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Research paper

Non-invasive *in vivo* methods for investigation of the skin barrier physical properties

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ABSTRACT

Skin as an organ of protection covers the body and accomplishes multiple defensive functions. The intact skin represents a barrier to the uncontrolled loss of water, proteins, and plasma components from the organism. Due to its complex structure, the epidermal barrier with its major component, stratum corneum, is the rate-limiting unit for the penetration of exogenous substances through the skin. The epidermal barrier is not a static structure. The permeability barrier status can be modified by different external and internal factors such as climate, physical stressors, and a number of skin and systemic diseases.

Today, different non-invasive approaches are used to monitor the skin barrier physical properties in vivo. The quantification of parameters such as transepidermal water loss, stratum corneum hydration, and skin surface acidity is essential for the integral evaluation of the epidermal barrier status. Novel methods such as *in vivo* confocal Raman microspectroscopy offer the possibility for precise and detailed characterization of the skin barrier.

This paper will allow the readership to get acquainted with the non-invasive, *in vivo* methods for the investigation of the skin barrier.

1. Introduction

Skin, being the largest and the outermost organ of the human body, accomplishes multiple defensive and regulatory functions [1]. The skin barrier function resides almost entirely in the epidermis and, in particular, in its superficial layer – stratum corneum (SC) [2]. According to Oxford advanced learner's dictionary, "barrier" is a term referred to as an object that separates two compartments and/or prevents or hinders the movement from one place to another [3]. Skin is not inertly covering the organism, but rather performs a number of important functions such as protection, excretion, absorption, thermoregulation, and hormone synthesis. In the ontogenesis, the epidermal barrier develops relatively late during embryogenesis (at approx. gestational age of 34 weeks in humans) [4,5]. However, skin barrier function is not completely developed in the early stages of postnatal life [6,7]. The immature epidermal barrier in infants is responsible not only for the disease susceptibility in this lifespan period, but also for the increased permeability of the barrier for exogenous substances [8].

The epidermal barrier protects the human body against many external stressors, namely, physical stress (e.g., mechanical, thermal, radiation), chemical stress (e.g., tensides, solvents, topical xenobiotics), and environmental conditions [9]. Skin as a barrier prevents the organism from loss of essential components such as ions, water and serum proteins. However, the epidermis is not completely impermeable for chemical substances directly applied on the skin surface. This phenomenon is used in topical dermatological therapy as well as in the transdermal drug delivery for the systemic drugs (e.g., hormones). Hence, the investigation of skin barrier functions is important not only for the clinical specialists, but also for the researchers working on (trans)cutaneous drug delivery.

In the past decades, a number of *in vitro, ex vivo, in silico* and mathematical models have been developed for studying and predicting skin barrier permeation and the penetration of exogenous agents [10–13]. However, none of these methodologies can simulate thoroughly the real life conditions in humans [14]. The *in vivo* assessment of skin bioavailability of xenobiotics is interesting for obtaining toxicology and transdermal drug delivery data. Current methods applied *in vivo* human studies, e.g., suction blister fluid, microdialysis, skin biopsy, and tape stripping exhibit certain disadvantages such as the need for standardization and/or the invasiveness of the test procedures [14]. Thus, the constant development of novel, non-invasive, *in vivo* methods for skin barrier research and transcutaneous penetrations is justified.

Abbreviations: CE, cornfied envelope; HSE, heat-separated epidermis; LB, lamellar bodies; NMF, natural moisturizing factors; SLS, sodium lauryl sulfate; SC, stratum corneum; TEWL, transepidermal water loss.

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2. The epidermal barrier – morphological basis

The mammalian skin has layered and complex organization including the SC (the most superficial part of the epidermis), the viable epidermis, and the vascularized dermis. The vast majority of skin barrier functions are attributed to the SC. Over the last decades the simple two-compartment model (“brick-and-mortar”) of the SC structure evolved to a concept presenting SC as a system with a regulated metabolic activity and as a biosensor for external factors (e.g., regulating proteolytic activity, DNA synthesis, and lipid synthesis). Previously considered as immunologically inert, the residential cells of the epidermis (keratinocytes and corneocytes) can secrete pre-formed cytokines (i.e., interleukin-1 alpha) upon skin barrier disruption [15]. SC with its main components, i.e., the corneocytes, the intercorneal bilamellar lipids and the cornified envelope (CE), are considered as the rate-controlling structures for the transcutaneous xenobiotic delivery [9,14].

The mechanical strength of the skin barrier is provided by the corneocytes, embedded in the CE consisting of extensively cross-linked proteins such as loricrin, involucrin and filaggrin. Filaggrin is a member of the S100 Calcium binding protein family. It is derived to the SC from the enzymatic transformation of its precursor, profilaggrin, packed in the keratohyalin granules of stratum granulosum [16,17]. A small percentage of filaggrin quantity is linked to the proteins of the CE (loricrin and involucrin), while the majority is degraded into free amino acids. These components form an important part of the highly hygroscopic complex, natural moisturizing factor (NMF), responsible for the skin hydration and elastic properties [18]. Recent findings revealed that mutations in the gene encoding for filaggrin result in the development of diseases characterized by dry skin and a defective skin barrier such as atopic dermatitis and ichthyosis vulgaris [19–21].

Corneodesmosomes (the desmosomes of the SC) accomplish the intercellular contact of the adjacent corneocytes in the SC. They comprise different proteins, i.e., desmosomal cadherins, desmogleins, and desmocollins, of desmosomal plaque proteins, and of extracellular proteins (corneodesmosin) [22]. Corneodesmosin plays a central role in SC cohesion, as an association between its degradation and the corneocyte shedding is observed [23]. It has been proposed that the sites of corneodesmosome hydrolysis correspond to the “aqueous pore pathway” for water, drug, and xenobiotic movement in the epidermis [24].

The lipid bilayers, adjacent to the corneocytes, are responsible for the protection against uncontrolled water loss from the viable epidermis and regulate the electrolyte movement in the SC. The lipids of SC comprise approximately 50% ceramides, 25% cholesterol, 15% free fatty acids, and some minor lipid components [25]. The three major classes of SC lipids originate from their precursors (phospholipids, glucoceramides, sphingomyelin and free sterols) delivered to the SC by ovoid, membrane-bilayer-enriched, secretory organelles named lamellar bodies (LBs) or Odland bodies. The LBs contain enzymes including lipid hydrolases and proteases important for further extracellular lipid processing and for physiologic desquamation. Once secreted into the intercellular space of the SC, the precursor lipids are transformed by the enzymes co-delivered from the LBs. This process is known as “lipid processing”. Different factors affect this step in lipid transformation, e.g., changes in surface acidity, Calcium gradient and barrier disruption. Inhibition of secretory phospholipase A_2, responsible for conversion of phospholipids to free fatty acids, results in a defect structure of the intercorneocyte lipid membranes [26]. Deficiency in beta-glucocerebrosidase and acidic sphingomyelinase activity, respectively, in Gaucher‘s and Niemann-Pick disease, leads to defects in the extracellular lipid bilayers and disturbance in the skin barrier function [27,28].

The structural organization of the SC lipids has been investigated utilizing different methods such as low- and wide-angle X-ray diffraction, Fourier transformed infrared spectroscopy, and electron microscopy. Early studies evidenced the existence of continuous lipid sheets in the extracellular space of the SC [29]. The use of ruthenium tetroxide as a fixative (instead of the routine osmium tetroxide) in electron microscopy further revealed the organization of the lipid membrane bilayer [30]. It became evident that the packing of the lipids in SC is different from the one in other phospholipid biomembranes. In the SC membrane bilayer, two lamellar phases are present with repeat distances of approximately 6 and 13 nm with mainly crystalline and hexagonal lateral packing of the lipids [31]. Ceramide 1 plays a key role in the formation of the periodicity phase as shown by X-ray diffraction studies [32]. In addition, cholesterol may provide some necessary fluidity to the membranes, thus facilitating the elastic properties of the skin [33]. Thus the structural organization of the lipid bilayers provides the optimal ratio between permeability and fragility of the epidermal barrier. A detailed review on the organization and the phase behaviour of the SC lipids are provided elsewhere [34].

3. Non-invasive *in vivo* methods for assessment of the skin barrier physical properties

In the era of evidence-based medicine, a precise quantification and standardization is requested in the scope of biomedical research. The scientific community is witnessing the development of novel techniques with greater descriptive and accuracy properties [35]. Different non-invasive methods for monitoring skin functions have been introduced, offering the advantages of precise and non-invasive methods – thus harmless investigation of the epidermal barrier properties *in vivo* (Table 1). Due to the complexity of its structure and functions, a single parameter is not sufficient to describe entirely the skin barrier. Thus a multi-parametric approach can be helpful in monitoring the epidermal barrier functions. A similar approach (multi-parameter classification tree), was proposed for the assessment evaluation of allergic patch reactions and skin irritancy *in vivo* by means of different non-invasive techniques [36,37]. The results revealed that there is not a single parameter efficient to embrace fully all pathophysiologic aspects of skin irritancy/contact allergic reaction. Considering and extrapolating the accumulated data, a multi-parametric approach in assessing the skin barrier function is proposed.

Table 2 summarizes the influence of environment and subject-related variables on the measurement of the epidermal barrier parameters.

3.1. Assessment of epidermal barrier function

The assessment of epidermal barrier routinely involves measurements of the transepidermal water loss (TEWL), thus providing information on permeability barrier status under normal, experimentally perturbed, or diseased conditions [38]. The validity of TEWL as a parameter reflecting permeability barrier status was proved by correlating *ex vivo* gravimetric measurements to absolute rates of water loss and determination of TEWL. A low TEWL is generally a characteristic feature of an intact skin function *in vivo* [38]. Hence, elevated TEWL values are observed in a number of diseases with skin barrier abnormalities (e.g., atopic dermatitis, ichthyosis vulgaris, and psoriasis), and in experimental barrier perturbation (e.g., application of detergents, solvents, physical stimuli, and cellophane tape stripping) [39–43].

TEWL measurements can be used to assess the homeostasis of the permeability barrier but also indirectly to predict the influence of topically applied substances on the skin [44]. Furthermore, it might

Table 1
An overview of the non-invasive methods and their applications in the investigation of the skin barrier physical properties *in vivo*.

Parameter	Method	Applications
TEWL	Open chamber	Epidermal barrier integrity under basal conditions and experimental disruption
	Closed chamber	Permeability barrier status
	Ventilated chamber	Disease monitoring (e.g., atopic dermatitis, OSAAD score)
		Effects of externally applied compounds on the skin barrier
SC hydration	Electrical methods (measurement of conductance, capacitance, or impedance)	Water content of the epidermis
	Microwave methods	Skin moisturization/hydration
	Spectroscopic methods	Objective disease evaluation (OSAAD score)
		Water gradient in the SC (only shown *in vivo* by Raman spectroscopy)
		Efficacy of topically applied products (drugs, emollients) and claim support (hydrating effect)
Skin surface pH	Flat glass electrode measurement	Monitoring of skin surface acidity in a defined lifetime range, specific populations (ethnic groups, sensitive skin subjects), anatomical sites
	Tape-stripping techniques	Studying the role of pH for perturbed barrier restoration (link to the metabolic processes in SC)
	Fluorescent lifetime imaging	pH in dermatological and systemic disorders
	Use of pH-sensitive fluorescent dyes	Effects of topically applied substances (medications, cosmetics) on pH
Skin surface lipids	Photometric methods	Estimation of different parameters (sebum casual level, sebum excretion rate, sebum replacement time, instant sebum delivery, follicular excretion rate, and sustainable rate of sebum excretion)
	Solvent extraction	Assessment of skin greasiness/ skin type
	Cigarette paper pads	Anti-seborrhoic efficacy of medications/cosmetics
	Bentonite clay	
	Lipid-sensitive tapes	
SC components (compounds)	Tape harvesting methods (sequential tape stripping, D-Squame, Sebutape)	Investigations in epidermal barrier recovery after perturbation
	Cyanoacrylate strip	SC components (lipids, proteins, enzymes, cytokines, DNA, RNA)
	Solvent extraction	Oxidative stress assessment
		Topical drug availability/pharmacokinetics
Molecular imaging of SC compounds	*In vivo* confocal Raman microspectroscopy	SC thickness (axial resolution of 2 μm)
		SC water gradient
		Semi-quantitative assessment of SC lipids, lactate, urea, urocanic acid and other components
		Topical drug penetration/concentration as a function of SC depth and time

Abbreviations: OSAAD, objective severity assessment of atopic dermatitis; SC, stratum corneum; TEWL, transepidermal water loss.

be an indicative parameter for the permeability of the barrier to externally applied compounds. The capacity of the TEWL to reflect skin barrier properties justifies its use in the efficacy testing of topically applied pharmaceuticals and cosmetic compounds [45,46].

Different methodology settings are used in measuring devices: unventilated chamber (closed) method, ventilated chamber method, and a method using an open chamber [38]. The unventilated chamber probes can potentially occlude the skin and are incapable of continuous measurement. Blocking of the normal evaporation of the skin can partly be solved by shortening the measurement time or by the use of water vapor absorber [47]. Ventilated chambers, using dry or moistened carrier gas, are capable of continuous TEWL measurements. The open chamber instruments are based on the Fick's diffusion law, indicating the quantity being transported per a defined area and period of time. By using the data obtained from thermo- and hydro-sensors, and after processing the information by an inbuilt microprocessor, a numerical value of the TEWL is obtained, commonly shown in $g/m^2/h$. A schematic overview of the measuring principle and the measure with a commercially available open chamber device is shown in Fig. 1.

Due to the discrepancies in the used principle, the results obtained from different devices should not be directly compared [48]. A comparison between three closed chamber instruments and four open chamber devices was performed in different models (human skin *in vivo*, hairless mice *in vivo*, *ex vivo* mouse skin model using gravimetric assay) and across a wide range of perturbations (mild, moderate, severe) [38]. TEWL measurements, with both open and closed systems, correlated significantly with absolute rates of water loss, assessed gravimetrically. A high Pearson correlation coefficient was detectable for data from all instruments vs. gravimetrically assessed TEWL. Nonetheless, all methods could be influenced by the microclimate changes near the skin surface. Hence, the measurements must be performed in climatized rooms with controlled air temperature and relative humidity without direct air flow into the test field [49]. Beyond instrument- and environment-related variables, a number of individual-originating factors influence the

Table 2
Influence of subject- and environment-related variables on the measurement of the epidermal barrier parameters. Extrinsic and intrinsic factors influence epidermal barrier, SC hydration, surface pH and surface lipidse.

Variable	Parameter			
	TEWL	SC hydration	Surface pH	Skin surface lipids
Age	+	+	+	+
Gender	–	–	±	+
Race/ethnicity	±	±	+	–
Anatomical site	+	+	+	+
Skin temperature	+	+	±	n.d.
Sweating	+	+	+	+
Air convection	+	+	n.d.	n.d.
Ambient temperature	+	+	n.d.	n.d.
Humidity	+	+	n.d.	n.d.
Direct light	+	n.d.	n.d.	n.d.
Season	+	+	+	+
Circadian rhythms	+	±	+	+

Abbreviations: SC, stratum corneum, TEWL, transepidermal water loss.
Symbols: "+" influencing; "–" no influence; "n.d." no data; "±" controversial data.

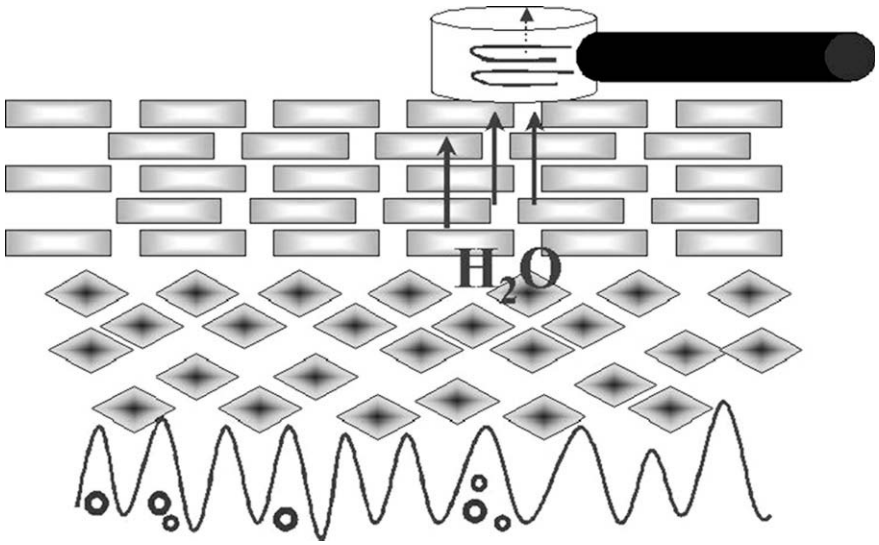

Fig. 1. Measuring principle of an open chamber method an open chamber device: The open chamber instruments are based on the Fick's diffusion law, indicating the quantity being transported per defined area and period of time. The water-containing air is moving along the two sensors within the measurement head. By using the data obtained from thermo- and hydro-sensors, and after processing the information by an inbuilt microprocessor, a numerical value of the TEWL is obtained, commonly shown in $g/m^2/h$.

measurement and should be taken into account, e.g., anatomical site, sweating, and skin surface temperature [48,49].

Measuring TEWL is used in studying skin irritancy. Estimation of TEWL under basal conditions is a good predictor for irritancy susceptibility [50]. A correlation between basal TEWL and the TEWL values post exposure to the model irritant sodium lauryl sulfate (SLS) was set [44,50]. Others could not confirm such a relation [51,52]. The measurement of TEWL is the most appropriate non-invasive modality to distinguish skin changes over time in irritation induced by SLS and tape stripping, compared to irritation models with dithranol, UV irritation and tretinoin [53]. Moreover, it is a very sensitive tool for studying subjective (sensory) in the complex problem of the sensitive skin syndrome [54,55]. Higher baseline TEWL values were measured on the nasolabial fold and cheek in the subject reacting positively to lactic acid (lactic acid sting test) [54].

In addition to the *in vivo* assessment of epidermal barrier properties, the measurement of TEWL was used in a number of *ex vivo* and *in vitro* experimental models [56–58]. Recently developed *in vitro* skin permeation model simulates atopic-like skin changes [59]. Pig ear skin was mounted to Franz-type diffusion cells. Skin barrier impairment characteristic for the disease was achieved by sequential tape stripping. Assessment of TEWL was performed to control and to follow up the barrier abnormalities. Subsequently, the permeation of the skin barrier for topical medications (fusidic acid and betamethasone-17-valerate) was investigated. No statistically significant difference between the penetration of both substances was found in barrier-impaired in comparison to intact skin. The results were in accordance to previously obtained data from an international multicentre clinical study suggesting the predictability of this *in vitro* test [59].

The reliability of TEWL as a barrier integrity parameter for human heat-separated epidermis (HSE) was investigated *in vitro* [60]. Human HSE and punctured HSE (with a needle, up to five holes) were compared and there was no significant difference in the TEWL values. Permeation experiments with flufenamic acid showed a significantly higher diffusion rate through punctured HSE. The authors concluded that TEWL has certain limitations when used in *in vitro* HSE systems as it cannot discriminate minor damage to the SC sufficient to alter the skin permeability. These findings challenge the data obtained from human *in vivo* studies and should be taken in consideration in the study planning phase.

3.2. Measurement of the stratum corneum hydration

Water content of the SC affects barrier permeability, its mechanical properties, as well as the regulation of hydrolytic enzymes involved in the process of normal corneocyte desquamation [61]. Failure of the SC to retain water induces dryness and impairs epidermal barrier function [62,63]. Different *in vivo* methods for the assessment of SC hydration have been described, namely, microwave, thermal, spectroscopic, including nuclear magnetic resonance spectroscopy, infrared and Raman spectroscopies [64,65]. However, most commonly applied methods are based on measuring the electrical conductance, capacitance, or impedance as an indirect indicator for SC water content. Low-frequency skin impedance measurement reflects rather the water content in the living tissues of the skin [66], whereas high-frequency conductance detects more selectively the hydration of SC [67]. The physical principle of a capacitance-based device is schematically presented in Fig. 2.

A micro-sensor, multi-cell technology, Skin Chip®, is useful in providing detailed capacitance mapping of the skin surface in vivo, thus providing an extended picture of the SC hydration [68]. The data on the skin capacitance acquired from each single cell of the sensor are processed by software, thus, resulting in a detailed "capacitance" map (interpreted in terms of skin hydration/dryness) of the investigated skin site.

In general, there is a correlation between SC hydration and TEWL values, so as lower TEWL (intact epidermal barrier function) corresponds to normal hydration state of the horny skin layer [53]. The opposite is valid for disease with epidermal barrier impediment (e.g., atopic dermatitis and ichthyosis vulgaris) i.e., higher proportion of water is being lost through the clinically dry and flaky skin [69]. Discrepancies in the correlation of TEWL and SC hydration are observed in specific anatomic regions such as the palmo-plantar skin as well as in the early stages of SLS-induced irritant reaction (state of hyperhydration is displayed) [70,71] or in extremely damaged skin barrier with elevated TEWL and high hydration values.

Estimation of the dynamics in SC hydration is used in efficacy claim studies on topically applied potentially hydrating (moisturizing) agents [72,73]. The increase in SC hydration correlated with the improvement of the skin barrier function (lowering TEWL) and to the clinical signs of inflammation in patients with atopic

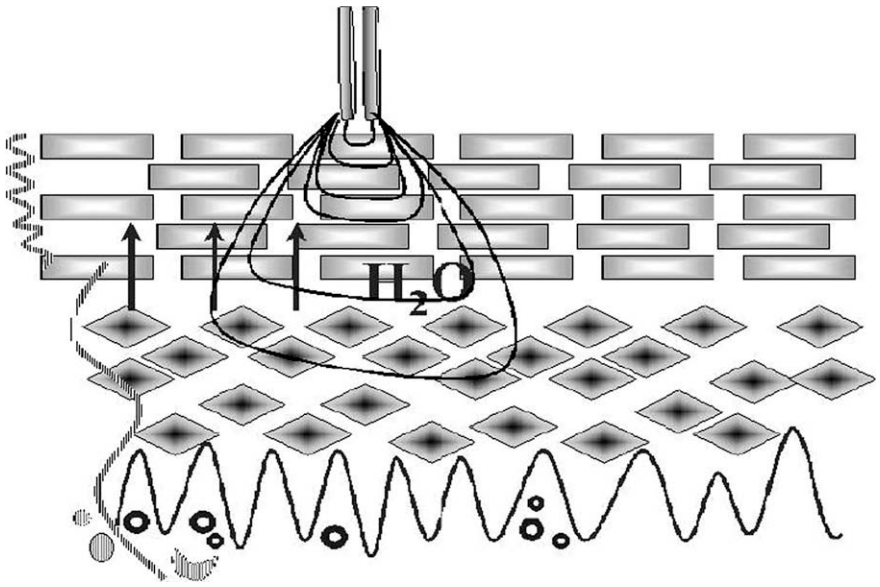

Fig. 2. Capacitance-based device for the assessment of stratum corneum hydration: The physical basis of capacitance-based devices is the difference of the dielectric constant of water and other substances brought in the electrical measurement field. The low operating frequency (40–75 Hz) of the probe makes it sensitive to relative dielectric changes of material placed in contact with the electrode surface and thus altering the electrical field intensity. Stratum corneum is a dielectric medium dependent on its water content, thus allowing different capacitance values to be detected.

dermatitis treated with glycerol-based emollient [72]. Considering the inter-individual variations in the SC hydration levels, the baseline value (before topical treatment introduction) should always be registered as an internal control of the experiment.

The electrical methods give an integrated value of the SC hydration, rather than the actual water distribution of the superficial epidermal layers. Novel methods such as *in vivo* confocal Raman microspectroscopy reveal the actual water gradient of the SC (Fig. 3) [74]. The principle of the method is described elsewhere [75,76]. In brief, the physical basis of the technique is the inelastic light scattering of different molecules. During the measurement, different Raman spectra are obtained specific to the chemical structure of the molecules. Considering the intensities of the Raman bands at certain shifts, the water to protein ratio is calculated. Raman microspectroscopy studies showed *in vivo* that the water content raises gradually from the skin surface to the lower parts of SC with a steep rise at the SC/stratum granulosum boundary [75]. These findings confirm the results of *in vitro* X-ray microanalysis [77]. The high axial resolution (max. 2 μm) and the specificity of Raman microspectroscopy are used in the semi-quantitative measurement of skin components (lipids, lactate, urea, urocanic acid) and exogenously applied substances (dimethyl sulfoxide, trans-retinol, carotenoids) as a function of the depth of the epidermis [61,75,76,78–80].

3.3. Skin surface pH measurement

The acidic milieu of the skin surface plays a central role for the epidermal permeability barrier homeostasis, the restoration of the disrupted barrier, and the non-specific antimicrobial defense of the skin [23,81–83]. The importance of the "acid mantel" of the skin was demonstrated in a number of diseases, e.g., in diaper dermatitis [82]. The incomplete acidification of the SC in neonates, the ammonia-induced alkalization, and the activated stool enzymes (trypsin, lipase) cause irritation and further perturbation of the skin barrier, thus forming the vicious circle in the diaper rash pathogenesis. Furthermore, the recovery of the perturbed barrier is delayed at a neutral pH, due to the disturbance in extracellular processing of the SC lipids [84]. This could be used in the therapeutic and prophylactic approach of diseases with skin barrier disturbances such as atopic dermatitis, psoriasis, and diaper dermatitis. Both non-alkaline detergents and soaps increase the skin surface pH (pH assessment – 10 min after washing) with the highest proportion for the alkaline soap [85]. The use of alkaline soaps can further impede the epidermal barrier restoration. Therefore, acidification of the skin is advised in diseases with skin barrier

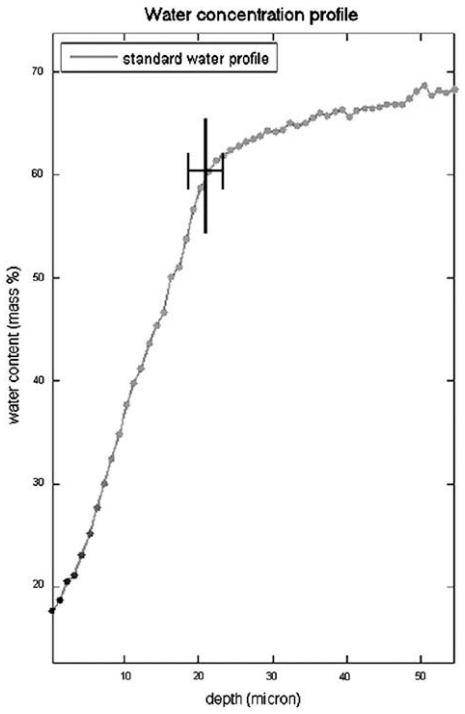

Fig. 3. A typical curve representing the water profile of stratum corneum obtained by *in vivo* confocal Raman microspectroscopy. The line at approximately 20 μm represents the stratum corneum/stratum granulosum interface (± error bars). The present curve is representative for healthy skin.

abnormalities (such as atopic dermatitis) by using cleansers and emollients with low pH and the application of topical salicylates [86]. Three-week treatment of chemotherapy patients with dry skin with an acidic washing and an acidic emollient (both pH 5.5) resulted in a significant improvement of the epidermal barrier (decrease in TEWL) and an increase in the SC hydration [87].

Innovative methods for the assessment of skin surface have been introduced, e.g., fluorescent lifetime imaging and pH-sensitive fluorescent dyes [88]. On the other hand, the flat glass electrode measurements are widely used for being simple, non-cumbersome, and reproducible. Furthermore, the pH values obtained by flat glass electrode assessment were concordant with the ones estimated by fluorescent dye marking [89]. Any commercialised pH meter devices with flat glass electrode can be applied in the measurement of skin surface pH [90].

The *in vivo* assessment of the skin surface acidity is influenced by endogenous factors (age, race, anatomical site, gender, and concomitant disease), exogenous factors (seasonal variations, washing procedures, use of cosmetics and topical medications) and the age of the pH-probe itself [91]. Hence, controlling the different subject- and environment-related variables is essential in performing studies involving the *in vivo* skin surface pH measurement [92].

Short-term exposure of intact skin to neutral pH does not affect the epidermal barrier integrity and functions [23]. However, repetitive and sustained elevation of surface pH (resembling more closely every-day and occupational exposure) resulted in an elevation of the basal TEWL values [93].

3.4. Assessment of skin surface lipids

The protective hydrolipid film on the skin surface was first described in 1928 by Schade and Marchionini [94]. Being a major component of this superficial layer, sebum lipids take part in the non-specific protective mechanisms of the skin barrier. Sebum is produced by the sebaceous glands (with higher density on the forehead, chest, and back) and consists predominantly of triglycerides, wax esters, and squalene. [95]. The role of sebum for the epidermal barrier was demonstrated in asebia mice (with profound sebaceous gland hypoplasia) [96]. Despite the unaffected permeability barrier, asebia mice displayed epidermal hyperplasia, inflammation, and decreased (>50%) SC hydration, associated with a reduction in sebaceous gland lipids. The barrier abnormalities were attributed to the insufficient glycerol levels, derived from the triglyceride hydrolysis. Application of exogenous glycerol, but not a mixture of synthetic, sebum-like lipids, restored the SC hydration [96].

Subjective evaluation of sebum content and excretion could be inconsistent. Several *in vivo* methods have been developed to quantify sebum secretion non-invasively: solvent extraction, cigarette paper pads, photometric assessment, bentonite clay, and lipid-sensitive tapes. The application of these techniques is used to assess different quantitative parameters, i.e., sebum casual level, sebum excretion rate, sebum replacement time, instant sebum delivery, follicular excretion rate, and sustainable rate of sebum excretion [97].

In the past decades, the photometric methods have been established in the diagnostic practise and in clinical trails, as they are time-saving and highly reproducible [97]. Sebumetry is an example of a photometric measurement technique. The principle is based on the fact that an opalescent medium (glass, sapphire plate, plastic strip) of a given opacity to light becomes translucent when its surface is covered with lipids. The value is derived from the difference in transparency of the media before and after the contact with the skin surface, as registered by a light-sensitive unit. Factors such as age, gender, race, anatomical site, and especially topically applied products interfere with the measurement and should be considered in the study planning and performance [97].

Skin surface lipids were evaluated as a function of age, sex and anatomical region [70]. Skin surface lipids did not differ statistically between age groups on all regions except for the ankle, where lipid content was lower in the elderly. This finding can serve as an argument of the epidermal barrier role in the pathogenesis of venous ulcers on the lower limbs of the elderly. A linear correlation between the dynamic friction coefficient and the surface lipids was revealed combining the measurements of all tested skin sites (forehead, upper arm, volar and dorsal forearm, post-auricular, palm, abdomen, upper and lower back, thigh, and ankle) [70]. However, plotting the data for each anatomical region, strong correlation was observed only for forehead and post-auricular area. It was concluded that sebum surface lipids have limited influence on the skin frictional properties.

Casual sebum levels as measured by sebumetry were estimated in 30 adult patients on chemotherapy with dry skin in a controlled, mono-centric, three-week treatment trial [87]. The efficacy of concomitant treatment for the epidermal barrier abnormality with an acidic washing and an acidic emollient (both pH 5.5) was evaluated. A significant increase in the casual sebum levels was revealed in correlation with reduced TEWL (improvement of the permeability barrier) and an increase in the SC hydration.

3.5. In vivo methods for harvesting SC material

Collecting material from the superficial skin layers is applied in studying the biochemical components of the epidermal barrier under basal conditions, in different provocation experiments, and in a number of diseases. From the pharmacological point of view, the SC harvesting methods, and tape stripping in particular, are applied in studies on topical drug bioavailability, absorption, and pharmacodynamics (details provided below in this section) [14].

Different techniques for sampling skin surface material exist such as scraping with a blade, use of topical extracting solutions, and the use of quick-setting cements or glues. Nonetheless, the application of adhesive methods for harvesting SC is commonly used in skin barrier investigations. The place of the "tape stripping" method among the other non-invasive techniques is questionable, as it represents a mechanical SC perturbation *per se*. However, neither previous anesthesia/analgesia/disinfection is required prior to performing the stripping, nor the blood-supplied layers of the skin are reached during the procedure. In addition, only the outermost layer (SC) is removed, comprising cells that had already lost their nuclei during the differentiation process. Nonetheless, due to deductive reasons and the strive for exhaustiveness, the tape stripping method will be shortly reviewed in this section. Tape stripping is a method based on sequential removal of thin layers of approximately 0.5–1 μm from the SC [98]. An adhesive tape is placed on the skin surface with gentle pressure and then it is sharply pulled in the upward direction. The procedure is generally well tolerated, relatively painless, and causes little discomfort to the volunteers. The sequential and repetitive performance of the stripping technique leads to the almost complete removal of the SC indicated visually by the glazing surface of the stripped skin. The perturbation of the epidermal barrier results in activation of restoration processes including the synthesis and delivery to SC of epidermal lipids, DNA synthesis, regulation of mitogen-activated protein kinases, and nuclear hormone receptor–ligand interactions [99–102].

The stripping procedure is influenced by the contact time, the applied pressure and the anatomical site [98]. Thus, a standardized protocol and defining the influencing factors are recommended when a study is initiated.

The integrity and cohesion of the SC can be investigated by adhesive tape methods. The number of tape strips required to induce a predefined degree of barrier disruption (e.g., to raise TEWL values to a certain level) is used to characterize the SC integrity [98]. On the other hand, the SC cohesion is evaluated by the amount of SC removed by sequential tape stripping. An increase in the removed mass indicates a decrease in the cohesion of SC, and in contrary, lower amount of SC removed with increasing tape number is due to the stronger cohesion between the cells in deeper SC layers. Generally the tape stripping removes cell layers maximally down to the glistening layer corresponding to approximately 90% of the SC.

The collected material by tape harvesting is object of further investigation with spectroscopic, cytological and qualitative/quantitative biochemical analysis. The processed data provide information on the compounds and the regulation of biochemical processes in the SC [83], oxidative stress [103], cytokine profiles [104], DNA and RNA synthesis [105], and tracing of chemical substances [106]. The method is efficient in the estimation of topical drug bioavailability as shown for topical antifungal agents [107], sunscreen filters [108], and glucocorticosteroids [109].

Tape stripping is partially validated as an *in vivo* method for estimating the extent of systemic absorption of topically applied substances [110,111]. A good correlation was found between the amount present in the SC 30 min after application of the drug (as estimated by tape stripping technique) and in the urinary excretion over the next 4 days. It was concluded that the method is useful in the estimation of systemic exposure risk, although no precise data on the absorption rate of the substance can be established. A correlation was set between the levels of the drug estimated in the tape strips and the pharmacodynamic effect of the drug topically applied on the skin [109,112]. Skin blanching effect is used to compare the potency of topically applied glucocorticosteroids. For topical betamethasone dipropionate 0.05%, the increasing amount of drug in the tape-stripped SC correlated with an increased skin blanching score [112]. Despite the promising results, tape stripping has certain limitations in its use in bioavailability assays. Further efforts are again needed for standardization to validate the tape harvesting as a method for the assessment of topical drug pharmacokinetics.

4. Conclusion

The availability of various non-invasive techniques for the *in vivo* investigation of skin barrier poses the question on which is the most appropriate method for the characterization of the barrier physical properties. A variety and not one single of the reviewed methods should be used to embrace the full range of biophysical functions of the epidermal barrier. Thus, an integrated and a multi-parametric approach in the evaluation of skin barrier properties should be implemented.

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