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METHODS REVIEW

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Noninvasive analysis and minimally invasive in vivo experimental challenges of the skin barrier

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Abstract

In this review, we aim to give a concise and selective overview of noninvasive biophysical analysis techniques for skin barrier analysis (transepidermal water loss, electrical methods, confocal Raman microspectroscopy, sebumeter, reflectance spectrophotometry, tristimulus colorimetry, diffuse reflectance spectroscopy and reflectance confocal microscopy), including advantages and limitations. Rather than giving an exhaustive description of the many techniques currently available, we show the usefulness of a representative selection of techniques in the functional and morphological evaluation of the skin barrier. Furthermore, we introduce human minimally invasive skin challenging models as a means to study the mechanisms regulating skin homoeostasis and disease and subsequently show how biophysical analysis techniques can be combined with these in vivo skin challenging models in the functional and morphological evaluation of the skin barrier in healthy human skin. We are convinced that the widespread application of biophysical analysis techniques in dermatological practice and in cosmetic sciences will prove invaluable in offering personalized and noninvasive skin treatment solutions. Furthermore, combining the human in vivo challenging models with these novel noninvasive techniques will provide valuable methodology and tools for detailed characterization of the skin barrier in health and disease.

KEYWORDS

biophysical analysis techniques, human in vivo challenging models, noninvasive, skin barrier characterization, skin disease

1 | INTRODUCTION

The major function of the skin is to form a barrier between the internal milieu and the hostile external environment, protecting against physical, chemical and microbial insults, as well as against the loss of water and electrolytes.[1,2] This barrier function has been termed "la raison d'être of the epidermis".[3] The barrier function of the skin localizes primarily to the stratum corneum (SC), and its structure can be described as a "brick and mortar" model, in which corneocytes (bricks) are embedded in a water-lipid matrix (mortar).[1,2] The latter is essential in providing the permeability barrier and is composed of ceramides (50%), cholesterol (25%) and free fatty acids (10%-20%), with very little phospholipid. Of note, the barrier function of the skin is not absolute, but it allows a physiological movement of water through the SC and into the atmosphere.[3] This process is known as transepidermal water loss (TEWL).

Several approaches can be used to analyse the physiology and pathology of the skin barrier function. Common to other skin layers, the gold standard in dermatology is the histological and immunohistochemical analysis of biopsies. This procedure

Abbreviations: CRS, Confocal Raman microspectroscopy; DRS, Diffuse reflectance spectroscopy; PASI, Psoriasis Area and Severity Index; PMN, Polymorphonuclear cells; RCM, Reflectance confocal microscopy; SCORAD, SCORing Atopic Dermatitis Index; SC, Stratum corneum; TEWL, Transepidermal water loss.

involves surgical removal of a skin sample following local anaesthesia; after preparatory processing, specimens are stained with dyes to label specific cells, cellular components or structures. While offering unrivalled sensitive and specific analysis of cells and tissue morphology, taking biopsies is invasive and discomfort during the procedure may be experienced. Another widely used method to analyse skin barrier status in dermatology is visual assessment. Being easily accessible, dermatologists can rely on their visual grading of clinical signs to evaluate the type and severity of skin diseases involving skin barrier impairment and inflammation. This has led to the definition of scores based on visual grading of clinical signs such as erythema, dryness and swelling. Examples include the Psoriasis Area and Severity Index (PASI) and the SCORing Atopic Dermatitis Index (SCORAD).[4,5] Despite their reliability when performed by trained physicians, justifying their use in clinical trials, visual assessments remain subjective and, most importantly, cannot appreciate the processes unfolding below the skin surface. Alternatively, removal of corneocytes by adhesive tape (tape stripping) to measure, for example, corneocyte size, maturation or adhesion could be used as a minimally invasive approach.[6,7]

A fourth approach to assess skin barrier status is represented by biophysical analysis techniques.[8,9] The interested reader is referred to dedicated books for an exhaustive description of the many techniques currently available.[10] The focus of this review is, rather, to show the usefulness of a representative selection of biophysical analysis techniques in the functional and morphological evaluation of the skin barrier. Firstly, we provide a concise overview of the biophysical analysis techniques most widely used in the assessment of the skin barrier, highlighting their advantages and pitfalls. Secondly, we introduce noninvasive skin challenging models as a means to study the mechanisms regulating skin homoeostasis and disease. We then show how biophysical analysis techniques can be combined with in vivo skin challenging models in the functional and morphological evaluation of the skin barrier in healthy human skin. Lastly, we conclude by discussing how biophysical analysis techniques for skin barrier analysis can make a meaningful impact in the wider dermatological context.

2 | NONINVASIVE TECHNIQUES FOR SKIN BARRIER ANALYSIS: OVERVIEW, ADVANTAGES AND LIMITATIONS

Over the last four decades, much (cosmetic) research has focused on the development of biophysical analysis techniques.[8,9] These techniques provide objective and noninvasive biophysical or optical measurements. For example, an indirect estimate of the hydration of the SC can be obtained by measurements of conductance or capacitance on the skin surface.[11] The barrier function can be indirectly evaluated by measurement of TEWL, with higher TEWL indicating an impaired skin barrier.[11] Other techniques are based on the interaction of light with various skin structures and on the subsequent measurement of the exiting photons. Examples of these techniques are optical coherence tomography, near-infrared spectroscopy and confocal Raman microspectroscopy (CRS). These have also been reported in the in vivo and noninvasive assessment of skin properties.[12–14] Among these, since the pioneering studies of Caspers et al[15–17] in 2000, CRS has emerged for the evaluation of SC barrier function and hydration at high spatial and temporal resolution. The technique exploits inelastic (Raman) scattering to measure the biochemical composition of the skin.[11] The addition of a confocal pinhole allows to obtain this information in a depth-resolved fashion, resulting in molecular concentration profiles from the skin surface into the dermis of amino acids, sweat constituents, intercellular lipids, proteins and water.[16–18] The penetration and transdermal delivery of topically applied substances can also be studied, provided the substances under investigation have a Raman signal and their amount is sufficient to be detected by currently available devices.[18] Recently, methods have been introduced, to determine the molecular properties of skin barrier function-related parameters, such as water bonding states, intercellular lipid conformation ordering and secondary and tertiary structures of keratin, both in in vivo and ex vivo SC.[19]

CRS has been recognized and widely accepted by scientists working on the frontier of skin research, as well as single cell research, including stem cell characterization.[20–24]A systematic review on CRS for the in vivo assessment of the skin barrier has been published recently.[25] However, being this technique complex and still expensive, it is not widely applied yet.

Reflectance confocal microscopy (RCM) uses the different refractive indexes between the cell structures and the surrounding tissue to provide images with morphological information at a resolution comparable to that of conventional light microscopy.[26,27] A representative selection of biophysical analysis techniques used in the functional and morphological evaluation of the skin barrier is reported in Table 1, and their illustrations are shown in Figure 1.

Given their noninvasiveness, biophysical analysis techniques are invaluable in cosmetic sciences, where the development of new products requires tests on human volunteers.[8,28] This is even more so since the entry into force of the new European Cosmetics Regulation (1223/2009) in 2013, which banned animal testing (art. 18) and introduced more stringent requirements for substantiation of claims about the efficacy and effects of cosmetics (art. 20).[8,28] On the other hand, biophysical analysis techniques have not had a great impact in dermatological practice yet,[9] with an exception for RCM, on its way towards implementation in clinical practice,[29] and dermoscopy, which continues to gain appreciation in general dermatology.[30] The late Professor Albert M. Kligman, one of the greatest exponents of experimental dermatology, was among the first and major advocates of the use of biophysical analysis techniques in dermatological practice.[9] He warned against relying solely on visual assessment, since even clinically normal-appearing skin could hide abnormal changes, a phenomenon he called “invisible dermatoses”.[31] Unfortunately,

biophysical analysis techniques have their limitations. In addition to their cost, which in the case of top-notch techniques can be extremely high, they can measure only one or a few parameters in the very complex environment which is the skin, and some of them are strongly influenced by external factors such as temperature and humidity.[9,11,28] As a consequence, it is frequently

TABLE 1 Overview of biophysical analysis techniques used in the functional and morphological evaluation of the skin barrier

	Measurement principle	Output	Measurement depth	Advantages	Limitations	Ref
Barrier function						
Transepidermal water loss	A measurement chamber is applied on the skin surface; the extremity in contact with the skin is open, the opposite extremity can be open or closed. The chamber contains sensors of relative humidity and temperature. In the open-chamber and closed condenser-chamber methods, the flux of water vapour from the skin surface is calculated from the humidity gradient between the chamber extremities. In the unventilated closed-chamber method, the flux is calculated from the rate of increase of the humidity and temperature readings.	Flux density of water vapour [g/m^2 h]	n.a.	Easy-to-use Small-sized probes for measurement in recessed body parts Relatively inexpensive	Indirect measurement Influenced by environment	[36,70]
Electrical methods	Electrical properties of the SC (mostly capacitance and conductance), dependent on the hydration status, are measured with probes placed in contact with the skin. Capacitance-based instruments apply an oscillating electric field to measure the dielectric constant of the SC. Conductance-based instruments apply an electric current to measure the conductance of the SC.	Estimate of the hydration of the SC expressed in arbitrary units [a.u.] or conductance [μS]	~ 45 μm (capacitance) ~ 15 μm (conductance)	Easy-to-use Small-sized probes for measurement in recessed body parts Relatively inexpensive	Indirect measurement Influenced by environment	[32,71]
Confocal Raman microspectroscopy	Monochromatic laser light is focused in the skin. A tiny proportion of photons undergoes inelastic (Raman) scattering with the endogenous molecules, releasing some energy. Of the photons that exit the skin, only the ones coming from the focus region are detected, thanks to the presence of a confocal pinhole. The photons which underwent frequency shifts due to the release of energy to molecules during the interaction are used to obtain (Raman) spectra. The position and intensity of each peak in the spectra are representative of the different molecules and their amounts.	Direct measurement of water, NMF, lipids, carotenoids and exogenously applied compounds + Estimate of SC thickness [μm]	200-500 μm	High spatial and temporal resolution (lateral: 1 μm, axial: 3-5 μm; 1-3 s/spectrum) High biochemical specificity	Expensive Needs trained personnel for interpretation of spectra Bulky set-up not suitable for measurements in recessed body parts	[16,22,72]

(*Continues*)

TABLE 1 (Continued)

	Measurement principle	Output	Measurement depth	Advantages	Limitations	Ref
Greasiness						
Sebumeter©	A probe containing an opaque plastic strip is pressed on the skin for 30 s. As lipids accumulate on its surface, the tape becomes transparent. Transparency is measured by transmitting light through the tape. The light transmission represents the sebum content.	Sebum content on the skin surface expressed in arbitrary units [a.u.]	n.a.	Easy-to-use Small-sized probes for measurement in recessed body parts Relatively inexpensive	Influenced by environment	[35]
Skin colour/erythema						
Reflectance spectrophotometry	LED light at two (eg *DermaSpectrometer*©) or three (eg *Mexameter*©) wavelengths or corresponding to the full visible spectrum (eg *Dermacatch*©) is emitted by a probe placed on the skin. The reflected light is detected and used to calculate the light absorbed by haemoglobin and melanin according to predefined formulae.	Erythema index [a.u.] Melanin index [a.u.]	n.a.	Easy-to-use Small-sized probes for measurement in recessed body parts Relatively inexpensive	Influenced by environment No information on extent of erythema or on perceived skin colour	[16,22,34–36,70–73]
Tristimulus colorimetry	LED light corresponding to the full visible spectrum (eg *Colorimeter*©) or light from a xenon lamp (eg *Chromameter*©) is emitted by a probe placed on the skin. The reflected light is detected and filtered according to the CIE standard observer curves centred in the blue, green and red. L*, a* and b* values are derived from the curves according to predefined formulae.	Colour expressed in the L*a*b* CIE colour space [a.u.]	n.a.	Easy-to-use Small/medium-sized probes for measurement in recessed body parts Relatively inexpensive	Influenced by environment No information on extent of erythema or on molecular origin of skin colour	[34,73–76]
Diffuse reflectance spectroscopy	Light from a xenon lamp (eg *Spectrophotometer CM*©) is emitted by a probe placed on the skin. The L*a*b* values are derived, and the addition of a spectrometer allows the measurement of the reflectance spectrum in the 400-700 nm range.	Colour expressed in the L*a*b* CIE colour space [a.u.] + Reflectance spectrum	n.a.	Easy-to-use Small/medium-sized probes for measurement in recessed body parts	Influenced by environment No information on extent of erythema	[76]

(*Continues*)

necessary to combine more than one method to obtain an overall clinical picture[28] and to follow guidelines to obtain reliable and reproducible measurements.[32–36] Besides careful measurements, attention must be paid to other aspects of the experiment, from the design of the study protocol, to the selection of volunteers and to the analysis and interpretation of the results, as all these aspects might hamper the meaningfulness of the study.[8] Professor Kligman was well aware of these aspects; as he notably remarked, "a fool with a tool is still a fool".[9]

3 | COMBINATION BETWEEN IN VIVO SKIN CHALLENGING MODELS AND NONINVASIVE TECHNIQUES FOR SKIN BARRIER ANALYSIS

Given the complexity of skin structure and functions, studying the mechanisms regulating skin homoeostasis and underlying skin diseases can be challenging. To investigate one specific process or cell type, in vivo skin challenging models can be used. These consist in

TABLE 1 (Continued)

	Measurement principle	Output	Measurement depth	Advantages	Limitations	Ref
Skin morphology						
Reflectance confocal microscopy	Monochromatic laser light is focused in the skin. The photons are reflected according to the different refractive indexes of the cells and structures present in the skin. Of the photons that exit the skin, only the ones coming from the focus region are detected, thanks to the presence of a confocal pinhole. The photons which are reflected from the skin are used to obtain images, where skin structures with a higher refractive index appear bright (eg melanin, keratin) and structures with a lower refractive index appear dark (eg skin folds).	En face binary images showing skin morphology	200-300 μm	High spatial and temporal resolution (lateral: 0.5-1 μm, axial: 2-5 μm) Real-time imaging Video mode (15-25 frames/s) Medium-sized probes for measurement in recessed body parts	Expensive Needs trained personnel for interpretation of images	[26,27]

a.u., arbitrary units; CIE, Commission Internationale de l'Éclairage; LED, light-emitting diode; n.a., not applicable; NMF, natural moisturizing factor; SC, stratum corneum.

the infliction of a minimally invasive and standardized challenge to the skin, and in the evaluation of cutaneous growth, differentiation and inflammation at one or several points in time. Depending on the time point of measurement, an effect of the challenge can be observed. Early effects are a flair reaction, vasodilation and later in time spongiosis, influx of infiltrating cells and epidermal proliferation occurs. At later stages, para- and hyperkeratosis can be observed. While in vivo challenging models provide a simplification of a complex situation, they allow to retain the interplays and cross-talks among the different cells and mediators in the skin. In this review, we describe three in vivo models: tape stripping, histamine iontophoresis and leukotriene B4.

4 | TAPE STRIPPING

Tape stripping consists in the repetitive application of adhesive tape to remove corneocytes from the stratum corneum. This procedure is used to abrogate, totally or partially, the barrier function of the skin without inducing cytopathic effects on the underlying epidermal keratinocytes.[37,38] Total abrogation can be assessed clinically with the appearance of a red and homogeneously glistening surface characterizing the viable epidermis. Since its introduction in the 1950s,[39] tape stripping has become an established model of acute as well as chronic barrier disruption to investigate homoeostatic repair responses.[37] In many studies, biopsies were taken to investigate these responses at the immunohistochemical level, revealing in a synchronized way the presence of a dermal inflammatory infiltrate, epidermal spongiosis, a stark increase in epidermal proliferation, as well as the presence of parakeratosis and hyperkeratosis.[40–42] Other studies used biophysical analysis techniques only,[43–46] or a combination of biophysical analysis techniques and skin biopsies.[47,48] At the functional level, the acute abrogation of the SC is mirrored by a sharp increase in TEWL and dermal blood concentration measured by diffuse reflectance spectroscopy (DRS)[43–47]; at the morphological level, parakeratosis, hyperkeratosis and epidermal thickness increase due to proliferation and spongiosis can be recognized in RCM images.[48] Representative images of the use of biophysical analysis techniques in the assessment of the skin barrier function after tape stripping are shown in Figure 2.

5 | HISTAMINE IONTOPHORESIS

Iontophoresis is a technique which uses a small and defined electric current to facilitate the transport of charged molecules across the skin.[49] Charged molecules are driven into the skin under the direct influence of an electric field, a process called electromigration.[49–51] Although iontophoresis cannot entirely avoid variability in passive diffusion, the delivery of molecules is less dependent on the condition of the skin.[49] Histamine is a well-known pruritogen and vasoactive substance.[52] The delivery of histamine in salt form (eg histamine dihydrochloride) to the skin via iontophoresis has been extensively performed in the last decades to study, among others mechanisms, local inflammatory skin responses and microcirculation[51,53] and differences in itch perception and vascular response between subjects with inflammatory skin diseases and controls.[54,55]

In a previous study, we analysed skin biopsies taken after histamine iontophoresis and we observed the absence of skin barrier disruption, parakeratosis and hyperkeratosis.[56] We therefore concluded that, in contrast to tape stripping, the impact of this model on the skin barrier function is mild (at least when low histamine concentration and current density are used). Accordingly, assessment with biophysical analysis techniques reveals only a slight increase in TEWL and in SC thickness as measured in RCM images.[45,56] The vasoactive effects of histamine are instead short-lived but prominent and are mirrored by a sharp increase in dermal blood concentration measured by DRS.[56] Representative images of the use of biophysical analysis techniques in the assessment of the skin barrier function after histamine iontophoresis are shown in Figure 2.

6 | LEUKOTRIENE B4

Epicutaneous application of human leukotriene B4 (LTB4) is an established in vivo model that induces skin inflammation locally, as histamine iontophoresis.[57,58] Leukotrienes are intracellular signalling molecules that are overproduced during an allergic and inflammatory response in several tissues, including the skin.[59,60] Leukotrienes are primarily released by inflammatory cells such as neutrophils, basophils, eosinophils, monocytes, macrophages and mast cells.[60,61] They are metabolites of arachidonic acid, derived from the 5-lipoxygenase pathway.[59–61] The analysis of skin biopsies taken after topical application

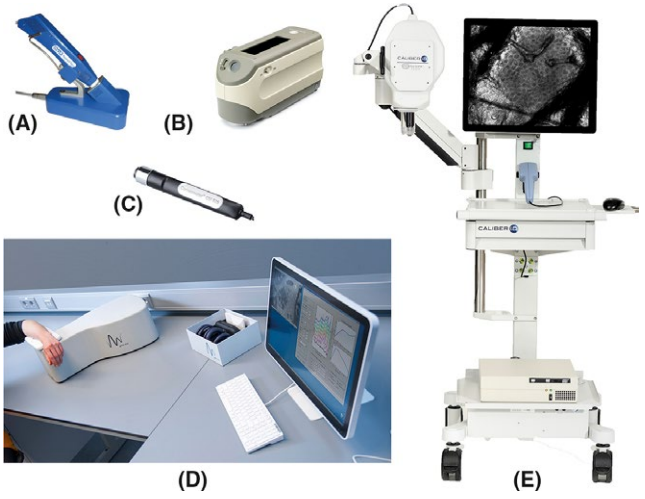

FIGURE 1 Biophysical analysis techniques for in vivo and noninvasive skin assessments. A, Aquaflux AF200 (Biox, UK) for measurement of TEWL; B, Spectrophotometer 2600d (Konica Minolta, Japan) for measurement of skin colour/eythema; C, Corneometer CM825 (Courage and Khazaka, Germany) for indirect measurement of stratum corneum hydration; D, gen2 Skin Composition Analyzer (RiverD International B.V., the Netherlands) for direct measurement of stratum corneum biochemical composition based on the principle of confocal Raman microspectroscopy; e) VivaScope 1500 (Lucid Inc., USA) for imaging of skin morphology based on the principle of reflectance confocal microscopy

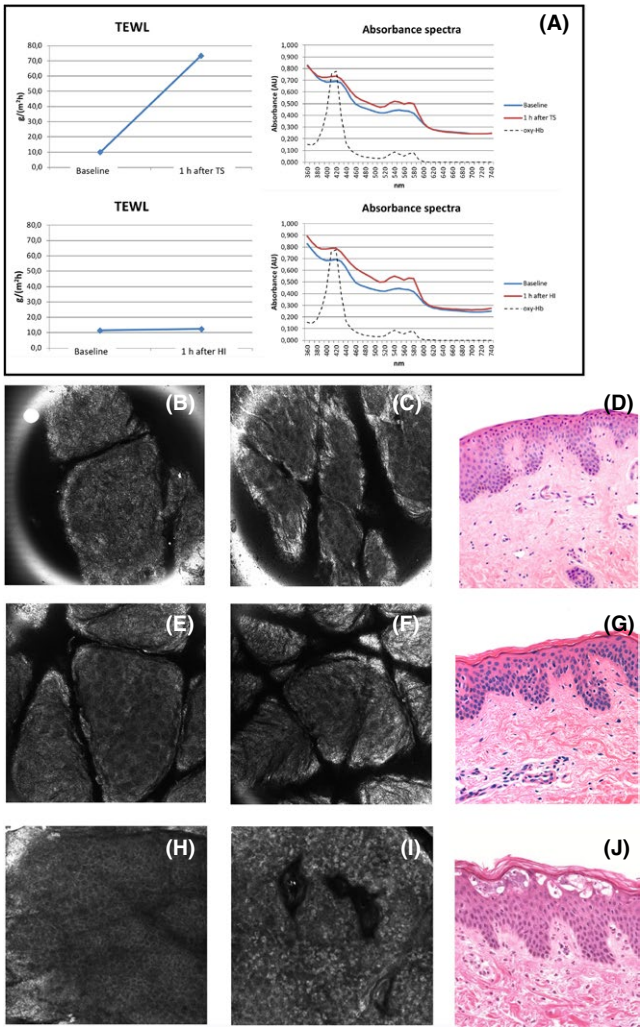

FIGURE 2 A, Measurement of TEWL and absorbance spectra by diffuse reflectance spectroscopy at 1 h after tape stripping (TS, above) and at 1 h after histamine iontophoresis (HI, below). Left: TEWL starkly increases after barrier removal by tape stripping, while it remains almost unchanged after histamine iontophoresis. Right: the absorbance in the 540-580 nm region, where the absorbance peaks of oxygen haemoglobin are located, increases after tape stripping and histamine iontophoresis, reflecting the inflammatory reaction following acute removal of the skin barrier (above) and the vasodilatory effects of histamine (below). The absorbance spectrum of oxygen haemoglobin is rescaled and added to the graphs for comparison. B, C, D, Tape stripping: RCM image at baseline, stratum corneum visible (B). RCM image immediately after tape stripping, nucleated cells of the stratum granulosum visible indicating removal of stratum corneum (C). HE histology confirms the absence of stratum corneum (D). E, F, G, Histamine iontophoresis: RCM image at baseline, nucleated cells at the boundary between stratum corneum and granulosum visible (E). RCM image immediately after histamine iontophoresis, decreased contrast in the nucleated cells at the boundary between stratum corneum and granulosum indicates spongiosis (F). HE histology confirms intact stratum corneum and moderate spongiosis (G). H, I, J, Epicutaneous application of LTB4: RCM image at baseline, honeycomb pattern in stratum spinosum (H). RCM image showing PMN infiltration into the skin 24 h after topical application (I). HE histology confirms the appearance of microabscesses containing PMNs (J)

of LTB4 showed that this model has potent chemoattractant activity for polymorphonuclear cells (PMN), resulting in PMN infiltration into the skin 24 hour after topical application, followed by a mononuclear infiltrate in the dermis between 48 hour and 72 hour.[62] Therefore, this model is useful in studying the specific role of PMN in inflammatory skin diseases like psoriasis.[62] Strikingly, RCM is able to clearly visualize PMN migration, accumulation and degeneration over time in the LTB4 model: PMN cells appear as highly reflective, ill-defined particles with a granular content, visible throughout the epidermis 24 hour after topical application of LTB4.[63] Over time, the appearance of these cells changes throughout the epidermis, corresponding to their degeneration. Representative RCM images are shown in Figure 2.

7 | FUTURE PERSPECTIVES ON THE USE OF NONINVASIVE TECHNIQUES FOR SKIN BARRIER ANALYSIS IN DERMATOLOGY

The sensitivity and specificity of the analysis of cells and tissue morphology offered by skin biopsies make them the gold standard to assess skin physiology and pathology. However, the importance of objective skin assessments offered by biophysical analysis techniques, obtained noninvasively and therefore without discomfort, cannot be underestimated. Recent developments have increased the specificity and sensitivity of information offered by these techniques. An example is CRS, which can directly measure the biochemical components and their distribution in the SC, as opposed to TEWL, which can only offer an indirect assessment of the status of the skin barrier. Besides CRS, successful attempts at directly measuring SC components have been reported with other top-notch techniques. A novel prototype based on near-infrared microspectroscopy has been recently proposed for simultaneous and quantitative measurement of water and lipid levels in the skin.[64] Another study has demonstrated, for the first time, the feasibility of time-of-flight secondary ion mass spectrometry (ToF-SIMS), a surface analysis technique characterized by high chemical sensitivity and specificity, to analyse SC lipids in tape-stripped samples collected in vivo.[65] Efforts have also been directed at overcoming the limitation of single-point measurements given by TEWL and electrical methods for estimating SC hydration. Information on the heterogeneity of the status and hydration of the skin barrier was obtained by imaging the skin with contact-based capacitance sensors[66] and by interpolating single-point measurements to generate continuous colour maps.[67] Another approach to offer a more thorough evaluation of the skin barrier could be to combine macroscopic assessments with biophysical analysis techniques with the assessment of molecular markers of inflammation with skin molecular diagnostic tests, such as Transdermal Analysis Patch.[45,46] To understand better the aberrant lipid organization in challenged or diseased skin, information on the relation between lipid composition and organization is crucial. Therefore, in the future, the use of multiple technologies, qualitative as well as quantitative, in combination with in vivo and in vitro skin barrier models will be needed to get an integrated measure of molecular content and structural morphology, to be able to get a better insight into barrier function.[68]

Despite their potential, biophysical analysis techniques still face some challenges before widespread application in clinical dermatological practice. A potential way forward could also be to promote collaborations between technology experts, dermatologists and skin scientists. The aim would be to determine how to include the information provided by the instrumentation in routine clinical or product testing protocols, and how it could affect the decision-making processes. This view is shared by other authors,[69] who also highlighted the necessity to raise awareness of the existing technologies within the medical community, standardize protocols, determine clinically relevant parameters such as sensitivity and specificity in large clinical trials and determine the cost-effectiveness of the technologies. This approach has been proven successful for RCM, for which a protocol for the diagnosis of chronic plaque psoriasis in dermatological practice has been proposed.[29] The protocol was based on the knowledge of disease features recognizable in RCM images, gained through a series of preliminary clinical studies in psoriasis expertise centres, and on a sound knowledge of relevant literature. As such challenges will be overcome, and more affordable technological implementations will be brought forward, chances are high that the widespread application of biophysical analysis techniques in dermatological practice and in cosmetic sciences will prove invaluable in offering personalized and noninvasive skin treatment solutions.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTIONS

MP and DF performed the research and contributed to the development of the methodology. PvE, MP and DF designed the study and wrote the manuscript. PvE supervised the studies described in the review.

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REFERENCES

[1] K. R. Feingold, P. M. Elias, *Biochim. Biophys. Acta* **2014**, *1841*, 280.
[2] D. J. Tobin, *Chem. Soc. Rev.* **2006**, *35*(1), 52.
[3] K. C. Madison, *J. Invest. Dermatol.* **2003**, *121*, 231.
[4] E. Puzenat, V. Bronsard, S. Prey, P. A. Gourraud, S. Aractingi, M. Bagot, B. Cribier, P. Joly, D. Jullien, M. Le Maitre, C. Paul, M. A. Richard-Lallemand, J. P. Ortonne, F. Aubin, *J. Eur. Acad. Dermatol. Venereol.* **2010**, *24*(Suppl 2), 10.
[5] J. Schmitt, S. Langan, S. Deckert, A. Svensson, L. von Kobyletzki, K. Thomas, P. Spuls, I. Harmonising, *J. Allergy. Clin. Immunol.* **2013**, *132*, 1337.

[6] R. J. Richters, L. D. Janssen, N. E. Uzunbajakava, P. C. van de Kerkhof, P. E. van Erp, Detailed analysis of the stratum corneum in sensitive skin, **2018**.
[7] Y. Naoe, T. Hata, K. Tanigawa, H. Kimura, T. Masunaga, *J. Dermatol. Sci.* **2010**, *57*, 192.
[8] B. Dreno, E. Araviiskaia, E. Berardesca, T. Bieber, J. Hawk, M. Sanchez-Viera, P. Wolkenstein, *J. Eur. Acad. Dermatol. Venereol.* **2014**, *28*, 1409.
[9] A. M. Kligman, Personal Perspectives on Bioengineering and The Skin, In Handbook Of Non-Invasive Methods and The Skin, 2nd ed., CRC press, Boca Raton, USA **2006**.
[10] J. Serup, G. Jemec, G. Grove, Handbook of Non-Invasive Methods and The skin, 2nd ed., CRC Press, Boca Raton, USA **2006**.
[11] R. Darlenski, S. Sassning, N. Tsankov, J. W. Fluhr, *Eur. J. Pharm. Biopharm.* **2009**, *72*, 295.
[12] E. M. Attas, M. G. Sowa, T. B. Posthumus, B. J. Schattka, H. H. Mantsch, S. L. Zhang, *Biopolymers* **2002**, *67*(2), 96.
[13] L. Franzen, M. Windbergs, *Adv. Drug Deliv. Rev.* **2015**, *89*, 91.
[14] F. M. Hendriks, D. Brokken, C. W. Oomens, F. P. Baaijens, *Skin Res. Technol.* **2004**, *10*, 231.
[15] P. J. Caspers, G. W. Lucassen, H. A. Bruining, G. J. Puppels, *J. Raman Spectrosc.* **2000**, *31*(8–9), *6*.
[16] P. J. Caspers, G. W. Lucassen, E. A. Carter, H. A. Bruining, G. J. Puppels, *J. Invest. Dermatol.* **2001**, *116*, 434.
[17] L. Chrit, C. Hadjur, S. Morel, G. Sockalingum, G. Lebourdon, F. Leroy, M. Manfait, *J. Biomed. Opt.* **2005**, *10*, 44007.
[18] J. Lademann, M. C. Meinke, S. Schanzer, H. Richter, M. E. Darvin, S. F. Haag, J. W. Fluhr, H. J. Weigmann, W. Sterry, A. Patzelt, *Int. J. Cosmetic. Sci.* **2012**, *34*, 551.
[19] C. Choe, J. Schleusener, J. Lademann, M. E. Darvin, *J. Biophotonics* **2018**, *11*, e201700355.
[20] M. Hoppel, K. Kwizda, D. Baurecht, M. Caneri, C. Valenta, *Exp. Dermatol.* **2016**, *25*, 390.
[21] F. C. Pascut, S. Kalra, V. George, N. Welch, C. Denning, I. Notingher, *Biochim. Biophys. Acta* **2013**, *1830*, 3517.
[22] P. D. Pudney, E. Y. Bonnist, P. J. Caspers, J. P. Gorce, C. Marriot, G. J. Puppels, S. Singleton, M. J. van der Wolf, *Appl. Spectrosc.* **2012**, *66*, 882.
[23] N. Uzunbajakava, A. Lenferink, Y. Kraan, E. Volokhina, G. Vrensen, J. Greve, C. Otto, *Biophys. J* . **2003**, *84*, 3968.
[24] L. Verzeaux, R. Vyumvuhore, D. Boudier, M. Le Guillou, S. Bordes, M. Essendoubi, M. Manfait, B. Closs, *Exp. Dermatol.* **2018**, *27*, 403.
[25] D. Falcone, N. E. Uzunbajakava, B. Varghese, G. R. de Aquino Santos, R. J. Richters, P. C. van de Kerkhof, P. E. van Erp, *Skin Pharmacol. Physiol.* **2015**, *28*, 307.
[26] P. Calzavara-Pinton, C. Longo, M. Venturini, R. Sala, G. Pellacani, *Photochem. Photobiol.* **2008**, *84*, 1421.
[27] M. Rajadhyaksha, S. Gonzalez, J. M. Zavislan, R. R. Anderson, R. H. Webb, *J. Invest. Dermatol.* **1999**, *113*, 293.
[28] P. Clarys, A.O. Barel, New European Legislation Concerning Efficacy Claims of Cosmetic Products, in Handbook of Cosmetic Science and Technology, 4th ed., CRC press, Baco Raton, USA **2014**.
[29] L. Hoogedoorn, M. J. Gerritsen, E. A. Wolberink, M. Peppelman, P. C. van de Kerkhof, P. E. van Erp, *J. Eur. Acad. Dermatol. Venereol.* **2016**, *30*, 1308.
[30] A. Lallas, J. Giacomel, G. Argenziano, B. Garcia-Garcia, D. Gonzalez-Fernandez, I. Zalaudek, F. Vazquez-Lopez, *Br. J. Dermatol.* **2014**, *170*, 514.
[31] A. M. Kligman, *Arch. Dermatol.* **1991**, *127*, 1375.
[32] E. Berardesca, European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO), *Skin Res. Technol.* **1997**, *3*, 126.
[33] E. Berardesca, J. L. Leveque, P. Masson, E. Grp, *Skin Pharmacol. Physiol.* **2002**, *15*, 442.
[34] G. E. Pierard, *J. Eur. Acad. Dermatol.* **1998**, *10*(1), 1.
[35] G. E. Pierard, C. Pierard-Franchimont, R. Marks, M. Paye, V. Rogiers, *Skin Pharmacol. Appl. Skin. Physiol.* **2000**, *13*, 372.
[36] V. Rogiers, EEMCO Group, *Skin Pharmacol. Appl. Skin Physiol.* **2001**, *14*, 117.
[37] B. J. Nickoloff, Y. Naidu, *J. Am. Acad. Dermatol.* **1994**, *30*, 535.
[38] P. C. van de Kerkhof, H. van Rennes, R. M. de Grood, G. J. de Jongh, F. W. Bauer, P. D. Mier, *Br. J. Dermatol.* **1983**, *109*, 287.
[39] H. Pinkus, *J. Invest. Dermatol.* **1952**, *19*, 431.
[40] M. J. Gerritsen, P. E. van Erp, I. M. van Vlijmen-Willems, L. T. Lenders, P. C. van de Kerkhof, *Arch. Dermatol. Res.* **1994**, *286*, 455.
[41] A. G. Hendriks, R. R. Keijsers, M. M. Seyger, P. E. van Erp, P. C. van de Kerkhof, *Dermatology* **2014**, *228*, 255.
[42] R. Keijsers, A. G. M. Hendriks, P. E. J. van Erp, B. van Cranenbroek, P. C. M. van de Kerkhof, H. Koenen, I. Joosten, *J. Invest. Dermatol.* **2014**, *134*, 1276.
[43] W. A. Boiten, T. Berkers, S. Absalah, J. van Smeden, A. P. M. Lavrijsen, J. A. Bouwstra, *J. Lipid Res.* **2018**, *59*, 250.
[44] R. Voegeli, A. V. Rawlings, B. Summers, *Int. J. Cosmet. Sci.* **2015**, *37*, 241.
[45] D. Falcone, P. Spee, K. Salk, M. Peppelman, P. C. M. van de Kerkhof, P. E. J. van Erp, *Skin Res. Technol.* **2017**, *23*, 336.
[46] D. Falcone, N. E. Uzunbajakava, F. van Abeelen, G. Oversluizen, M. Peppelman, P. E. J. van Erp, P. C. M. van de Kerkhof, *Photodermatol. Photoimmunol. Photomed.* **2018**, *34*, 184.
[47] R. J. Richters, N. E. Uzunbajakava, D. Falcone, J. C. Hendriks, E. J. Jaspers, P. C. van de Kerkhof, P. E. van Erp, *Br. J. Dermatol.* **2016**, *174*, 1126.
[48] M. Peppelman, W. A. van den Eijnde, E. J. Jaspers, M. J. Gerritsen, P. E. van Erp, *Skin Res. Technol.* **2015**, *21*, 474.
[49] A. Sieg, V. Wascotte, *J. Drug Target.* **2009**, *17*, 690.
[50] Y. N. Kalia, A. Naik, J. Garrison, R. H. Guy, *Adv. Drug Deliv. Rev.* **2004**, *56*, 619.
[51] E. Tesselaar, F. Sjoberg, *Microvasc. Res.* **2011**, *81*(1), 88.
[52] A. Ikoma, M. Steinhoff, S. Stander, G. Yosipovitch, M. Schmelz, *Nat. Rev. Neurosci.* **2006**, *7*, 535.
[53] W. Magerl, R. A. Westerman, B. Mohner, H. O. Handwerker, *J. Invest. Dermatol.* **1990**, *94*, 347.
[54] G. Heyer, O. P. Hornstein, H. O. Handwerker, *J. Invest. Dermatol.* **1989**, *93*, 492.
[55] G. Heyer, W. Koppert, P. Martus, H. O. Handwerker, *Acta Derm. Venereol.* **1998**, *78*, 123.
[56] D. Falcone, N. Uzunbajakava, R. Richters, P. C. M. van de Kerkhof, P. E. J. van Erp, *Skin Pharmacol. Physiol.* **2017**, *30*, 246.
[57] F. W. Bauer, P. C. van de Kerkhof, R. M. Maassen-de, *Br. J. Dermatol.* **1986**, *114*, 409.
[58] A. M. Lammers, P. C. van de Kerkhof, *Br. J. Dermatol.* **1987**, *116*, 521.
[59] R. A. Lewis, K. F. Austen, R. J. Soberman, *N. Engl. J. Med.* **1990**, *323*, 645.
[60] C. D. Funk, *Science* **2001**, *294*, 1871.
[61] M. Luo, S. Lee, T. G. Brock, *Histol. Histopathol.* **2003**, *18*, 587.
[62] A. G. Hendriks, R. R. Keijsers, M. M. Seyger, P. C. van de Kerkhof, P. E. van Erp, *Skin Pharmacol. Physiol.* **2014**, *27*, 120.
[63] M. Peppelman, E. A. Wolberink, M. J. Gerritsen, P. C. van de Kerkhof, P. E. van Erp, *Skin Res. Technol.* **2015**, *21*, 232.
[64] A. Ezerskaia, S. F. Pereira, H. P. Urbach, R. Verhagen, B. Varghese, *Biomed. Opt. Express* **2016**, *7*, 2311.
[65] N. J. Starr, D. J. Johnson, J. Wibawa, I. Marlow, M. Bell, D. A. Barrett, D. J. Scurr, *Anal. Chem.* **2016**, *88*, 4400.
[66] D. Batisse, F. Giron, J. L. Leveque, *Skin Res. Technol.* **2006**, *12*(2), 99.
[67] R. Voegeli, A. V. Rawlings, P. Seroul, B. Summers, *Int. J. Cosmet. Sci.* **2015**, *37*, 595.
[68] J. van Smeden, M. Janssens, G. S. Gooris, J. A. Bouwstra, *Biochim. Biophys. Acta* **2014**, *1841*, 295.
[69] H. J. Byrne, M. Baranska, G. J. Puppels, N. Stone, B. Wood, K. M. Gough, P. Lasch, P. Heraud, J. Sule-Suso, G. D. Sockalingum, *Analyst* **2015**, *140*, 2066.

[70] B. a. M. Imhof, G. McFeat, Evaluation of the Barrier Function of Skin Using Transepidermal Water Loss (TEWL), in Handbook of Cosmetic Science and Technology, 4th ed., CRC Press, Boca Raton, USA **2014**.
[71] P. Clarys, R. Clijsen, J. Taeymans, A. O. Barel, *Skin Res. Technol.* **2012**, *18*, 316.
[72] J. C. Lademann, P. J. Caspers, A. van der Pol, H. Richter, A. Patzelt, L. Zastrow, M. Darvin, W. Sterry, J. W. Fluhr, *Laser Phys. Lett.* **2008**, *6*(1), 76.
[73] M. Baquie, B. Kasraee, *Skin Res. Technol.* **2014**, *20*, 218.
[74] P. Clarys, K. Alewaeters, R. Lambrecht, A. O. Barel, *Skin Res. Technol.* **2000**, *6*, 230.
[75] A. R. Matias, M. Ferreira, P. Costa, P. Neto, *Skin Res. Technol.* **2015**, *21*, 346.
[76] G. N. Stamatas, B. Z. Zmudzka, N. Kollias, J. Z. Beer, *Pigment Cell Res.* **2004**, *17*, 618.

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