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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