compartments, such as the skin layers (Heisig et al., 1996; Naegel et al., 2008). Such models inherently include the assumption of transport in a homogenous medium, which can be the whole tissue or a tissue compartment. Other models based on finite element analysis (Barbero and Frasch, 2006; Naegel et al., 2011) address some issues of spatial heterogeneity but continue to assume a static tissue architecture. The proposed agent-based model avoids such assumptions at the tissue level. The assumption of a homogeneous medium is made only at the cellular level. This allows for spatial variations within the epidermal layers. Moreover, the model considers the dynamic nature of the epidermis and the keratinocyte proliferation, upward movement, differentiation, and desquamation processes, all of which are adding an element of counterflow to the direction of skin penetration by

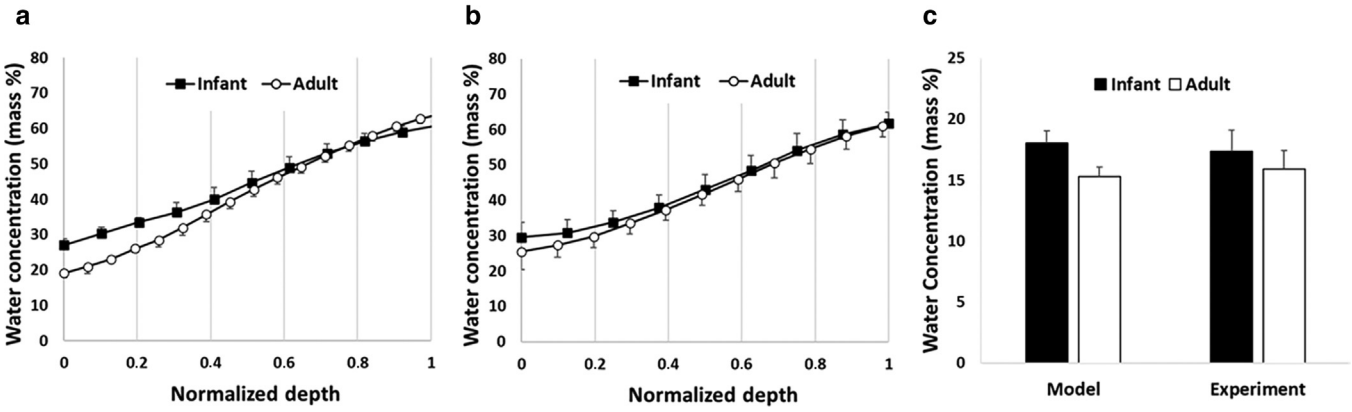

Figure 2. The computational models of infant and adult epidermis recapitulate the shape of the water concentration profiles and the differences between infant and adult. (**a**) Comparison of the water concentration profiles calculated by the infant and adult epidermal models. (**b**) Experimental water concentration profiles in infant and adult SC from published data (Nikolovski et al., 2008). In both panels, for easier comparison between infant and adult profiles, the x-axis is shown as the depth normalized to the SC thickness. (**c**) Comparison of the average water content in the top half of the SC for infant and adult data from the model and the experiment. The computational data points shown correspond to the average, and the error bars correspond to the SD of five independent runs of the model. SC, stratum corneum.

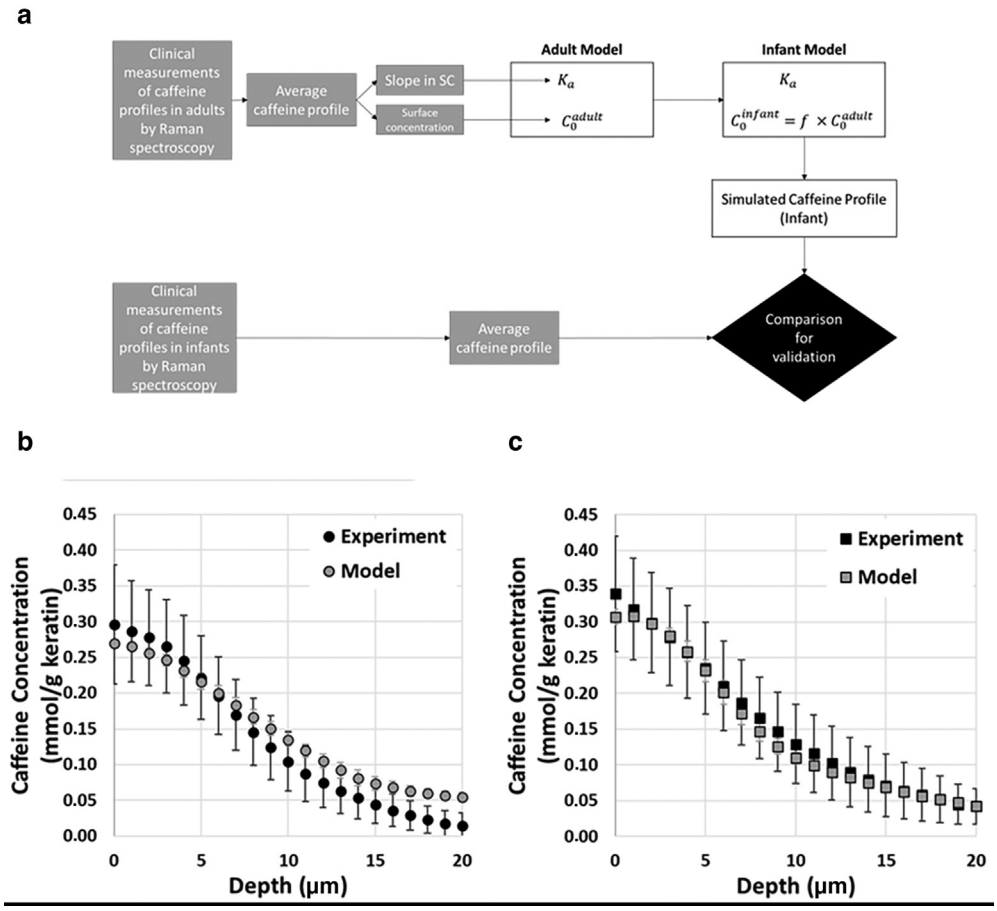

Figure 3. Validation of the infant permeability model. (**a**) Flowchart of the validation process. (**b**) The experimental caffeine concentration profiles collected in adults are used to adjust the penetration kinetic constants in the adult model. (**c**) These constants are passed to the infant model, and the resulting caffeine profile is compared with the data collected on the infants. The computational data points shown correspond to the average, and the error bars correspond to the SD of five independent runs of the model. C_0, caffeine concentration at the skin surface; f, hydrophilicity scaling factor; K_a, exchange rate of caffeine molecules between neighboring agents at each time step; SC, stratum corneum.

an external substance. The latter point becomes very relevant for substances with permeation kinetics in the order of hours (Reddy et al., 2000).

Infant skin evolves its structural and functional characteristics as the child grows. The rate of change of skin maturation is not constant throughout infancy. Newborn skin (defined from birth up to 3 months) is undergoing rapid changes to adapt to the demands of the dry atmospheric environment. In this study, we are considering the skin of babies aged >3 months, an age when the maturation process, although still active, becomes relatively more stable. The computational model of infant skin has been based on functional and structural data from studies of infant skin (forearm) in the age range of 3−24 months (Nikolovski et al., 2008; Stamatas et al., 2010).

Moreover, infant skin is characterized by increased proliferation rate at the basal layer (Stamatas et al., 2010), combined with increased enzymatic activity at the skin surface (Liu et al., 2018), which translates into a faster desquamation rate than that of the adult skin. Taken together, this enhanced cell-turnover rate leads, among others, to the thinning of the epidermal layers, something that is indeed recapitulated by the described infant model compared with that of the adult model.

The SC is the primary layer responsible for the skin's capacity to resist inside-out as well as outside-in transport. An example of the first is the SC resistance to water loss, and an example of the second is the SC resistance to external irritant penetration. The fact that infant SC is 30% thinner than the adult SC (Stamatas et al., 2010) has important implications on water transport, as evidenced by the water concentration profiles in the SC (Nikolovski et al., 2008). The infant skin model proposed in this work is capable of qualitatively capturing the relative differences in the water concentration

Table 1. Infant Skin Barrier Is More Permeable to an External Topical Agent than Adult Skin Barrier

Parameter	Infant (n = 20)	Adult (n = 20)	P-Value
Caffeine concentration at the skin surface (mmol/g keratin)	0.339 ± 0.081	0.296 ± 0.083	0.018
Caffeine concentration gradient in the SC (mmol/g keratin/μm)	0.019 ± 0.004	0.019 ± 0.005	n.s.
The relative amount of permeated caffeine (AU)	3.28 ± 1.00	2.78 ± 0.79	0.027

Abbreviations: AU, arbitrary unit; n.s., not significant; SC, stratum corneum.
Experimentally determined caffeine permeability parameters for infant and adult skin.

profiles between infant SC and the adult SC. These two observations on the structural and water-barrier properties reinforce the validity of the infant skin model.

In this work, we considered caffeine as a useful permeability marker in a clinical setting. Caffeine has been used as a model compound for the delivery of hydrophilic compounds having similar properties (Luo and Lane, 2015). It has a well-known safety profile in adults and infants. It has been used systemically in premature infant wards to successfully treat apnea for >20 years (Dobson and Hunt, 2018; Kreutzer and Bassler, 2014). Moreover, it has a characteristic Raman signature that can easily be included in a chemometric analysis of in vivo Raman spectra of the skin (Caspers et al., 2019). Taking advantage of a confocal setup, one can obtain noninvasively caffeine concentration profiles into the epidermis as a function of distance from the skin surface.

Using the experimental results of caffeine penetration in adult skin, we adjusted the permeation parameters of the adult computational model to match the caffeine concentration profiles in the SC. We then used these parameters to simulate the caffeine concentration profiles in the infant model. We finally compared these profiles with those obtained experimentally from the infant model. The good fit of the computational predictions to the experiment reinforces the validity of the infant skin permeability model. Because the two models differ in their cell-turnover rates and their resulting structural differences, one can conclude that the higher absorbed caffeine concentration in infants than that absorbed in adults is due to these two differences. It is important to note that the flow of cells in a high-turnover model counters the flow of the permeation of an externally applied substance; in other words, the faster the cells are removed from the top by desquamation, the more difficult it would be for the substance to permeate, which is true for the case of poorly permeable compounds (Reddy et al., 2000). The fact that we still observe a higher permeability in the case of infant skin than that observed in the case of adult skin, despite the higher cell-turnover rate in the infant skin, highlights the importance of structural differences between the two models.

In conclusion, a dynamic infant skin permeability model has been developed and has shown permeation predictions of the model in congruence with in vivo experimental data. The model can be used to study the effects of everyday skin care, such as cleansers and emollients, on infant skin permeability barrier. Moreover, it provides a computational tool in the study of the role of infant skin permeability barrier in irritant and allergic contact dermatitis (Jakasa et al., 2018; Tsakok et al., 2019).

MATERIALS AND METHODS

Computational modeling

In this work, we used a two-dimensional version of the model described earlier (Sütterlin et al., 2017). Briefly, the agent-based model is created in the multicellular modeling and simulation platform EPISIM (Sütterlin et al., 2013). Each agent represents a cell and its immediate extracellular space. The model consists of (i) a biomechanical model based on ellipsoid cell shapes with sizes corresponding to the reported values characteristic of the cell differentiation state and (ii) a cell behavioral model embodying regulatory feedback loops between the epidermal barrier, water loss to the environment, and water and calcium flow within the tissue. The model begins with a small number of stem cells that initiate the generation and self-organization of the epidermal stratification, yielding at a steady state the emergence of water and calcium gradients comparable with experimental data. For the adult model, the user-defined constants relating to the rates of (i) basal cell division, (ii) suprabasal cell differentiation, and (iii) surface cell desquamation were assigned values that corresponded to published cell-turnover rates for adult skin (Sütterlin et al., 2017). To simulate the infant epidermis, the values of these parameters were adjusted to correspond to the increased epidermal cell-turnover rates and shorter residence time of the keratinocytes in the infant epidermis (Liu et al., 2018; Stamatas et al., 2010) (Supplementary Materials and Methods).

Finally, to simulate topical substance penetration, we introduced a variable corresponding to the concentration of the substance within a given agent of the model and equations expressing the interagent exchange. The SC and the viable epidermis have different permeation kinetics owing to their different compositions: the first one being rich in lipids and the latter mostly represented by an aqueous phase. To account for these differences, we used two distinct values of the exchange rate. The external substance is introduced at a specified point in time at the surface of the tissue as a step function with the external surface concentration changing from zero to a specified value. The exchange rate parameter and the concentration of the substance at the SC surface were both adjusted to the experimentally defined concentration profiles of the substance in the epidermis at a steady state, as described later.

Clinical study

The clinical study was approved by an Independent Review Board (IntegReview, Austin, TX; protocol number: CS2019-22488) and was conducted in accordance with the ethical principles of the Declaration of Helsinki and after obtaining written informed consent from the study volunteers (for mothers) or their legal guardians (for infants). A total of 20 infant and mother pairs (mothers aged 20–40 years and infants aged 3–6 months) were recruited, and the study was completed. All subjects were of Fitzpatrick skin types I–III with no dermatologic conditions and were generally in good health. Excluded from the study were subjects with known allergies or sensitivity to common topical skincare products (including adhesives present in transdermal patches, bandages, medical-grade adhesives, caffeine-containing products), pre-existing dermatologic conditions, and chronic illness, or subjects undergoing treatment. The mothers were instructed not to use any lotions, creams, or ointments on their forearms or on their children for 24 hours before the test.

All measurements were performed after 15 minutes of acclimatization in an environmentally controlled room (20–25 °C, 40% relative humidity). All measurements were performed on the skin in the middle of the ventral side of the lower forearm. Confocal Raman microspectroscopy (Skin Analyzer Model 3510, RiverD International B.V., Rotterdam, The Netherlands) was used to assess the concentration profiles of caffeine that permeated the SC. Initially, the skin test site was outlined with a pen, and baseline Raman measurement was performed. Then a padded 25-mm Hill Top chamber patch (Cliantha Research, St. Petersburg, FL) with 350 μl solution of 1.8% caffeine (Sigma-Aldrich, St. Louis, MO) in United States Pharmacopeia– and European Pharmacopoeia–purified water was applied on the test site for 30 minutes and was secured with medical tape

(Band-Aid Secure Comfort Cloth Tape, Johnson & Johnson Consumer, Skillman, NJ). The patch was gently removed, and the skin site was patted dry with absorbing paper and was allowed to acclimate to ambient conditions for 10 minutes before the final Raman measurements were performed.

Depth-resolved confocal Raman spectroscopic measurements were acquired in the Raman fingerprint region (400−1,800 cm^{-1}) through the SC at sequential depths ranging from 0 to 32 μm every 4 μm. The instrument was set to acquire spectra through the SC, starting from a point just outside of the skin surface to allow for the analysis algorithm to find the skin surface by interpolation. A depth series of Raman spectra were acquired from 7−10 different areas of the same skin site for each volunteer. The Raman instrument was calibrated once at the beginning of each experiment day according to the manufacturer's instructions. Before each measurement at a different skin site, the calcium fluoride window was cleaned with a single-use alcohol tissue wipe.

Spectral analysis was performed using the SkinTools software (RiverD International B.V.). The acquired spectra were fitted with the primary spectra of 18 components of the SC. To this database, the spectrum of caffeine was added. Each spectral series resulted in a depth caffeine concentration profile (given in mmol caffeine per gram of keratin). Caffeine concentration profiles were averaged for each time point. Before averaging, outliers were removed, including unstable values, owing to subject movement or pushing down too hard on the instrument window. For each subject, the average profiles at baseline were subtracted from those after the patch application. Then the baseline-corrected average concentration profile for the infants was compared with that for adults. All data points in the graphs are shown as mean ± one SEM. Statistical comparison of two distributions was performed after Anderson–Darling normality test and test of variance (F-test) to select the appropriate *t*-test. Statistical significance was accepted at the level of $\alpha = 0.05$. All data processing was performed using MATLAB routines and Excel software.

The caffeine concentration profiles in the SC were used to calculate the following parameters: (i) the caffeine concentration at the SC surface; (ii) the gradient of the profile in the first 15 μm of the SC, which relates to the diffusion coefficient; and (iii) the area under the curve representing the total amount of caffeine that penetrated the SC.

Data availability statements

The executable jar file of the model and the relating instructions ("Readme.txt" file) are provided in the Supplementary Materials and Methods. The jar file runs on the EPISIM platform simulator that can be downloaded using the following link: http://www.tiga.uni-hd.de/downloads.html. The model is available for research purposes and for any commercial application of the model directed to the use of the computational model of adult skin penetration to evaluate the potential impact of systems on infant skin and is covered by a patent application. The authors are open to licensing and/or collaboration opportunities.

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CONFLICT OF INTEREST

GNS, JB, EG, SK, HW, and TO are employees of Johnson & Johnson Group of Consumer Companies. MVD has served in the Global Advisory Board of Johnson & Johnson. MJC is/has been an investigator and/or consultant for the following organizations: Astellas, Boots, Dermavant, Galapagos, Galderma, Hyphens, Johnson & Johnson, Kymab, Leo Pharma, L'Oreal, Menlo, Novartis, Oxagen, Pfizer, Procter & Gamble, Reckitt Benckiser, Regeneron, and Sanofi Genzyme. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: GNS, TO, MVD, MJC, AJF; Data Curation: SK, HW; Formal Analysis: GNS, JB, EG, SK, HW; Funding Acquisition: GNS, TO; Investigation: GNS, JB, EG, SK, HW; Methodology: GNS, JB, EG; Project Administration: GNS, TO; Resources: GNS, SK, HW, TO; Software: JB, EG; Supervision: GNS, JB, SK, TO; Validation: GNS, JB, EG, SK, HW, TO, MVD, MJC, AJF; Visualization: GNS, JB, EG, SK, HW; Writing - Original Draft Preparation: GNS; Writing - Review and Editing: GNS, JB, EG, SK, HW, TO, MVD, MJC, AJF

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.02.012.

REFERENCES

Barbero AM, Frasch HF. Effect of stratum corneum heterogeneity, anisotropy, asymmetry and follicular pathway on transdermal penetration. J Control Rel 2017;260:234−46.

Barbero AM, Frasch HF. Transcellular route of diffusion through stratum corneum: results from finite element models. J Pharm Sci 2006;95:2186−94.

Blank IH. Cutaneous barriers. J Invest Dermatol 1965;45:249−56.

Caspers PJ, Nico C, Bakker Schut TC, de Sterke J, Pudney PD, Curto PR, et al. Method to quantify the in vivo skin penetration of topically applied materials based on confocal Raman spectroscopy. Transl Biophotonics 2019;1:e201900004.

Chen L, Han L, Lian G. Recent advances in predicting skin permeability of hydrophilic solutes. Adv Drug Deliv Rev 2013;65:295−305.

Chilcott RP, Dalton CH, Emmanuel AJ, Allen CE, Bradley ST. Transepidermal water loss does not correlate with skin barrier function in vitro. J Invest Dermatol 2002;118:871−5.

Chiou YB, Blume-Peytavi U. Stratum corneum maturation. A review of neonatal skin function. Skin Pharmacol Physiol 2004;17:57−66.

Dias M, Farinha A, Faustino E, Hadgraft J, Pais J, Toscano C. Topical delivery of caffeine from some commercial formulations. Int J Pharm 1999;182:41−7.

Dobson NR, Hunt CE. Caffeine: an evidence-based success story in VLBW pharmacotherapy. Pediatr Res 2018;84:333−40.

Elias PM, Feingold KR, editors. Skin barrier. Boca Raton: CRC Press; 2005.

Hadgraft J, Lane ME. Transepidermal water loss and skin site: a hypothesis. Int J Pharm 2009;373:1−3.

Harding CR. The stratum corneum: structure and function in health and disease. Dermatol Ther 2004;17(Suppl. 1):6−15.

Heisig M, Lieckfeldt R, Wittum G, Mazurkevich G, Lee G. Non steady-state descriptions of drug permeation through stratum corneum. I. The biphasic brick-and-mortar model. Pharm Res 1996;13:421−6.

Jakasa I, Thyssen JP, Kezic S. The role of skin barrier in occupational contact dermatitis. Exp Dermatol 2018;27:909−14.

Kasting GB, Miller MA, LaCount TD, Jaworska J. A composite model for the transport of hydrophilic and lipophilic compounds across the skin: steady-state behavior. J Pharm Sci 2019;108:337−49.

Kreutzer K, Bassler D. Caffeine for apnea of prematurity: a neonatal success story. Neonatology 2014;105:332−6.

Liu Q, Zhang Y, Danby SG, Cork MJ, Stamatas GN. Infant skin barrier, structure, and enzymatic activity differ from those of adult in an East Asian cohort. BioMed Res Int 2018;2018:1302465.

Luo L, Lane ME. Topical and transdermal delivery of caffeine. Int J Pharm 2015;490:155–64.

Madison KC. Barrier function of the skin: "la raison d'etre" of the epidermis. J Invest Dermatol 2003;121:231–41.

Naegel A, Hansen S, Neumann D, Lehr CM, Schaefer UF, Wittum G, et al. In-silico model of skin penetration based on experimentally determined input parameters. Part II: mathematical modelling of in-vitro diffusion experiments. Identification of critical input parameters. Eur J Pharm Biopharm 2008;68:368–79.

Naegel A, Heisig M, Wittum G. Computational modeling of the skin barrier. Methods Mol Biol 2011;763:1–32.

Nemes Z, Steinert PM. Bricks and mortar of the epidermal barrier. Exp Mol Med 1999;31:5–19.

Nikolovski J, Stamatas GN, Kollias N, Wiegand BC. Barrier function and water-holding and transport properties of infant stratum corneum are different from adult and continue to develop through the first year of life. J Invest Dermatol 2008;128:1728–36.

Reddy MB, Guy RH, Bunge AL. Does epidermal turnover reduce percutaneous penetration? Pharm Res 2000;17:1414–9.

Stamatas GN, Boireau-Adamezyk E. Development of a non-invasive optical method for assessment of skin barrier to external penetration. In: Biomedical Optics and 3-D Imaging. Washington, DC: OSA Technical Digest (Optical Society of America); 2012. p. JM3A–42.

Stamatas GN, Nikolovski J, Luedtke MA, Kollias N, Wiegand BC. Infant skin microstructure assessed in vivo differs from adult skin in organization and at the cellular level. Pediatr Dermatol 2010;27:125–31.

Stamatas GN, Nikolovski J, Mack MC, Kollias N. Infant skin physiology and development during the first years of life: a review of recent findings based on in vivo studies. Int J Cosmet Sci 2011;33:17–24.

Sütterlin T, Kolb C, Dickhaus H, Jäger D, Grabe N. Bridging the scales: semantic integration of quantitative SBML in graphical multi-cellular models and simulations with EPISIM and COPASI. Bioinformatics 2013;29:223–9.

Sütterlin T, Tsingos E, Bensaci J, Stamatas GN, Grabe N. A 3D self-organizing multicellular epidermis model of barrier formation and hydration with realistic cell morphology based on EPISIM. Sci Rep 2017;7:43472.

Tsakok T, Woolf R, Smith CH, Weidinger S, Flohr C. Atopic dermatitis: the skin barrier and beyond. Br J Dermatol 2019;180:464–74.

Tsakovska I, Pajeva I, Al Sharif M, Alov P, Fioravanzo E, Kovarich S, et al. Quantitative structure-skin permeability relationships. Toxicology 2017;387:27–42.

Visscher MO, Burkes SA, Adams DM, Hammill AM, Wickett RR. Infant skin maturation: preliminary outcomes for color and biomechanical properties. Skin Res Technol 2017;23:545–51.

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SUPPLEMENTARY MATERIALS AND METHODS

From adult model to infant model

Starting from the two-dimensional version of the adult model described previously (Sütterlin et al., 2017), we aimed to create a model that would correspond closely to the structure of infant epidermis, targeting a reduction in stratum corneum thickness by 30% and in suprapapillary epidermal thickness by 20% to match the experimental observations (Stamatas et al., 2010). To achieve this target, we reduced the time between cell divisions (defined by the value of the model parameter cell cycle). This move reflects the experimentally observed higher cell-turnover rate of the infant epidermis than those of the adult epidermis (Stamatas et al., 2010). We assumed a reduction by 33% in the time between cell divisions. This corresponds to a decrease from 120 simulation steps to 80 simulation steps. The resulting simulation yielded a significantly thicker epidermis. To obtain a thinner tissue, the resident cell time (time from formation to disappearance by desquamation) had to be reduced. To achieve this, the model had to be adjusted to reflect increased desquamation and differentiation rates. Increased desquamation rates in infants can be inferred from experimental observations (Liu et al., 2018). An increased differentiation rate is then required to avoid accumulation of spinous cells that would otherwise dominate the epidermis. Practically, to increase the differentiation rates, we lowered the calcium threshold required for spinous cells to differentiate into granular cells (parameter c_minCaGranu) and accelerated the rate of cornification by increasing the amount of FLG produced in one step by granular cells (parameter co_filagProduction). Corneocyte desquamation in the model is a consequence of loss of adhesion to the neighboring cells. Therefore, to increase the desquamation rate, we reduced the number of simulation steps until corneocyte adhesion started decreasing (parameter adh_increase_max_time) and increased the degradation of adhesion proteins. (parameter adh_k2_dec). The process of identifying the appropriate values that would result in a stable infant model, reflecting the 30% and 20% reduction compared with those of the adult model, was to test the combinations within the range of 0.3-fold to 3-fold of the original value. The chosen values of the corresponding parameters for the adult and the infant models are shown in Supplementary Table S1.

Substance penetration model

We introduced in the model a variable representing the substance intracellular concentration. At a given step, the user-defined initial surface concentration is applied to the outermost cells. The interagent substance exchange is then driven by the concentration gradient and weighted by a coefficient representing both partition and diffusion, taking values between 0 and 1. The stratum corneum and the viable epidermis have different permeation kinetics owing to their different compositions: the first one being rich in lipids, and the latter being mostly represented by an aqueous phase. To account for these differences, we used two separate permeation coefficient values corresponding to the two conditions. The substance is allowed to diffuse through the epidermis driven by the concentration gradient until the basal layer. The dermis is considered a permeability sink, and at each step, a constant proportion of the substance concentration in the basal cells is lost to the dermis.

Finally, to fit the caffeine concentration profile in adult skin, the intra-agent exchange rate was calculated as the experimentally determined concentration gradient in adult skin multiplied by 20. The same value for this parameter was used for the adult and infant models. To calculate the infant surface concentration, a scaling factor was used on the experimentally derived adult value. This scaling factor was predetermined on the basis of the higher hydrophilicity (15% higher water content) of infant skin surface than that of the adult skin office from published experimental data (Figure 2b).

SUPPLEMENTARY REFERENCES

Liu Q, Zhang Y, Danby SG, Cork MJ, Stamatas GN. Infant skin barrier, structure, and enzymatic activity differ from those of adult in an East Asian cohort. BioMed Res Int 2018;2018:1302465.

Stamatas GN, Nikolovski J, Luedtke MA, Kollias N, Wiegand BC. Infant skin microstructure assessed in vivo differs from adult skin in organization and at the cellular level. Pediatr Dermatol 2010;27:125–31.

Sütterlin T, Tsingos E, Bensaci J, Stamatas GN, Grabe N. A 3D self-organizing multicellular epidermis model of barrier formation and hydration with realistic cell morphology based on EPISIM. Sci Rep 2017;7:43472.

Supplementary Table S1. Adjustment of Parameter Values of the Adult Model for the Development of the Model of Infant Epidermis

Parameters	Adult	Baby
Time steps between two divisions (cellCycle)	120	80
Calcium threshold for the differentiation step from spinosum to granulosum (c_minCaGranu)	575	470
Rate of FLG synthesis (co_filagProduction)	0.35	0.65
Rate of decay and/or degradation of cell–cell adhesion (adh_k2_dec)	0.009	0.025
Maximum number of time steps until corneocyte adhesion starts decreasing (adh_increase_max_time)	360	130

The corresponding variable names in the EPISIM modeler are shown in parenthesis.