A Review of Atomic-Force Microscopy in Skin Barrier Function Assessment

Article *in* Journal of Investigative Dermatology · June 2024

DOI: 10.1016/j.jid.2024.04.005

14 authors, including:

Chia-Yu Chu
National Taiwan University Hospital
324 PUBLICATIONS **9,257** CITATIONS

SEE PROFILE

Christoph Riethmüller
University of Münster
91 PUBLICATIONS **2,767** CITATIONS

SEE PROFILE

Hsien-Shun Liao
National Taiwan University
58 PUBLICATIONS **821** CITATIONS

SEE PROFILE

Benjamin Ungar
Icahn School of Medicine at Mount Sinai
117 PUBLICATIONS **5,537** CITATIONS

SEE PROFILE

Please use this PDF proof to check the layout of your article. If you would like any changes to be made to the layout, you can leave instructions in the online proofing interface. First, return to the online proofing interface by clicking "Edit" at the top page, then insert a Comment in the relevant location. Making your changes directly in the online proofing interface is the quickest, easiest way to correct and submit your proof.

Please note that changes made to the article in the online proofing interface will be added to the article before publication, but are not reflected in this PDF proof.

A Review of Atomic-Force Microscopy in Skin Barrier Function Assessment

Jorge Pereda[1], Casper Milde Khatib[2], Sanja Kezic[3], Maria Oberländer Christensen[2], Sara Yang[4], Jacob P. Thyssen[2], Chia-Yu Chu[5], Christoph Riethmüller[6], Hsien-Shun Liao[7], Imtisal Akhtar[1], Benjamin Ungar[8], Emma Guttman-Yassky[8], Merete Hædersdal[9] and En-Te Hwu[1]

Skin barrier function (SBF) disorders are a class of pathologies that affect a significant portion of the world population. These disorders cause skin lesions with intense itch, impacting patients' physical and psychological well-being as well as their social functioning. It is in the interest of patients that their disorder be monitored closely while under treatment to evaluate the effectiveness of the ongoing therapy and any potential adverse reactions. Symptom-based assessment techniques are widely used by clinicians; however, they carry some limitations. Techniques to assess skin barrier impairment are critical for understanding the nature of the disease and for helping personalize treatment. This review recalls the anatomy of the skin barrier and describes an atomic-force microscopy approach to quantitatively monitor its disorders and their response to treatment. We review a panel of studies that show that this technique is highly relevant for SBF disorder research, and we aim to motivate its adoption into clinical settings.

Keywords: Atomic-force microscopy, Atopic dermatitis, Corneocyte morphology, Skin barrier function

Journal of Investigative Dermatology (2024) ■, ■–■; doi:10.1016/j.jid.2024.04.005

INTRODUCTION

Skin barrier function (SBF) disorders are a common class of pathologies that present a major burden on global health. Atopic dermatitis (AD) is an SBF disorder of particular interest, affecting roughly 790 million people (10 % of adults and 20% of children globally [Silverberg et al, 2021]). It is an inflammatory skin disease that causes eczematous lesions and intense itch (Nutten, 2015). The typical onset of AD is in infancy to early childhood, and its increasing prevalence presents serious concerns (Charman et al, 2004; Nutten, 2015). It is a distressing and chronic condition characterized by skin barrier abnormalities, immune deregulation, cutaneous inflammation, and microbiome alteration. Similar to other SBF disorders, AD is sometimes accompanied by atopic conditions such as food allergies, allergic asthma, and allergic rhino-conjunctivitis.

Its symptoms can be severe enough to traumatize and impair the psychological well-being, development, and social functioning (Rønnstad et al, 2018; Urban et al, 2020), not only of the patients but also of their family (Vittrup et al, 2022). Moreover, AD brings a significant economic burden: the total cost per patient with severe AD can reach €15,000 per year (Bieber, 2022).

Although there is no known cure for AD, appropriate treatment can ease symptoms and keep it under control. For moderate to severe AD, physicians have prescribed oral corticosteroids, cyclosporine, or methotrexate (Simpson et al, 2011), which can carry severe dose-dependent side effects, notably nephrotoxicity. Recently, mAbs and Jak inhibitors have emerged as the preferred course for AD management (Dattola et al, 2019). mAbs dupilumab and tralokinumab are approved in the United States and Europe, whereas nemolizumab and lebrikizumab are in clinical trials (Ratchataswan et al, 2021). Jak inhibitors upadactinib and abrocitinib are approved (Kamata and Tada, 2023). However, these treatments represent a significant economic burden, with a lifetime drug cost of up to €268,000 (Zimmermann et al, 2018). Therefore, methods to score the severity of AD—and quantitatively evaluate the efficacy of the interventions—are critical for clinicians and researchers.

[1]*The Danish National Research Foundation and Villum Foundation's Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Health Technology, Technical University of Denmark, Lyngby, Denmark;* [2]*Department of Dermatology, Bispebjerg and Frederiksberg Hospital, University of Copenhagen, Copenhagen, Denmark;* [3]*Amsterdam UMC, Coronel Institute of Occupational Health, Amsterdam, The Netherlands;* [4]*Department of Dermatology, Allergology and Venereology, University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany;* [5]*Department of Dermatology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan;* [6]*Center for Nanotechnology, Serend-ip GmbH, Münster, Germany;* [7]*Department of Mechanical Engineering, National Taiwan University, Taipei, Taiwan;* [8]*The Kimberly and Eric J. Waldman Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, USA; and* [9]*Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark*

Correspondence: En-Te Hwu, Department of Health Technology, Technical University of Denmark, Ørsteds Plads, Building 345C, Lyngby 2800, Denmark. E-mail: etehw@dtu.dk

Abbreviations: ACD, allergic contact dermatitis; AD, atopic dermatitis; AFM, atomic-force microscopy; AK, actinic keratosis; CNO, circular nano-object; DTI, dermal texture index; ICD, irritant contact dermatitis; KC, keratinocyte; LoF, loss-of-function; MCI, methylchloroisothiazolinone; MI, methylisothiazolinone; NMF, natural moisturizing factor; OBD, optical beam deflection; OPU, optical pickup unit; PSPD, position-sensitive photodiode; SB, stratum basale; SBF, skin barrier function; SC, stratum corneum; SCORAD, Severity Scoring of Atopic Dermatitis; SG, stratum granulosum; SLS, sodium lauryl sulfate; SS, stratum spinosum; TEWL, transepidermal water loss

Received 5 May 2023; revised 2 April 2024; accepted 13 April 2024; corrected proof published online XXX

Current scoring methods, such as Severity Scoring of Atopic Dermatitis (SCORAD) (Kunz et al, 1997), Eczema Area and Severity Index (Hanifin et al, 2001; Leshem et al, 2015), Patient-Oriented Eczema Measure (Charman et al, 2004), and Investigator global assessment (Futamura et al, 2016; Simpson et al, 2020, 2011), provide researchers and clinicians with well-tested standards to assess AD severity. However, they rely on visual observation and interrogation of the patients or their parents, which makes subjectivity unavoidable. Moreover, because they are based on symptomatic manifestation of the disease, they cannot be used to study its pre or postsymptomatic evolution. Analytical methods exist (Drutis et al, 2014; Xing et al, 2017), but these are costly, requiring specialized equipment with highly trained operators, and have a turnaround time of several days to weeks. Because of these limitations, current scoring methods could be improved on to make frequent observations, to monitor the early onset and evolution of the disease, to routinely evaluate the effectiveness of a prescribed treatment, or to proactively manage the QOL of the patient in the long term.

To this end, we recall a method that uses a noninvasive skin tape-stripping method for ex vivo analysis. Atomic-force microscopy (AFM) analysis of the corneocyte texture provides an objective and quantitative metric related to SBF able to detect physiological changes before, during, and after the SBF disorder is manifest. This review will discuss the formation of the stratum corneum (SC) and introduce AFM in the field of dermatology. We aim to show that nanotexture analysis is a useful tool to study AD and other skin barrier dysfunctions, and we hope to motivate its adoption in clinical, patient-facing use.

HUMAN SKIN

The skin contains 3 distinct layers: epidermis, dermis, and subcutis or hypodermis (Montagna and Parakkal, 1974; Wong et al, 2016). The epidermis is the outermost barrier and protects the body against chemicals, allergens, and pathogens while maintaining balance of fluids with the outside environment (Koster, 2009; Simpson et al, 2011). The epidermis is divided into 5 layers or strata: SC, stratum lucidum (only present in thick hairless skin [Yousef et al, 2022]), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB) (Fuchs and Raghavan, 2002). The lower strata are predominated by keratinocyte (KC) cells, which differentiate and migrate upward from the SB to the SS. The KCs are connected mechanically by desmosomes and adherens junctions (Hwa et al, 2011).

When KCs reach the SG, they develop intracellular keratohyalin granules containing pro-FLG as the precursor protein of FLG (Thyssen and Kezic, 2014). The SG is also responsible for producing the lipids that complete the skin barrier when reaching the SC. At the border between SG and SC, pro-FLG is dephosphorylated into FLG monomers. In the SC, the FLG monomers aggregate the keratin filaments inside the cell, flattening the KCs and creating macrofibrils that mechanically tether them. The FLG monomers are further hydrolyzed to amino acids and their derivatives by proteases, including caspase-14, bleomycine hydrolase, and calpain 1. The proliferation rate of KCs is equal to the desquamation rate of the SC on the outer surface (Engebretsen et al, 2016). When KCs reach the SC, they differentiate into corneocytes, losing their nucleus and collapsing, to eventually shed off. The entire epidermal differentiation process takes approximately 28 days (Regnier et al, 1993).

The SC

The essential function of the SC is to control water loss and protect against stressors, but it also acts as an absorption route for environmental compounds (Grandjean, 1990). The SC is typically described by a bricks and mortar model of Q7 corneocytes embedded into lipids. The keratin-filled interior of corneocytes is enveloped by cornified lipid layers. The cornification process comprises 3 main events: forming an intracellular keratin network, assembly of cornified lipid envelopes, and selective degradation of corneodesmosomes (Harding et al, 2000).

The maturation of corneocytes and desquamation of superficial cells are well-controlled processes that depend on several parameters such as pH gradient, proteases and their inhibitors, and hydration of the SC. The interior of corneocytes contain components such as lactic acid, various amino acids, pyrrolidone carboxylic acid, urocanic acid, and hyaluronic acid. These molecules constitute the natural moisturizing factors (NMFs) that maintain SC hydration and low pH to prevent cracking and protect against pathogens (Boireau-Adamezyk et al, 2021).

The mature corneocyte has a diameter of approximately 25−35 μm and a surface area of 700−900 μm^2, which increases as they mature (Evans and Roth, 2014; Naoko et al, 2013). Structural changes are visible between young and aged skin because aged skin is characterized by an increase in single-cell surface area (753 ± 120 μm^2; n = 14) compared with young skin (surface area: 555 ± 80 μm^2; n = 10) (Gorzelanny et al, 2006). An increase in corneocyte roughness and a prominent intercellular gap are also characteristic of aged skin.

Skin barrier dysfunction

The SBF resides largely in the highly organized lipid lamellae, composed of ceramides, free fatty acids, and cholesterol. The SBF is commonly determined by measuring transepidermal water loss (TEWL). The upkeep of an adequate skin barrier is also dependent on the skin's NMF concentration, which is essential for skin hydration, desquamation, and plasticity. Loss-of-function (LoF) mutations in *FLG* gene are determinant Q8Q9 of low NMF concentration, but T helper 2−mediated inflammation and exposure to skin irritants can also lead to reduced NMF levels (Thyssen and Kezic, 2014). The NMF concentration can be quantified in vivo or ex vivo by chromatographic or spectrometric methods (Caspers et al, 2001; Drutis et al, 2014; Soltanipoor et al, 2018).

Ex in vivo analysis of SC

The most common method to assess SC composition in vivo is confocal Raman spectroscopy (Caspers et al, 2001; Drutis et al, 2014), which can be used to determine NMF, lipids, and keratin concentrations. However, this method requires expensive instrumentation with highly trained personnel who must convene by the instrument with the patient (Riethmüller, 2018). Ex vivo methods involve skin sample

REV 5.6.0 DTD ■ JID4266_proof ■ 13 May 2024 ■ 11:18 am ■ ce

collection and separate analysis of the sample. These methods allow for repeatability, centralization of analysis, and better convenience to the patient.

A noninvasive way to collect skin tissue is the tape-stripping method (Lademann et al, 2009). It involves pressing a circular adhesive tape for a few seconds onto the surface of the skin and then carefully removing the tape. The outermost layers of corneocytes adhere to the tape and are peeled off. On average, depending on SC cohesion, type of the adhesive tape, and applied pressure, 1 SC layer is removed per tape, but variability is high. For detailed discussion on the amount of corneocytes removed, see Jacobi et al (2005), Keurentjes et al (2021), Simon et al (2023), and Sølberg et al (2019). Successive peels of the same area can be used to study corneocytes at different depths of the SC (Lademann et al, 2009). The skin tape is small (<30 mm in diameter) and lightweight (<1 g), which makes it practical to ship to laboratories for centralized analysis.

The tape-stripped corneocyte samples can be studied either morphologically or biochemically. For biochemical analysis, the tape is soaked in a solvent or buffer to extract the compound of interest, which is then determined by an appropriate method (Hulshof et al, 2019; Riethmuller et al, 2015). In dermatological research, the tape-stripping technique is often used to determine NMF levels in the corneocyte as a biomarker of LoF mutations in *FLG* gene, which is one of the main risk factors in AD development. Furthermore, tape-stripped corneocytes have also been used to determine immunological profiles (Keurentjes et al, 2021).

Beyond biochemical assays, tape-stripped corneocytes lend themselves to morphological characterization. Because they are affected by the condition of the skin during their 4-week maturation from the SB, they carry information about skin abnormalities, which is revealed by imaging. There are 2 main corneocyte imaging methods: scanning electron microscopy and AFM. Sufficient-resolution scanning electron microscopy requires a vacuum environment and metal coating of the sample, which makes it impractical for routine research and clinical applications. In contrast, AFM can achieve atomic resolution without the need for vacuum or special sample preparation. In addition, AFM can probe the sample's mechanical properties such as elasticity and friction. Therefore, AFM is an ideal tool to image (Braet et al, 1998; Fredonnet et al, 2014; Gorzelanny et al, 2006; Kashibuchi et al, 2002) nanotextures and characterize the mechanical properties (Fredonnet et al, 2014; Gaikwad et al, 2010; Tang and Bhushan, 2010) of the corneocyte.

AFM

Introduction to AFM

AFM has demonstrated its potential in biological science, affording high-resolution topography of biological samples under physiological conditions (Danzberger et al, 2018; Franz et al, 2016; Fredonnet et al, 2014; Gorzelanny et al, 2006; Rankl et al, 2010), used to determine the mechanical properties of various tissues (Braet et al, 1998; Grandbois et al, 2000; Hwang et al, 2013); analyze protein structure (Hansma and Hoh, 1994); and image living cells (Kasas et al, 1993), chromatin structures (Lohr et al, 2007), and biological membranes (Frederix et al, 2009).

AFM is a scanning-probe technique, in which the sample is fixed on a translation stage that uses piezoelectric transducers to move the sample on the XY plane. The probe is a microscale cantilever with a sharp tip (apex diameters range from nm to μm scale depending on the application) carefully brought to contact with the sample. The sample surface exerts a force on the tip in the Z direction, which deflects the cantilever. A raster image of the sample surface topography is produced by scanning the XY plane and recording the cantilever deflection as a function of XY position. In conventional AFMs, cantilever deflection is detected by an optical beam deflection (OBD) system, where a laser beam is focused on the upside of the cantilever and the reflected beam is made to strike a position-sensitive photodiode (PSPD). The position of the laser spot on the photodiode is calculated by comparing the electrical current produced by each quadrant of the PSPD. The sensitivity is calibrated, so the probe deflection—and therefore the sample surface height Z—can be directly calculated from the spot displacement.

The mechanical properties of the sample can also be obtained: if the cantilever stiffness is known, the instantaneous tip−sample force is deduced from the deflection (Cappella and Dietler, 1999). From the time-dependent relationship between the tip−sample force and the Z-displacement, properties such as elasticity, adhesion force, and energy dissipation can be derived (Bosco et al, 2013; Braet et al, 1998; Grandbois et al, 2000; Harding et al, 2000; Peñuela et al, 2018; Riethmüller et al, 2007). The mechanical properties of the corneocyte have been shown to correlate with SBF parameters and are likely to have a causative effect on its reduced function (Haftek et al, 2020). By choosing an AFM probe with suitable spring constant and tip material, this measurement can be performed on soft (Dokukin and Sokolov, 2012), hard (Zeng et al, 2018), and elastic (Radmacher, 1997) materials.

General-purpose laboratory AFMs equip many different measurement modes with multiple parameters and have a typical scan rate of 0.5−2 lines per second, which gives 4−17 minutes per image. Usually, this complex system must be tuned and operated by experienced technicians. Moreover, optical table and thermal/acoustic isolation are needed for eliminating environmental interference. The whole AFM setup, including vacuum chambers, delicate optics, electronics, and surrounding accessories, occupy a large space (~1 m^3), bringing the cost of a laboratory AFM up to hundreds of thousands of United States dollars.

Optical pickup unit−based AFM

State-of-the-art AFM has found widespread use in scientific research, but it has not been adopted into clinical dermatology, mainly owing to the cost and complexity of the OBD system and its need for highly trained operators. Optical pickup units (OPU) of consumer CD (Compact Disc), DVD (Digital Versatile Disc), and Blu-ray players have been repurposed for different scientific applications, such as biosensing (Bache et al, 2013; Bosco et al, 2013, 2011, 2010; Hwu and Boisen, 2018; Hwu et al, 2013), physical parameter characterization (Chang et al, 2022; Hwu et al, 2012, 2008; Liao et al, 2018, 2012), and micro/nanoscale 3-dimensional

REV 5.6.0 DTD ■ JID4266_proof ■ 13 May 2024 ■ 11:18 am ■ ce

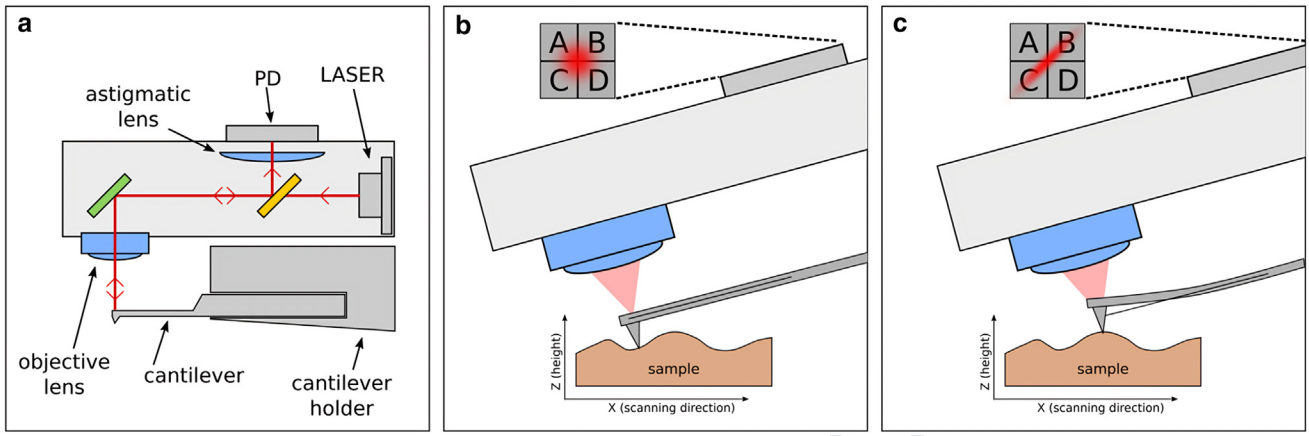

Figure 1. Schematic and operating principle of an OPU-based AFM system. **(a)** Simplified schematic of an OPU-based AFM, not to scale. Green, mirror; yellow, beam splitter; and blue, lenses. The circular beam emitted by the integrated LASER is guided to a lens that focuses the light on the back of an AFM cantilever mounted under a commercial OPU. The light is reflected and made to strike an integrated PD through an astigmatic lens. **(b)** When the cantilever is not deflected, its back remains on the focal plane, and the LASER spot on the PD remains circular. **(c)** Deflection of the cantilever moves its reflective back outside of the focal plane, causing the LASER spot on the PSPD to be elongated. The deflection of the cantilever is calculated by comparing the current produced by each quadrant of the PD. AFM, atomic-force microscopy; OPU, optical pickup unit; PD, photodetector; PSPD, position-sensitive photodiode.

printing (Chang et al, 2021). Hwu et al (2009, 2008) realized that the OPUs can be used to measure cantilever deflection of the AFM probe (Grey, 2015; Lopez Martinez et al, 2016), replacing the traditional optical system. They constructed an OPU-based AFM (Figure 1) (Hwu et al, 2009, 2007), which senses cantilever deflection without the need for external optics (Liao et al, 2014) and is capable of topographic imaging and calibrated force–displacement imaging (Chang et al, 2022; Liao et al, 2012). Hwu et al (2008) demonstrated that this microscope affords a resolution in air (Hwu et al, 2008) and liquid environments (Hwang et al, 2013; Liao et al, 2013) comparable with conventional AFM.

The optics and electronics of this microscope are integrated in the OPU, a mass-produced inexpensive and robust module, which requires no user tuning. Because OPU sensitivity does not depend on the optical distance between the cantilever and the photodetector, these microscopes are extremely compact. These advantages make OPU-based AFM accessible to clinical settings and allow for the adoption of AFM as a routine diagnostic technique. Hwu et al (XXX) are actively developing open-source hardware and clinic-ready user interfaces that require minimal training to produce nanometer-resolution images (Liao et al, 2022).

AFM in dermatology

Despite its cost and complexity, traditional AFM has become a principal tool in ex vivo dermatological research (Alsteens et al, 2017; Gaikwad et al, 2010; Iravanimanesh et al, 2017; Kashibuchi et al, 2002; Olejnik and Nowak, 2017; Peñuela et al, 2018; Qassem and Kyriacou, 2019; Rankl et al, 2010; Rinnov et al, 2023; Stylianou, 2017; Tang and Bhushan, 2010) used to analyze the morphology, presence of antibodies, response to irritants and treatments, and mechanical properties of KCs (Boyle et al, 2019; Ramms et al, 2013; Laly et al, 2021; Miroshnikova et al, 2018; Connelly et al, 2021). The fast-advancing field of low-cost OPU-based AFM has opened the opportunity to bring AFM techniques from research into clinics (Liao et al, 2022).

The following section discusses nanotexture analysis, a promising application of AFM, for both AD research and AD health care.

CORNEOCYTE NANOTEXTURE ANALYSIS

The deep side of tape-stripped corneocytes displays widely varying nanotextures. One of these is a circular nanotexture, commonly found on inflammatory skin and mostly absent in healthy skin (Fredonnet et al, 2014), which is thought to be a result of irregular maturation of corneocytes (Engebretsen et al, 2018a). This circular nanotexture has been called villous-like projections (Naoko et al, 2013; Zeng et al, 2018), bead- or nipple-like elevation (Radmacher, 1997), nanoscale protrusion (Gaikwad et al, 2010), and circular nano-objects (CNOs) (Franz et al, 2016). Because a biological function has not yet been definitively ascertained, this document prefers the term CNO, a strictly phenotypic nomenclature (Riethmüller, 2018).

A dermal texture index (DTI) can be obtained by counting CNOs in nanometer-resolution images of tape-stripped corneocytes. This CNO count was described by Riethmüller (2018) and Franz et al (2016) and thoroughly discussed in Riethmüller 2018). Franz et al (2016) found that skin of healthy subjects had a count of 24 ± 21, nonlesional skin from AD-affected subjects had a count of 116 ± 53, and AD lesional skin had a count of 529 ± 277, regardless of skin pigmentation, making the number of CNOs a quantitative score for AD severity assessment. The research that studies its significance in AD-related conditions can be found in Engebretsen et al (2018a, 2018b), Franz et al (2016), Hulshof et al (2019), Koppes et al (2017), Riethmuller et al (2015), Rüther et al (2021), Soltanipoor et al (2018), Thyssen et al (2020), and Vater et al (2021).

Nanotexture-based research in AD

Riethmuller et al (2015) investigated corneocyte topography in children with AD and related the measured DTI with *FLG* LoF mutations, TEWL, NMF, and AD severity measured by SCORAD. Subjects with LoF *FLG* mutations (−/− or −/+) had

REV 5.6.0 DTD ■ JID4266_proof ■ 13 May 2024 ■ 11:18 am ■ ce

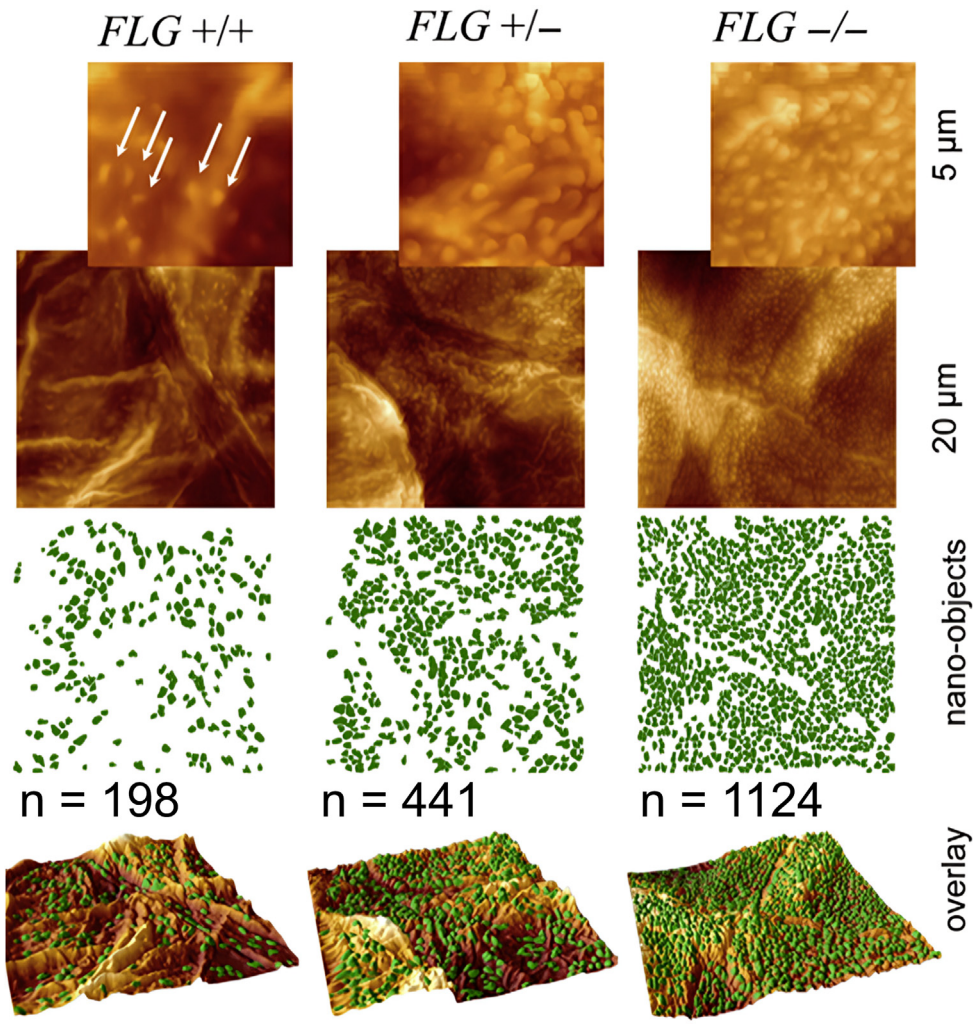

Figure 2. AFM images of skin samples from patients with AD with different *FLG* mutations. Figure was reprinted from Riethmuller et al (2015) with permission from Elsevier. AD, atopic dermatitis; AFM, atomic-force microscopy.

considerably higher DTI than wild-type (+/+) subjects with AD (Figure 2), with a correlation to disease severity (SCORAD). A significant (negative) correlation was observed between DTI and NMF. DTI was found to correlate more strongly with NMF (r = 0.80) (Riethmuller et al, 2015) than with SCORAD, suggesting that the absence of FLG rather than the presence of inflammation drives the formation of CNOs (villous-like projections in their article).

Nanotexture analysis can also help investigate the correlation between different skin phototypes, immune response biomarkers, and NMF. Hulshof et al (2019) found that immune-response biomarkers (IL-1β, CXCL8, and CCL22, etc) varied significantly between healthy skin and nonlesional AD skin but were independent of skin phototype. In contrast, NMF and CNO-count correlations with AD varied between phototypes.

Nanotexture-based SBF assessment

Engebretsen et al (2018a) studied healthy controls and subjects with a history of AD who were asymptomatic or only mildly symptomatic. Their barrier function parameters—TEWL, monomeric FLG levels, and NMF concentrations—were measured as well as their DTIs. A negative correlation between NMF and TEWL was found. The participants with a history of AD had different nanotexture and lower levels of monomeric FLG and NMF than the healthy controls. In subjects with a history of AD, low levels of monomeric FLG and NMF correlated with the presence of *FLG* mutations. In a separate study, Engebretsen et al (2016) found that DTI and TEWL were positively correlated in both healthy skin and skin with compromised barrier function.

AFM-based research in contact dermatitis

Irritant contact dermatitis (ICD) is a common inflammatory skin disease that occurs when exposed to chemicals such as detergents, alkaline agents, acids, and organic solvents. When skin is exposed to a common detergent, the surface topography of corneocytes changes, as do TEWL and NMF, followed by a significant increase in DTI within 72–96 hours (Koppes et al, 2017; Rüther et al, 2021; Soltanipoor et al, 2018; Vater et al, 2021).

Allergic contact dermatitis (ACD) is clinically similar to ICD. For diagnosis, patch testing, a well-established technique, is used. It is performed by exposing patients to suspected allergens on a small skin area (<1 cm^2). Inflammation at the application site of a particular substance is considered a positive test and proof of sensitization. However, many allergens often exert irritant properties, which makes

interpretation of the patch test difficult. To explore DTI as a tool to distinguish between ICD and ACD, Koppes et al (2017) studied ex vivo the changes in NMF and corneocyte morphology in skin exposed to common allergens: chromium, nickel, methylchloroisothiazolinone (MCI), methylisothiazolinone (MI), and p-phenylenediamine (Koppes et al, 2017). As an irritant compound, sodium lauryl sulfate (SLS) was included. Elevated DTI was observed when the skin was exposed to SLS but also to MCI/MI, which caused a remarkable change in NMF levels and had a profound effect on corneocyte morphology. They speculate that MCI and MI exert skin irritation by damaging the cornified envelope, resulting in leakage of NMF.

Soltanipoor et al (2018) investigated the effect of different skin irritants on corneocyte surface topography. They applied acetic acid, n-propanol, SLS, and sodium hydroxide and measured several skin parameters 24 and 96 hours after application. SLS had the most pronounced impact on corneocyte surface topography. They concluded that SLS may increase the SC pH value and play a predominant role in the degradation of FLG into NMF compounds. SLS denatures cornified envelope proteins, pro-FLG processing, and desquamatory SC enzymes and may denature NMF compounds. The remaining irritants significantly increased the CNO count and induced prominent topographical changes such as thinning of fibers, elongated spots, and wrinkles, which appeared after 24 hours and became abundant after 96 hours of exposure (most abundant with SLS and n-propanol). CNO count was inversely related to NMF levels, which agrees with previous studies (Koppes et al, 2017). This suggests that skin texture analysis by AFM is a reliable method to discern true allergic reactions from simple irritation (Corsini and Galbiati, 2019).

AFM-based seasonal change assessment

Seasonal factors significantly affect NMF and corneocyte morphology (Engebretsen et al, 2018a). Low humidity and low temperature have an adverse effect on SBF and increase the risk of dermatitis in general. The degradation of FLG into NMF components increases when the ambient humidity decreases, which causes a reduction of NMF level on human cheeks (Scott and Harding, 1986).

Engebretsen et al (2018a) studied the seasonal impact on skin morphology and NFM levels of 80 healthy subjects. Relative to those of summer, winter NMF levels and DTI were reduced and elevated, respectively. However, UV exposure elevated the DTI and caused changes in the corneocyte morphology on the exposed area of the hand and cheek. Although low doses of UVB irradiation have positive effects on SBF (Engebretsen et al, 2016), high-dose irradiation of UVB negatively affects the corneodesmosomes and intercellular lipids, leading to decreased skin integrity and skin barrier impairment (Engebretsen et al, 2018a). UV reduces the SC hydration necessary to maintain NMF levels on the cheeks. The reduced skin hydration could initiate FLG degradation, altering corneocytes. Other factors that could be responsible for alterations on corneocyte morphology are an immature cornified envelope and a disorganized cytoskeleton. In contrast to winter, the higher temperature in summer stimulates sweating, which could contribute to higher NMF levels. An age dependency of NMF was observed: younger participants had less NMF than older participants and less NMF in winter than in summer. This is consistent with previous studies by high-performance liquid chromatography (Engebretsen et al, 2016) and Raman spectroscopy (Egawa and Tagami, 2008). The lower NMF levels in young skin could be explained by the difference in the level of FLG and its metabolites: a Japanese study (Takahashi and Tezuka, 2004) had previously investigated FLG degradation in those aged 60−81 years compared with that in those aged 1−10 years. It found that age markedly reduces FLG levels and markedly increases byproducts of FLG degradation, which are amino acids and lipids that contribute to NMF. A similar increase in NMF levels was previously observed in cheek and arm skin of aged subjects compared with that in young subjects (Egawa and Tagami, 2008). Contrary to NFM, many other SC components (eg, lactates, ceramides, urea) were lower in aged skin, although the increased NFM levels in aged subjects could be possibly explained by a larger cell surface (Gorzelanny et al, 2006) and longer SC transit time (Grove, 1983; Mohammed et al, 2012).

AFM-based actinic keratosis assessment

Actinic keratosis (AK) is a frequent premalignant skin lesion that develops in light skin phototype (types I and II) subject to chronic sun exposure (Keurentjes et al, 2020). Perhaps motivated by the clinical similarity of inflammatory disease and keratosis conditions and by the success of AFM in investigating inflammatory disease, Keurentjes et al (2020) studied the morphology, DTI, and NFM in lesional AK and surrounding skin. They found that a change in the general topography of corneocytes was marked, concomitant with a marked increase in DTI. They found that DTI decreases and that NMF increases gradually with distance to the lesion, remaining affected even in regions with no visible changes.

DISCUSSION

Although AFM has shown great promise in skin research, some limitations remain. The high cost and complexity of traditional AFM have hindered clinical adoption. Furthermore, data acquisition and analysis currently require specialized expertise. Imaging artifacts can arise from factors such as tip contamination, especially in biological samples. Only surface topology is directly accessible, whereas mechanical data require additional analysis. Finally, skin sampling by tape stripping is sometimes difficult in patients with damaged skin, and high-resolution throughput is relatively low.

To address these limitations, various AFM technologies are emerging. Low-cost, compact OPU-based AFMs could enable point-of-care use. Hwu et al (XXX) are developing user interfaces that allow a minimally trained user to produce[Q11] and analyze corneocyte images. High-speed mechanical mapping through force−displacement scanning is being implemented (Liao et al, 2019), and automated imaging workflows and machine learning analysis will aid standardization. These developments have the potential to make AFM more accessible, streamlined, and informative.

Looking ahead, AFM has prospects for even greater impact in dermatology, with significant potential to provide

REV 5.6.0 DTD ■ JID4266_proof ■ 13 May 2024 ■ 11:18 am ■ ce

nanoscale insights into skin health and disease. As a quantitative biomarker of skin barrier impairment, AFM could enable diagnostic screening, treatment selection, and therapeutic monitoring. Personalized medicine may be advanced by correlating nanotexture to genetic, immune, and molecular profiles in precision skin phenotyping. Beyond AD, AFM could provide insights into other inflammatory and age-related conditions affecting skin barrier and integrity. Point-of-care AFMs could allow rapid assessment during clinic visits to inform therapy.

SUMMARY

SBF deregulations such as the ones found in AD have a significant and mounting impact on the well-being of the global population. In this review, we briefly described the anatomy involved with SBF. We introduced the use of AFM in dermatology and how it is used in scientific research to establish a quantitative ex vivo measurement by counting CNOs per unit area. We introduced the OPU-based AFM, a technology that can potentially make AFM-based measurements accessible to routine clinical applications. We reviewed a panel of articles that use corneocyte nanotexture to study the correlations between surface nanotexture and other parameters of skin health. We hope to show that AFM is a valuable tool in our understanding of dermatological diseases associated with impaired SBF, and we hope to motivate its adoption in clinical settings to provide caregivers and patients a powerful practical tool in the diagnosis and management of AD and other skin barrier dysfunctions.

ORCIDs

Jorge Pereda: http://orcid.org/0000-0002-4802-0591
Casper Milde Khatib: http://orcid.org/0000-0002-4973-3745
Sanja Kezic: http://orcid.org/0000-0002-1063-4547
Maria Oberländer Christensen: http://orcid.org/0000-0001-7177-1054
Sara Yang: http://orcid.org/0000-0001-8854-0965
Jacob P. Thyssen: http://orcid.org/0000-0003-3770-1743
Chia-Yu Chu: http://orcid.org/0000-0002-9370-3279
Christoph Riethmüller: http://orcid.org/0000-0002-0116-984X
Hsien-Shun Liao: http://orcid.org/0000-0003-1338-0332
Imtisal Akhtar: http://orcid.org/0000-0001-9960-1110
Benjamin Ungar: http://orcid.org/0000-0003-0882-8163
Emma Guttman-Yassky: http://orcid.org/0000-0002-9363-324X
Merete Hædersdal: http://orcid.org/0000-0003-1250-2035
En-Te Hwu: http://orcid.org/0000-0002-5971-4978

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the Villum Experiment (grant number 23116), the Danish National Research Foundation (grant number DNRF122), the Villum Foundation (grant number 9301) for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), the LEO Foundation (grant number LF-OC-20-000370) project: Rapid Clinical Assessment of Skin Barrier Function by Corneocytes Nanotexture; and the Novo Nordisk Foundation (grant number NNF22OC0076607) project: Rapid Skin Barrier Function Diagnostic Assessment.

AUTHOR CONTRIBUTIONS

Conceptualization: E-TH, JP; Funding Acquisition: E-TH; Investigation: JP, CMK, SK, MOC, SY, JPT, C-YC, CR, H-SL, BU, EG-Y, MH, E-TH; Methodology: JP, E-TH; Project Administration: E-TH; Supervision: CMK, SK, MOC, SY, JPT, C-YC, CR, H-SL, BU, EG-Y, MH, E-TH; Validation: CMK, SK, MOC, SY, JPT, C-YC, H-SL, BU, EG-Y, MH, E-TH; Writing — Original Draft Preparation: JP, E-TH; Writing — Review and Editing: CMK, SK, MOC, SY, JPT, C-YC, H-SL, IA, BU, EG-Y, MH, E-TH

REFERENCES

Alsteens D, Gaub HE, Newton R, Pfreundschuh M, Gerber C, Müller DJ. Atomic force microscopy-based characterization and design of biointerfaces. Nat Rev Mater 2017;2:17008.

Bache M, Bosco FG, Brøgger AL, Frøhling KB, Alstrøm TS, Hwu ET, et al. Nanomechanical recognition of prognostic biomarker suPAR with DVD-ROM optical technology. Nanotechnology 2013;24:444011.

Bieber T. Atopic dermatitis: an expanding therapeutic pipeline for a complex disease. Nat Rev Drug Discov 2022;21:21–40.

Boireau-Adamezyk E, Baillet-Guffroy A, Stamatas GN. The stratum corneum water content and natural moisturization factor composition evolve with age and depend on body site. Int J Dermatol 2021;60:834–9.

Bosco FG, Bache M, Yang J, Chen CH, Hwu ET, Lin Q, et al. Micromechanical PDGF recognition via lab-on-a-disc aptasensor arrays. Sens Actuators A Phys 2013;195:154–9.

Bosco FG, Hwu ET, Chen CH, Keller S, Bache M, Jakobsen MH, et al. High throughput label-free platform for statistical bio-molecular sensing. Lab Chip 2011;11:2411–6.

Bosco FG, Hwu ET, Keller S, Greve A, Boisen A. Self-aligned cantilever positioning for on-substrate measurements using DVD pickup head. Microelectron Eng 2010;87:708–11.

Boyle CJ, Plotczyk M, Villalta SF, Patel S, Hettiaratchy S, Masouros SD, et al. Morphology and composition play distinct and complementary roles in the tolerance of plantar skin to mechanical load. Sci Adv 2019;5:eaay0244.

Braet F, Seynaeve C, De Zanger R, Wisse E. Imaging surface and submembranous structures with the atomic force microscope: a study on living cancer cells, fibroblasts and macrophages. J Microsc 1998;190:328–38.

Cappella B, Dietler G. Force-distance curves by atomic force microscopy. Surf Sci Rep 1999;34:1–104.

Caspers PJ, Lucassen GW, Carter EA, Bruining HA, Puppels GJ. In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. J Invest Dermatol 2001;116:434–42.

Chang TJ, Nielsen LH, Boisen A, Hwu ET. Open-source force analyzer with broad sensing range based on an optical pickup unit. HardwareX 2022;11:e00308.

Chang TJ, Vaut L, Voss M, Ilchenko O, Nielsen LH, Boisen A, et al. Micro and nanoscale 3D printing using optical pickup unit from a gaming console. Commun Phys 2021;4:23.

Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure: development and initial validation of a new tool for measuring atopic eczema severity from the patients' perspective [published correction appears in Arch Dermatol 2005;141:381]. Arch Dermatol 2004;140:1513–9.

Connelly JT, Gavara N, Sliogeryte K, Blowes LM. Research techniques made simple: analysis of skin cell and tissue mechanics using atomic force microscopy. J Invest Dermatol 2021;141:1867–18671.e1.

Corsini E, Galbiati V. Contact allergy to fragrances: in vitro opportunities for safety assessment. Cosmetics 2019;6:3.

Danzberger J, Donovan M, Rankl C, Zhu R, Vicic S, Baltenneck C, et al. Glycan distribution and density in native skin's stratum corneum. Skin Res Technol 2018;24:450–8.

Dattola A, Bennardo L, Silvestri M, Nisticò SP. What's new in the treatment of atopic dermatitis? Dermatol Ther 2019;32:e12787.

Dokukin ME, Sokolov I. Quantitative mapping of the elastic modulus of soft materials with HarmoniX and PeakForce QNM AFM modes. Langmuir 2012;28:16060–71.

Drutis DM, Hancewicz TM, Pashkovski E, Feng L, Mihalov D, Holtom G, et al. Three-dimensional chemical imaging of skin using stimulated Raman scattering microscopy. J Biomed Opt 2014;19:111604.

Egawa M, Tagami H. Comparison of the depth profiles of water and water-binding substances in the stratum corneum determined in vivo by Raman spectroscopy between the cheek and volar forearm skin: effects of age, seasonal changes and artificial forced hydration. Br J Dermatol 2008;158:251–60.

Engebretsen KA, Bandier J, Kezic S, Riethmüller C, Heegaard NHH, Carlsen BC, et al. Concentration of filaggrin monomers, its metabolites and corneocyte surface texture in individuals with a history of atopic dermatitis and controls. J Eur Acad Dermatol Venereol 2018a;32:796–804.

Engebretsen KA, Johansen JD, Kezic S, Linneberg A, Thyssen JP. The effect of environmental humidity and temperature on skin barrier function and dermatitis. J Eur Acad Dermatol Venereol 2016;30:223–49.

Engebretsen KA, Kezic S, Riethmüller C, Franz J, Jakasa I, Hedengran A, et al. Changes in filaggrin degradation products and corneocyte surface texture by season. Br J Dermatol 2018b;178:1143–50.

Evans ME, Roth R. Shaping the skin: the interplay of mesoscale geometry and corneocyte swelling. Phys Rev Lett 2014;112:038102.

Franz J, Beutel M, Gevers K, Kramer A, Thyssen JP, Kezic S, et al. Nanoscale alterations of corneocytes indicate skin disease. Skin Res Technol 2016;22:174–80.

Frederix PL, Bosshart PD, Engel A. Atomic force microscopy of biological membranes. Biophys J 2009;96:329–38.

Fredonnet J, Gasc G, Serre G, Séverac C, Simon M. Topographical and nanomechanical characterization of native corneocytes using atomic force microscopy. J Dermatol Sci 2014;75:63–5.

Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. Nat Rev Genet 2002;3:199–209.

Futamura M, Leshem YA, Thomas KS, Nankervis H, Williams HC, Simpson EL. A systematic review of Investigator Global Assessment (IGA) in atopic dermatitis (AD) trials: many options, no standards. J Am Acad Dermatol 2016;74:288–94.

Gaikwad RM, Vasilyev SI, Datta S, Sokolov I. Atomic force microscopy characterization of corneocytes: effect of moisturizer on their topology, rigidity, and friction. Skin Res Technol 2010;16:275–82.

Gorzelanny C, Goerge T, Schnaeker EM, Thomas K, Luger TA, Schneider SW. Atomic force microscopy as an innovative tool for nanoanalysis of native stratum corneum. Exp Dermatol 2006;15:387–91.

Grandbois M, Dettmann W, Benoit M, Gaub HE. Affinity imaging of red blood cells using an atomic force microscope. J Histochem Cytochem 2000;48:719–24.

Grandjean P. European countries discuss environmental health. Arch Environ Health 1990;45:198.

Grey F. Creativity unleashed. Nat Nanotechnol 2015;10:480.

Grove GL, Kligman AM. Age-associated changes in human epidermal cell renewal. J Gerontol 1983;38:137–42.

Haftek M, McAleer MA, Jakasa I, McLean WI, Kezic S, Irvine AD. Changes in nano-mechanical properties of human epidermal cornified cells in children with atopic dermatitis. Wellcome Open Res 2020;5:97.

Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group. Exp Dermatol 2001;10:11–8.

Hansma HG, Hoh JH. Biomolecular imaging with the atomic force microscope. Annu Rev Biophys Biomol Struct 1994;23:115–39.

Harding CR, Watkinson A, Rawlings AV, Scott IR. Dry skin, moisturization and corneodesmolysis. Int J Cosmet Sci 2000;22:21–52.

Hulshof L, Hack DP, Hasnoe QCJ, Dontje B, Jakasa I, Riethmüller C, et al. A minimally invasive tool to study immune response and skin barrier in children with atopic dermatitis. Br J Dermatol 2019;180:621–30.

Hwa C, Bauer EA, Cohen DE. Skin biology. Dermatol Ther 2011;24:464–70.

Hwang IS, Yang CW, Su PH, Hwu ET, Liao HS. Imaging soft matters in water with torsional mode atomic force microscopy. Ultramicroscopy 2013;135:121–5.

Hwu EE, Boisen A. Hacking CD/DVD/Blu-ray for biosensing. ACS Sens 2018;3:1222–32.

Hwu ET, Chen CH, Bosco FG, Wang WM, Ko HC, Hwang IS, et al. High-performance spinning device for DVD-based micromechanical signal transduction. J Micromech Microeng 2013;23. article id 045016.

Hwu ET, Hung SK, Yang CW, Huang KY, Hwang IS. Real-time detection of linear and angular displacements with a modified DVD optical head. Nanotechnology 2008;19:115501.

Hwu ET, Hung SK, Yang CW, Hwang IS, Huang KY. Simultaneous detection of translational and angular displacements of micromachined elements. Appl Phys Lett 2007;91:221908.

Hwu ET, Illers H, Jusko L, Danzebrink HU. A hybrid scanning probe microscope (SPM) module based on a DVD optical head. Meas Sci Technol 2009;20:084005.

Hwu ET, Liao HS, Bosco FG, Chen CH, Keller SS, Boisen A, et al. An astigmatic detection system for polymeric cantilever-based sensors. J Sens 2012;2012. Article ID 580939.

Iravanimanesh S, Nazari MA, Mahjoob M, Sohi MA. Human skin micromechanics measured in-vivo using atomic force microscopy(AFM). Paper presented at: 24th National and 2nd International Iranian Conference on Biomedical Engineering (ICBME). Tehran, Iran: Amirkabir University of Technology; 2017. 30 November–1 December.

Jacobi U, Weigmann HJ, Ulrich J, Sterry W, Lademann J. Estimation of the relative stratum corneum amount removed by tape stripping. Skin Res Technol 2005;11:91–6.

Kamata M, Tada Y. Optimal use of jak inhibitors and biologics for atopic dermatitis on the basis of the current evidence. JID Innov 2023;3:100195.

Kasas S, Gotzos V, Celio MR. Observation of living cells using the atomic force microscope. Biophys J 1993;64:539–44.

Kashibuchi N, Hirai Y, O'Goshi K, Tagami H. Three-dimensional analyses of individual corneocytes with atomic force microscope: morphological changes related to age, location and to the pathologic skin conditions. Skin Res Technol 2002;8:203–11.

Keurentjes AJ, de Witt KD, Jakasa I, Rüther L, Kemperman PMJH, Kezic S, et al. Actinic keratosis and surrounding skin exhibit changes in corneocyte surface topography and decreased levels of filaggrin degradation products. Exp Dermatol 2020;29:462–6.

Keurentjes AJ, Jakasa I, Kezic S. Research techniques made simple: stratum corneum tape stripping. J Invest Dermatol 2021;141:1129–11233.e1.

Koppes SA, Ljubojević Hadžavdić S, Jakasa I, Franceschi N, Riethmüller C, Jurakić Tončic R, et al. Effect of allergens and irritants on levels of natural moisturizing factor and corneocyte morphology. Contact Dermatitis 2017;76:287–95.

Koster MI. Making an epidermis. Ann N Y Acad Sci 2009;1170:7–10.

Kunz B, Oranje AP, Labrèze L, Stalder JF, Ring J, Taïeb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. Dermatology 1997;195:10–9.

Lademann J, Jacobi U, Surber C, Weigmann HJ, Fluhr JW. The tape stripping procedure–evaluation of some critical parameters. Eur J Pharm Biopharm 2009;72:317–23.

Laly AC, Sliogeryte K, Pundel OJ, Ross R, Keeling MC, Avisetti D, et al. The keratin network of intermediate filaments regulates keratinocyte rigidity sensing and nuclear mechanotransduction. Sci Adv 2021;7:eabd6187.

Leshem YA, Hajar T, Hanifin JM, Simpson EL. What the Eczema Area and Severity Index score tells us about the severity of atopic dermatitis: an interpretability study. Br J Dermatol 2015;172:1353–7.

Liao HS, Akhtar I, Werner C, Slipets R, Pereda J, Wang JH, et al. Open-source controller for low-cost and high-speed atomic force microscopy imaging of skin corneocyte nanotextures. HardwareX 2022;12:e00341.

Liao HS, Chen YH, Ding RF, Huang HF, Wang WM, Hwu ET, et al. High-speed atomic force microscope based on an astigmatic detection system. Rev Sci Instrum 2014;85:103710.

Liao H-S, Huang G-T, Tu H-D, Lin T-H, Hwu E-T. A novel method for quantitative height measurement based on an astigmatic optical profilometer. Meas Sci Technol 2018;29:107002.

Liao HS, Huang KY, Hwang IS, Chang TJ, Hsiao WW, Lin HH, et al. Operation of astigmatic-detection atomic force microscopy in liquid environments. Rev Sci Instrum 2013;84:103709.

Liao HS, Juang BJ, Huang KY, Hwu ET, Chang CS. Spring constant calibration of microcantilever by astigmatic detection system. Jpn J Appl Phys 2012;51:08KB13.

Liao HS, Lei KK, Tseng YF. High-speed force mapping based on an astigmatic atomic force microscope. Meas Sci Technol 2019;30:027002.

Lohr D, Bash R, Wang H, Yodh J, Lindsay S. Using atomic force microscopy to study chromatin structure and nucleosome remodeling. Methods 2007;41:333–41.

Lopez Martinez D, Lombraña D, Grey F, Hwu E. A crowdsourcing-based air pollution measurement system using DIY atomic force microscopes. HCj 2016;3:235–41.

Miroshnikova YA, Le HQ, Schneider D, Thalheim T, Rübsam M, Bremicker N, et al. Adhesion forces and cortical tension couple cell proliferation and differentiation to drive epidermal stratification. Nat Cell Biol 2018;20:69–80.

Mohammed D, Matts PJ, Hadgraft J, Lane ME. Variation of stratum corneum biophysical and molecular properties with anatomic site. AAPS J 2012;14:806–12.

Montagna W, Parakkal PF. The Structure and Function of Skin. 3d ed. New York: Academic Press; 1974.

Naoko O, Satoshi H, Fukuyoshi M, Mitsuyoshi H. Changes in villus-like projections of corneocytes from the facial skin in normal infants with or without infantile eczema; useful parameter to assess barrier function. Skin Res Technol 2013;19:361–7.

Nutten S. Atopic dermatitis: global epidemiology and risk factors. Ann Nutr Metab 2015;66:8–16.

Olejnik A, Nowak I. Application of atomic force microscopy in skin related research. 2017. Q18

Peñuela L, Negro C, Massa M, Repaci E, Cozzani E, Parodi A, et al. Atomic force microscopy for biomechanical and structural analysis of human dermis: a complementary tool for medical diagnosis and therapy monitoring. Exp Dermatol 2018;27:150–5.

Qassem M, Kyriacou P. Review of modern techniques for the assessment of skin hydration. Cosmetics 2019;6:19.

Radmacher M. Measuring the elastic properties of biological samples with the AFM. IEEE Eng Med Biol Mag 1997;16:47–57.

Ramms L, Fabris G, Windoffer R, Schwarz N, Springer R, Zhou C, et al. Keratins as the main component for the mechanical integrity of keratinocytes. Proc Natl Acad Sci U S A 2013;110:18513–8.

Rankl C, Zhu R, Luengo GS, Donovan M, Baghdadli N, Hinterdorfer P. Detection of corneodesmosin on the surface of stratum corneum using atomic force microscopy. Exp Dermatol 2010;19:1014–9.

Ratchataswan T, Banzon TM, Thyssen JP, Weidinger S, Guttman-Yassky E, Phipatanakul W. Biologics for treatment of atopic dermatitis: current status and future prospect. J Allergy Clin Immunol Pract 2021;9:1053–65.

Regnier M, Caron D, Reichert U, Schaefer H. Barrier function of human skin and human reconstructed epidermis. J Pharm Sci 1993;82:404–7.

Riethmüller C. Assessing the skin barrier via corneocyte morphometry. Exp Dermatol 2018;27:923–30.

Riethmuller C, McAleer MA, Koppes SA, Abdayem R, Franz J, Haftek M, et al. Filaggrin breakdown products determine corneocyte conformation in patients with atopic dermatitis. J Allergy Clin Immunol 2015;136:1573–80.e2.

Riethmüller C, Schäffer TE, Kienberger F, Stracke W, Oberleithner H. Vacuolar structures can be identified by AFM elasticity mapping. Ultramicroscopy 2007;107:895–901.

Rinnov MR, Halling AS, Gerner T, Ravn NH, Knudgaard MH, Trautner S, et al. Skin biomarkers predict development of atopic dermatitis in infancy. Allergy 2023;78:791–802.

Rønnstad ATM, Halling-Overgaard AS, Hamann CR, Skov L, Egeberg A, Thyssen JP. Association of atopic dermatitis with depression, anxiety, and suicidal ideation in children and adults: a systematic review and meta-analysis. J Am Acad Dermatol 2018;79:448–56.e30.

Rüther L, Kezic S, Riethmüller C. Corneocyte nanotexture as biomarker for individual susceptibility to skin irritants. Ann Work Expo Health 2021;65:201–5.

Scott IR, Harding CR. Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment. Dev Biol 1986;115:84–92.

Silverberg JI, Thyssen JP, Fahrbach K, Mickle K, Cappelleri JC, Romero W, et al. Comparative efficacy and safety of systemic therapies used in moderate-to-severe atopic dermatitis: a systematic literature review and network meta-analysis. J Eur Acad Dermatol Venereol 2021;35:1797–810.

Simon K, Oberender G, Roloff A. Continuous removal of single cell layers by tape stripping the stratum corneum - a histological study. Eur J Pharm Biopharm 2023;188:48–53.

Simpson CL, Patel DM, Green KJ. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. Nat Rev Mol Cell Biol 2011;12:565–80.

Simpson E, Bissonnette R, Eichenfield LF, Guttman-Yassky E, King B, Silverberg JI, et al. The Validated Investigator Global Assessment for Atopic Dermatitis (vIGA-AD): the development and reliability testing of a novel clinical outcome measurement instrument for the severity of atopic dermatitis. J Am Acad Dermatol 2020;83:839–46.

Sølberg J, Ulrich NH, Krustrup D, Ahlström MG, Thyssen JP, Menné T, et al. Skin tape stripping: which layers of the epidermis are removed? Contact Dermatitis 2019;80:319–21.

Soltanipoor M, Stilla T, Riethmüller C, Thyssen JP, Sluiter JK, Rustemeyer T, et al. Specific barrier response profiles after experimentally induced skin irritation in vivo. Contact Dermatitis 2018;79:59–66.

Stylianou A. Atomic force microscopy for collagen-based nanobiomaterials. J Nanomater 2017;2017:1–14.

Takahashi M, Tezuka T. The content of free amino acids in the stratum corneum is increased in senile xerosis. Arch Dermatol Res 2004;295:448–52.

Tang W, Bhushan B. Adhesion, friction and wear characterization of skin and skin cream using atomic force microscope. Colloids Surf B Biointerfaces 2010;76:1–15.

Thyssen JP, Jakasa I, Riethmüller C, Schön MP, Braun A, Haftek M, et al. Filaggrin expression and processing deficiencies impair corneocyte surface texture and stiffness in mice. J Invest Dermatol 2020;140:615–23.e5.

Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. J Allergy Clin Immunol 2014;134:792–9.

Urban K, Chu S, Giesey RL, Mehrmal S, Uppal P, Nedley N, et al. The global, regional, and national burden of atopic dermatitis in 195 countries and territories: an ecological study from the Global Burden of Disease Study 2017. JAAD Int 2020;2:12–8.

Vater C, Apanovic A, Riethmüller C, Litschauer B, Wolzt M, Valenta C, et al. Changes in skin barrier function after repeated exposition to phospholipid-based surfactants and sodium dodecyl sulfate in vivo and corneocyte surface analysis by atomic force microscopy. Pharmaceutics 2021;13:436. Q19

Vittrup I, Droitcourt C, Andersen YMF, Skov L, Egeberg A, Delevry D, et al. Family burden of hospital-managed pediatric atopic dermatitis: a nationwide registry-based study. Pediatr Allergy Immunol 2022;33:e13693.

Wong R, Geyer S, Weninger W, Guimberteau JC, Wong JK. The dynamic anatomy and patterning of skin. Exp Dermatol 2016;25:92–8.

Xing H, Zhang X, Zhai Q, Li J, Wang E. Bipolar electrode based reversible fluorescence switch using Prussian Blue/Au nanoclusters nanocomposite film. Anal Chem 2017;89:3867–72.

Yousef H, Alhajj M, Sharma S. Anatomy, skin (integument), epidermis. Treasure Island: StatPearls; 2022.

Zeng G, Dirscherl K, Garnæs J. Toward accurate quantitative elasticity mapping of rigid nanomaterials by atomic force microscopy: effect of acquisition frequency, loading force, and tip geometry. Nanomaterials (Basel) 2018;8:616.

Zimmermann M, Rind D, Chapman R, Kumar V, Kahn S, Carlson J. Economic evaluation of dupilumab for moderate-to-severe atopic dermatitis: a cost-utility analysis. J Drugs Dermatol 2018;17:750–6.