Deposition/Penetration Kinetics in the Stratum Corneum

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Number of Graduate Students/Postdoc: 1 GS/ 0.5 Postdoc

Timespan: 2 years

Budget: $200,000

This proposal is aiming to measure deposition and penetration of surfactants and moisturizers into the skin in vivo and ex vivo. We propose to quantify the adsorbents via in vivo/ex vivo confocal microscopy and correlate with dehydration rate.

Background/Rationale: Diffusion cell and the tape stripping methods are widely used to quantify deposition or penetration on skin. However, these methods may not be ideal to achieve accurate short-term kinetics of diffusion coefficient parameters. Recent studies have shown in vivo/ex vivo confocal microscopy was able to quantify nanoparticle deposition and penetration. [1] We hypothesize that real-time ex vivo imaging will make it possible to determine the short-term kinetics of penetration of various surfactants and parameters needed for in silico, such as diffusion coefficients. [2-3]

Objective: The objective of this proposal is to determine the short-term and long-term kinetics of surfactant penetration and deposition after washing via ex vivo confocal microscopy. In addition, we will correlate the penetration/deposition results with dehydration rate and investigate interaction between stratum corneum and surfactant at the molecular level.

Research Strategy:

Aim 1: *Measure the real-time penetration kinetics of surfactants ex vivo.* [4,5] This aim determines the short-term and long-term kinetics of surfactant penetration in human cadaver stratum corneum via confocal microscopy. We will compare the values with literatures to verify the technique.

Rationale/Preliminary Results: Kraeling et al. have shown dye penetration over time in the stratum corneum via confocal scanning microscopy (**Figure 1**).[1] Free dye molecules have been widely used to visualize location of the dye and determine the amount semi-quantitatively. Thus, we hypothesize that fluorescence dye tethered to surfactant of interest will be quantitatively shown in confocal imaging in real-time.

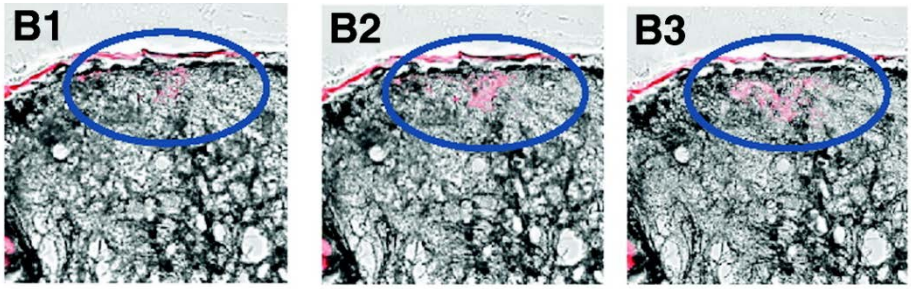

Figure 1. *Penetration of the fluorescence appears to go deeper into the skin at the low-dose solution. Adapted from Kraeling et al. [1].*

Research Design: We will use surfactant with fluorescent dye either chemically conjugated[6] or electrostatically attached[7] to visualize the penetration over time. [1] We will utilize an in vivo confocal microscope located in the core facility at UC with resolution of 1 μm to track fluorescence molecules over time. The facility has an environmental chamber for temperature and humidity control and capability of differential interference contrast imaging with fluorescence overlay in real-time. [8] We will validate our technique by comparing the results with literature. Morris et al. [5, 9] demonstrated that short-term kinetics was a function of monomer concentration. We will vary the concentrations to see the effect on penetration. For the long-term kinetics, after

the penetration studies, we will wash the surface of the skin cadaver and measure the residue to quantify the deposition at the z-direction.
Expected Outcomes: The milestone for Aim 1 is to show feasibility of the new technique in determining reliable short-term penetration kinetic parameters. The results also can be compared with available internal in vivo data. We believe the approach is very powerful because it is visualizing, quantitative and versatile.
Alternative approaches: Conjugation of the fluorescence dye may alter the total surfactant structure and affect the total penetration kinetics. In this case, we will calibrate the effect of conjugation by utilizing shorter alkyl chain lengths as suggested in Smith et al.[6]

Aim 2. *Correlate the data with dehydration level and investigate molecular structure alteration of protein and lipids in the stratum corneum***.** This aim will advance the understanding of dehydration and skin irritation in relationship with penetration and deposition of surfactant.
Rationale/Preliminary Results: Mechanisms determining the effect of surfactants on the human skin have been investigated extensively. [10] However, direct comparison between penetration and dehydration has not been systemetically studied yet. We have measured dehydration rate using our stratum corneum model which consists of lipids and keratin via a microbalance method.[11] We have confirmed that the method was very reproducible to conclude the effect of surfactant concentration and keratin on the normalized water weight remaining percent over time (%wwr/min). We discovered that dehydration rate for the short-term (minutes) and long-term (hours) were affected differently by hydrophobicity on the surface and structure alteration of protein, respectively. Morris et al. also mentioned that long-term penetration was affected by skin structure alteration which is due to surfactant deposition.[5] Thus, we hypothesize that penetration and deposition is correlated with dehydration. In this Aim, we will investigate the correlation and structure alteration at the molecular level.
Research Design: We will perform the dehydration rate measurement as described in our protocol in the same platform, using the same materials and substrates in Aim 1.[11] Briefly, 10 µL of deionized (DI) water will be added on the sample (human cadaver and fluorescence dye + surfactant) and the dehydration rate will be obtained by calculating % weight of water remaining (mass of water remaining on substrate/mass of water added*100) per time (minutes), %wwr/min, using a microbalance (Mettler Toledo XSE105 Dual range).
For the further characterization, we will measure contact angle and ATR-FTIR to understand structural changes in the skin samples. [12-13]
Expected Outcomes: The milestone for completion of Aim 2 will be providing better understanding on the mechanism of dehydration in relationship with surfactant penetration and deposition and validate the new hypotheses.
Alternative approaches: We will also characterize the skin structure alteration thoroughly optically by comparing images over time.

Future Plan: We will extract essential parameters that affect significant differences in deposition and penetration by varying (1) environmental conditions and (2) samples with different treatment histories in the future. Because different environmental conditions play a significant rol in penetration and deposition, [14] we will investigate the trend by varing temperature to further validate the new technique.

References

1. In Vitro Skin Penetration of Dendrimer Nanoparticles. *Applied In Vitro Toxicology* **2019,** *5* (3), 134-149.
2. Ghafourian, T.; Fooladi, S., The effect of structural QSAR parameters on skin penetration. *International journal of pharmaceutics* **2001,** *217* (1), 1-11.
3. Mitragotri, S.; Anissimov, Y. G.; Bunge, A. L.; Frasch, H. F.; Guy, R. H.; Hadgraft, J.; Kasting, G. B.; Lane, M. E.; Roberts, M. S., Mathematical models of skin permeability: An overview. *International journal of pharmaceutics* **2011,** *418* (1), 115-129.
4. Catanoiu, G.; Carey, E.; Patil, S. R.; Engelskirchen, S.; Stubenrauch, C., Partition coefficients of nonionic surfactants in water/n-alkane systems. *Journal of Colloid and Interface Science* **2011,** *355* (1), 150-156.
5. Morris, S. A. V.; Thompson, R. T.; Glenn, R. W.; Ananthapadmanabhan, K. P.; Kasting, G. B., Mechanisms of anionic surfactant penetration into human skin: Investigating monomer, micelle and submicellar aggregate penetration theories. *International Journal of Cosmetic Science* **2019,** *41* (1), 55-66.
6. Ashleigh D. Smith, M., Selin Ergülen, Meredith M. Ogle, Carlos A. de los Reyes, Matteo Pasquali, Angel A. Martí, Fluorescent surfactants from common dyes – Rhodamine B and Eosin Y. *Purue and Applied Chemistry* **2019**.
7. Yamaguchi, S.; Minbuta, S.; Matsui, K., Rhodamine B adsorption on anodic porous alumina in sodium dodecyl sulfate solutions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2018,** *555*, 209-216.
8. Cincinnati, U. o., https://www.med.uc.edu/psp/about/livecore.
9. Morris, S. A. V.; Ananthapadmanabhan, K. P.; Kasting, G. B., Anionic Surfactant-Induced Changes in Skin Permeability. *Journal of pharmaceutical sciences* **2019**.
10. Seweryn, A., Interactions between surfactants and the skin – Theory and practice. *Advances in Colloid and Interface Science* **2018,** *256*, 242-255.
11. Nithianandam, P., Das, S. Park, Y, Effect of Surfactant-Keratin Interactions on Hydration Properties of a Stratum Corneum Substitute. *Under revision*.
12. Yarovoy, Y.; Drutis, D. M.; Hancewicz, T. M.; Garczarek, U.; Ananthapadmanabhan, K. P.; Misra, M., Quantification of Lipid Phase Order of In Vivo Human Skin Using Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) Spectroscopy and Multivariate Curve Resolution Analysis. *Appl. Spectrosc.* **2019,** *73* (2), 182-194.
13. Arrondo, J. L.; Goni, F. M., Structure and dynamics of membrane proteins as studied by infrared spectroscopy. *Progress in biophysics and molecular biology* **1999,** *72* (4), 367-405.
14. Vizserálek, G.; Balogh, T.; Takács-Novák, K.; Sinkó, B., PAMPA study of the temperature effect on permeability. *European Journal of Pharmaceutical Sciences* **2014,** *53*, 45-49.