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Real-time, High-resolution, In Vivo Characterization of Superficial Skin With Microscopy Using Ultraviolet Surface Excitation (MUSE)

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Abstract

Background: Skin care products make up the largest part (36%) of the cosmetic market globally, of which the United States plays the largest role. In 2015, approximately 115 billion USD was spent globally on skin care products. Skin care products, in contradistinction to pharmaceuticals, are not strictly regulated by the FDA. A key factor for evaluation of a skin care product ortopical drug is skin barrier function and effect on superficial skin. Thus, it is critical to have quantitative and qualitative methods to study the effects of skin care products on skin barrier and the superficial skin. Currently, no imaging method exists that can evaluate and track superficial skin changes visually in real-time.

Objective: To report using a novel imaging modality, Microscopy using Ultraviolet Surface Excitation (MUSE), to provide real-time, high-resolution, in vivo characterization of superficial skin and moisturizing properties of topical moisturizer, and to highlight key benefits of using MUSE to visualize the superficial skin and serve as an excellent complementary tool to current quantitative methods.

Methods and Materials: The methodology of MUSE is based upon two main principles inherent to ultraviolet (UV) light and fluorescent staining agents. In this study, the author's (JJ) index fingertip was imaged using the MUSE instrument without and with moisturizer.

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DISCLOSURES

Dr. Levenson is co-founder and CEO of MUSE Microscopy, Inc., a start-up that intends to commercialize this technology. Mr. Ho, Dr. Fereidouni, and Dr. Jagdeo have no relevant conflicts of interest to disclose. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

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Results: Dermatoglyphics of the fingertip consists of ridges (cristae superficiales) and grooves (sulci superficiales] proved to be straightforward to visualize at high resolution. Desquamation of superficial corneocytes and opening of an acrosyringium (the most superficial portion of eccrine ducts) were visualized in high-resolution. Post-application of a moisturizer, a uniform layer of moisturizer could be seen superficial to the corneocytes along the ridges and grooves, and indicating that the superficial skin was well-moisturized.

Conclusions: Real-time, high-resolution, in vivo characterization of superficial skin and moisturizing properties of moisturizer using MUSE is feasible. Its utility can be enhanced with downstream quantification using imaging software.

INTRODUCTION

Skin care products make up the largest part (36%) of the cosmetic market globally, of which the United States plays the largest role.[1] In 2015, approximately 115 billion USD was spent globally on skin care products.[2]The skin care industry has shifted its consumer focus from the oiderto younger population, commercializing environmentally friendly, organic anti-aging products to achieve youthfulness and delay signs of aging.[2] Skin care products, in contradistinction to pharmaceuticals, are not strictly regulated by the FDA.[3] Manufacturers invest significant resources into research and development to demonstrate efficacy, namely to ensure that marketed products yield the desired outcome.

Superficial skin is important because it serves as environmental barrier protecting against external (physical, chemical/biochemical) stress and is regulating transdermal water loss.[4] A key factor for evaluation of a skin care product or topical drug is skin barrier function and other effects on superficial skin, which includes the ability of a product to penetrate the stratum corneum and to affect the status of skin hydration.[5]Thus, it is critical to have quantitative and qualitative methods to study the effects of skin care products on skin barrier and the superficial skin. Various methods (non-invasive, invasive, in vitro) and mathematical models have been developed to study skin barrier function, primarily focusing on transepidermal water loss (TEWL) and skin conductance measurements.[6]These measurements are currently employed to assess skin barrier function; however, limitations exist including factors associated with changes in humidity, temperature, circadian rhythms, stress, and device used.[7] In addition, penetration properties of topically applied products are related to the vehicle used, and conventional in vitro measurements from tape stripping, skin biopsies, and skin models may not necessarily reflect the actual *in vivo* condition. More importantly, a significant limitation is that none of these methods capture the visual appearance of superficial skin. Currently, there exists no imaging method that can evaluate and track skin barrier function visually in real-time.

Here, we report using a novel imaging modality. Microscopy using Ultraviolet Surface Excitation (MUSE), to provide real-time, high-resolution, in vivo characterization of superficial skin and moisturizing properties of a topical moisturizer. We highlight key benefits of using MUSE to visualize the skin barrier and to serve as an excellent complementary tool to current quantitative methodology.

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METHODS

The methodology of MUSE is based upon two main principles inherent to ultraviolet (UV) light and fluorescent staining agents. Due to wavelength-dependent absorption and scattering, UV light at 290 nm intensity at 70 μm epidermal thickness is reduced to 1.6% of incident power. The resulting highly surface-weighted signals provide excellent optical sectioning without the requirement of complex optics, reconstruction mathematics, and computational resources associated with other imaging techniques.[8,9] Additionally, due to varying amounts of photon absorption and emission, some fluorescent staining agents when excited with sub 300-nm UV light emit signals in the visible range that can be captured using conventional color cameras.[10] Individual frames can be captured rapidly, and large fields-of-view can be generated using the movable stage and computer stitching software. The entire process, from fluorescent staining of the skin to imaging, takes less than two minutes and requires minimal training, which may encourage widespread adoption by clinicians and researchers.

The MUSE instrument operates with four UV-emitting light-emitting diodes (LEDs, MTE280H32-UV, Marktech, Latham, NY) with maximum emission wavelength centered between 275 to 285 nm, and maximum power output per LED of 0.9 mW. The LED light is focused using a ball lens with a short focal length onto the surface of the skin to provide oblique-angle (approximately 30°) *cis*-illumination. The skin rests against a UV transparent sapphire window (GT Advanced Technologies, Salem, MA), and the emitted light is collected by a standard microscope objective and transmitted via tube lens to a conventional color camera. For capturing of a series of individual frames to generate large fields-of-view, the MUSE instrument is equipped with an XY2 translation stage for positioning of the specimen. Custom software written in Visual Basic .Net (VB.NET, Microsoft Corp., Mountain View, CA) is utilized for image acquisition and flat-fielding to correct for uneven illumination and optical system vignetting.

In this study, the author's (JJ) index fingertip was imaged using the MUSE instrument without and with moisturizer. The fingertip was stained with Rhodamine B (Sigma-Aldrich, St. Louis, MO) for ten seconds, and washed under running water prior to imaging. Repeat staining and imaging was performed post-application of a thin layer of moisturizer applied evenly onto the fingertip.

RESULTS

Figure 1A and 1B are grayscale MUSE images of the fingertip without and with moisturizer, respectively. In figure 1A, dermatoglyphics of the fingertip consists of ridges (cristae superficiales) and grooves (sulci superficiales) proved to be straightforward to visualize at high resolution. Desquamation of superficial corneocytes and opening of an acrosyringium (the most superficial portion of eccrine ducts) were visualized in high-resolution. In figure 1B, a uniform layer of moisturizer could be seen superficial to the corneocytes along the ridges and grooves, and indicating that the superficial skin was well-moisturized.

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DISCUSSION AND FUTURE DIRECTIONS

We have described real-time, high-resolution, *in vivo* characterization of superficial skin and moisturizing properties of topical moisturizer using MUSE. We demonstrate a fast, low-cost, non-labor-intensive imaging modality that may assist in the evaluation of skin barrier function. An advantage with an *in vivo*, non-invasive imaging modality, compared to conventional histology performed with tape stripping and invasive skin biopsies, is that it avoids alterations from fixation and extraction of water and other components. Moreover, repeat visualization of superficial skin can be tracked at the same site over time that may be important for outcome assessment. Current limitations include lack of correlation with conventional histology and evaluation of other staining agents to better visualize superficial skin. There is significant potential for MUSE to assist with efficacy outcome measures for skin care and cosmetic products, in addition to topical drug deliveries and assessment of skin pathologies.

MUSE has several key benefits compared to current imaging modalities.[6] Confocal laser microscopy, in combination with Raman spectroscopy, provides *in vivo* measurements of water content and is beneficial for understanding the structure and function of the skin as a water barrier and water transport in the stratum corneum. Major constituents of the skin, such as lipids and proteins, can be studied using this method.[11,12] Direct and indirect fluorescence spectroscopy and fluorescence-lifetime imaging microscopy have been utilized to track *in vivo* penetration of fluorescent substances as a surrogate model to evaluate penetration of topical cream or drug.[6] Optical coherence tomography may provide good image resolution; however, individual cells cannot be distinguished and details regarding the molecular and structural composition are often difficult to identify.[6,13] Near-infrared imaging has been studied for measuring skin hydration but provides only quantitative information.[14] Sonography has been previously investigated; however, sonography has poor resolution due to the required ultrasound gel for eliminating air between the transducer and skin that interferes with evaluation of superficial skin.[6] While the above mentioned techniques may be informative, they are nevertheless often complex, expensive, requires trained and skilled personnel.

MUSE has significant advantages that can complement current quantitative methodology of using TEWL and skin conductance for evaluation of superficial skin. TEWL and skin conductance only provide quantitative values of measured skin samples, and these values may not directly correspond to clinical efficacy and clinical relevance from the skin care product or topical drug.[15] For example, when information is required regarding restoration of normal skin morphology of the epidermis or monitoring of the wound healing process, skin visualization with MUSE can provide additional critical information to quantitative measurements. Additionally, there may be inherent limitations with TEWL measurements, including differences among sampled subjects, and exogenous/environmental factors. MUSE can serve as an excellent complementary tool to current quantitative methods, as imaging provides visualization and qualitative assessment of skin. A disadvantage of MUSE is that the images it generates are highly surface-weighted, so it reports on the status of the superficial epidermis and visible appendages.

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Real-time, high-resolution, in vivo characterization of superficial skin and moisturizing properties of topical moisturizer using MUSE may prove of value as it allows visualization and possible quantification using imaging software. We envision the MUSE instrument may become a useful tool for both clinicians and researchers, as it has the potential to increase our understanding of skin physiology, transdermal drug delivery, wound healing, and repair of the skin barrier function and superficial skin using skin care products and/or topical therapeutics.

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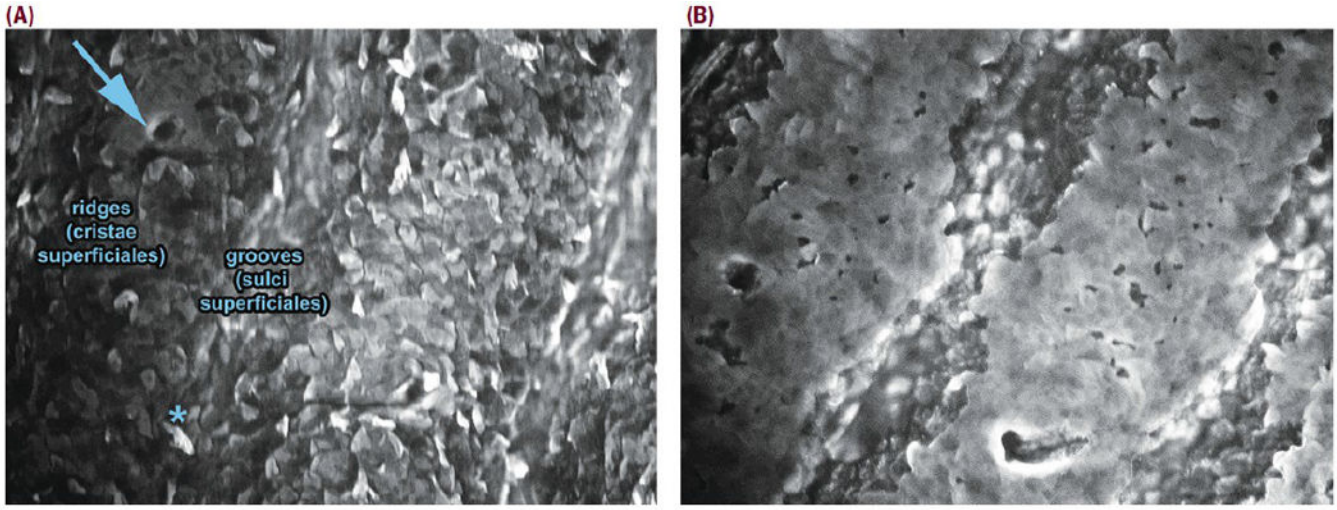

FIGURE 1.

Grayscale MUSE images of the fingertip without moisturizer **(A)** and with moisturizer **(B)**; original magnification × 10. In **(A)**, dermatoglyphics of the fingertip consists of ridges (cristae superficiales) and grooves (sulci superficiales) proved to be straightforward to visualize at high resolution. Desquamation of superficial corneocytes (asterisk) and opening of an acrosyringium (the most superficial portion of eccrine ducts, arrow) were visualized in high-resolution. In **(B)**, a uniform layer of moisturizer could be seen superficial to the corneocytes along the ridges and grooves, and indicating that the superficial skin was well-moisturized.