Send Orders for Reprints to reprints@benthamscience.ae

Current Pharmaceutical Design, 2016, *22*, 1-9 1

REVIEW ARTICLE

Pharmacological Targeting of the Epidermal Barrier

L. Kemény[1,2], N. Nagy[2,3], Zs.Csoma[2], K. Szabó[1] and G. Erős[2]

[1]*MTA-SZTE Dermatological Research Group, Szeged, Hungary;* [2]*Department of Dermatology and Allergology, University of Szeged, Hungary;* [3]*Department of Medical Genetics, University of Szeged, Hungary*

ARTICLE HISTORY

Received: June 7, 2016
Accepted: July 27, 2016

DOI: 10.2174/1381612822666160726094947

Abstract: The most important function of the skin is to form a barrier between the body and the external environment. The epidermal barrier prevents transepidermal water loss from the skin, but also serves as a barrier to the entry of harmful environmental allergic, toxic or infectious substances. Inherited defects in the genes encoding the components of the epidermal barrier result in the development of rare genetic disorders, whereas polymorphisms in these genes together with environmental factors cause frequent inflammatory skin diseases, such as atopic dermatitis. In this review, components of the skin-barrier function will be reviewed with special emphasis on how the altered epidermal barrier might be repaired. The different strategies to increase the transdermal penetration of drugs is also discussed.

G. Eros

Keywords: Cutaneous barrier, barrier development, barrier impairment, barrier repair.

INTRODUCTION

The human skin forms a complex barrier between our bodies and the external environment [1,2]. It has been known for a long time that epidermal keratinocytes, making up the outermost anatomical structure the epidermis, form an important physical barrier. These cells are the bricks in the outer wall of our bodies; they are very strongly attached to one another by specialized organelles called tight junctions, and the extracellular space between them is very small [3]. This is an important feature of this tissue, as it prevents the loss of water and other important chemicals. In addition, when this layer is intact, it makes it very difficult for environmental molecules and other harmful invaders (e.g., different microbes) to enter our bodies through this strong mechanical boundary.

The epidermis is also a stratified squamous epithelium in which continuously proliferating basal cells in the stratum basale give rise to new keratinocytes that gradually move to the upper epidermal layers (stratum granulosum, spinosum and lucidum). During these events the cells undergo natural differentiation processes. The keratinocytes become flattened, and their cytoplasm is gradually filled with keratin filaments, which are deposited into a matrix composed of mostly filaggrin and its breakdown products, to the point of the death of cells. In the resulting specialized tissue called stratum corneum (SC), dead keratinocytes (corneocytes) are held together by a 'mortar' composed of a lipid-enriched extracellular matrix containing ceramides (50%), cholesterol (30–35%) and free fatty acids (10–15%) [2,4-6]. These lipids are synthesized and released from lamellar bodies at the stratum granulosum – SC-interface, and their final processing is done by hydrolytic enzymes also released in parallel with the lipid precursors [7]. Together with the corneocytes, they form a dry, completely insoluble and nearly impermeable physical structure.

Apart from the formation of the mechanical or physical barrier, another level of protection is the special acidic environment (chemical barrier) established in the first few weeks after birth and maintained on our skins throughout life [8]. This acidic 'mantle' is important for permeability barrier formation and also for antimicrobial defense. It is a result of various acidic components of eccrine and sebaceous secretions, proton pumps, and breakdown products of processed lamellar body lipids [9].

Finally, our skin cells are in close connection with a specialized microbial flora, called the cutaneous microflora or microbiota [10]. Together with the skin cells, they form an immunological barrier, provide efficient protection from harmful pathogenic microorganisms and also, when in a balanced state, help to maintain the integrity and the healthy homeostasis of our bodies. Even though their role has long been suggested, the exact cellular and molecular mechanism contributing to these functions is just being uncovered. In this paper, we summarize what is currently known on this topic.

The cutaneous barrier is very complex, highly organized and strictly regulated. Its structure and function is, in part, genetically programmed. This is proven by the existence of inherited factors, mutations and polymorphisms that affect the development, as well as the structure and thereby also the function of our skins, often leading to the pathogenesis of various cutaneous disorders.

GENETICS OF THE EPIDERMAL BARRIER AND ASSOCIATED DISEASES

Large-scale transcriptome profiling of granular keratinocytes revealed genes (n=330) that are involved in the formation of the epidermal barrier and in the maintenance of its physiological functions [11]. Among the identified genes are several that are implicated in the transformation of granular keratinocytes into corneocytes, in lipid metabolism and transport or in composition and degradation of cell–cell junctions (summarized in Table **1**) [11]. Corneocytes contain abundant keratin: approximately 60% of their dry weight is keratin proteins [12,13]. In epidermal barrier formation, proteins encoded by the *keratin (KRT) 1, 2* and *10* genes are implicated with pivotal roles [13]. The cornified envelopes of the corneocytes contain a large amount of filaggrins, which are associated with keratin intermediate filaments and packed into bundles [14,15]. The keratin-filaggrin bundles contribute to the insolubility

*Address correspondence to this author at the Department of Dermatology and Allergology, University of Szeged, Hungary; Tel: +36-62-545 799; Fax: +36-62-545 799; E-mail: gabor.eros@med.u-szeged.hu

Table 1. Genes involved in the development of the epidermal barrier and their associated diseases.

	Genes involved in the formation of the epidermal barrier	Associated diseases	References
Encoding components of keratinocytes and corneocytes	*KRT 1*	Epidermolytic hyperkeratosis, palmoplantar keratodermas	[12, 13, 50]
	KRT2	Epidermolytic hyperkeratosis	[12, 13, 52]
	KRT10	Epidermolytic hyperkeratosis, ichthyosis with confetti	[12, 13, 52,53]
	FLG	Ichthyosis vulgaris, atopic dermatitis	[14,15, 16, 17,48,49]
	LOR	Loricrin keratoderma	[18]
	TGM1	Autosomal recessive congenital ichthyosis	[17,44,45,47]
	POMP	Keratosis linearis with ichthyosis congenita and sclerosing keratoderma	[19]
	ERCC2	Trichothiodystrophy	[20,21]
	ERCC3	Trichothiodystrophy	[20,21]
	C7ORF11	Trichothiodystrophy	[20,21]
Encoding components of lipid metabolism	*FALDH*	Sjögren-Larsson syndrome	[22]
	PHYH	Refsum syndrome	[23,24]
	ALOX12B	Autosomal recessive congenital ichthyosis	[25]
	ALOXE3	Autosomal recessive congenital ichthyosis	[25]
	CYP4F22	Autosomal recessive congenital ichthyosis	[26,27]
	NIPAL4	Autosomal recessive congenital ichthyosis	[26,27]
	LIPN	Autosomal recessive congenital ichthyosis	[26,27]
	STS	X-linked ichthyosis	[28,29,30]
	EBP	Conradi-Hünermann-Happle syndrome, MEND syndrome	[28,29,30,54]
	MBTPS2	Ichthyosis follicularis-alopecia-photophobia syndrome, keratosis follicularis spinulosa decalvans, olmsted syndrome	[28,29,30]
	SLC27A4	Ichthyosis prematurity syndrome	[31,32]
	ABCA12	Autosomal recessive congenital ichthyosis	[31,32]
	AP1S1	MEDNIK syndrome	[33,34,35]
	SNAP29	CEDNIK syndrome	[33,34,35]
	VPS33B	Arthrogryposis-renal dysfunction-cholestasis syndrome	[33,34,35]
Encoding components of cell–cell junctions	*CDSN*	Epidermolysis bullosa simplex, hypotrichosis simplex, peeling skin syndrome	[36]
	SPINK5	Netherton syndrome	[37,38]
	ST14	Autosomal recessive congenital ichthyosis	[37,38]
	CTSA	Peeling skin syndrome	[39,55]
	GJB2	Erythrokeratodermia variabilis, keratitis-ichthyosis-deafness syndrome, palmoplantar keratodermas, ectodermal dysplasias, deafness syndromes	[40,41,42,43]
	GJB3	Erythrokeratodermia variabilis, deafness syndromes	[40,41,42,43]
	GJB4	Erythrokeratodermia variabilis	[40,41,42,43]
	CLDN1	Neonatal sclerosing cholangitis with ichthyosis syndrome	[40,41,42,43]

of the epidermal barrier significantly [14,15]. The precursor protein of filaggrin, profilaggrin, is encoded by the *filaggrin (FLG)* gene, which contains several tandem filaggrin repeats [16,17]. The segregation of these repeats results in polymorphic variation in the size of the *FLG* gene due to simple allelic differences between individuals [16,17]. Another major component of the cornified cell envelope is the protein encoded by the *loricrin (LOR)* gene [18]. The cornified envelope proteins are crosslinked with disulfide and gamma-glutamyl-lysine isodipeptide bonds by transglutaminase enzymes such as the one encoded by the *transglutaminase 1 (TGM1)* gene [17]. Other clinically relevant genes contributing to the proper functioning of corneocytes are the following: the *proteasome maturation protein (POMP)* gene, encoding a protein pivotal in proteasome assembly [19], the *excision repair complementing defective in Chinese hamster (ERCC) 2* and *3* genes and the *chromosome 7 open reading frame 11 (C7ORF11)* gene, encoding proteins important in DNA transcription and excision repair [20,21].

Other genes contribute to epidermal barrier formation either through the metabolism of fatty acids, triglycerides and cholesterol or through lipid transport and secretion. Long-chain fatty aldehydes are oxidized to fatty acids by the enzyme encoded by the *fatty aldehyde dehydrogenase (FALDH)* gene [22]. Branched fatty acids are broken down by another enzyme encoded by the *phytanoyl-CoA hydroxylase (PHYH)* gene [23,24]. The *12R-lipoxygenase (ALOX12B)* and the *epidermis-type lipoxygenase (ALOXE3)* genes encode enzymes catalyzing the conversion of arachidonic acid to 12R-hydroxyeicosatetraenoic acid [25]. The proteins encoded by the *cytochrome P450 family 4 subfamily f polypeptide 22 (CYP4F22)*, the *NIPA-like domain-containing 4 (NIPAL4)* and the *lipase family member N (LIPN)* genes also participate in the signaling pathways associated with fatty acids and triglycerides [26,27]. The enzymes encoded by the *steroid sulfatase (STS)*, the *emopamil-binding protein (EBP)* and the *membrane-bound transcription factor protease site 2 (MBTPS2)* genes are implicated in epidermal barrier dysfunction through the metabolism of cholesterol [28-30]. The *solute carrier family 27 member 4 (SLC27A4)* and the *ATP-binding cassette subfamily A member 12 (ABCA12)* genes encode transporter proteins [31,32]. Proteins encoded by the *adaptor-related protein complex 1 sigma-1 subunit (AP1S1)*, the *synaptosomal-associated protein 29-kd (SNAP29)* and the *vacuolar protein sorting 33 B (VPS33B)* genes are involved in trafficking [33-35].

In the development of the epidermal barrier, other genes are also implicated through coding either structural components of the cell–cell junctions or proteolytic enzymes regulating the digestion of these junctions. The protein encoded by the *corneodesmosin (CDSN)* gene participates in the formation of corneodesmosomes [36], while the proteins encoded by the *serine protease inhibitor Kazal-type 5 (SPINK5)* and the *membrane-type serine protease 1 (ST14)* genes are involved in the degradation of these structures [37,38]. These proteolytic enzymes are regulated by inhibitors, such as the cysteine proteinase inhibitor encoded by the *cystatin A (CTSA)* gene [39]. Some of the genes involved in the development of other cell–cell junctions, such as the *gap junction protein beta (GJB) 2, 3* and *4* genes and the *claudin 1 (CLDN1)* gene, also contribute to the proper function of the epidermal barrier [40-43].

The above reviewed genes have clinical significance, since their mutations result in the development of rare, monogenic diseases. Many mutations result in genodermatoses, characterized by epidermal barrier dysfunction due to abnormalities in corneocytes, epidermal lipids or cell–cell junctions. Disease-causing genetic variations of the genes implicated in the development of corneocytes are associated with high clinical heterogeneity. *TGM1* mutations have been linked to several clinical variants of autosomal recessive congenital ichthyosis, such as the well known lamellar ichthyosis phenotype and the self-healing collodion baby phenotype, in which the condition is present at birth, but spontaneously improves [44,45]. A very rare form of this latter clinical variant is the acral self-healing collodion baby, in which the membrane is located on the extremities only [46]. *TGM1* mutations can also lead to development of bathing suit ichthyosis, another clinical form of autosomal recessive congenital ichthyosis, in which scaling is pronounced in the bathing suit area and is less pronounced on extremities [47]. *FLG* mutations have been associated with the development of the most common and mildest form of hereditary non-syndromic ichthyosis, ichthyosis vulgaris [17,48]. Of note, among common diseases, *FLG* variations are major predisposing factors for atopic dermatitis [49]. *KRT1* mutations impairing the interaction and network formation of intermediate filaments are frequently associated with epidermolytic hyperkeratosis [50]. *KRT1* mutations are also involved in the development of palmoplantar keratodermas [51]. *KRT2* and *KRT10* disease-causing genetic variations can also be associated with epidermolytic hyperkeratosis [52]. *KRT10* mutations can also contribute to ichthyosis with confetti, a rare form of ichthyosis with a reticulated pattern [53]. *LOR* mutations result in loricrin keratoderma [18]. *POMP* causative variants are associated with keratosis linearis with ichthyosis congenita and sclerosing keratoderma [19]. *ERCC2*, *ERCC3* and *C7ORF11* mutations are pivotal in trichothiodystrophy; additionally, *ERCC2* and *ERCC3* causative variants are implicated in xeroderma pigmentosum [20,21].

Causative variations of the genes linked with abnormal lipid metabolism and transport and, thus consequently epidermal barrier dysfunction, can contribute to the development of clinical variants of the same disease with overlapping symptoms, since *ALOX12B, ALOXE3, CYP4F22, NIPAL4, ABCA12* and *LIPN* mutations are frequently associated with the different clinical forms of autosomal recessive congenital ichthyosis [23,25-27,32]. *FALDH* mutations result in Sjögren-Larsson syndrome [22]. *PHYH* causative variants are associated with Refsum syndrome [24]. *STS* disease-causing variants contribute to the development of X-linked ichthyosis [28]. *EBP* mutations are linked with Conradi-Hünermann-Happle or MEND syndromes [29,54]. *MBTPS2* mutations can lead to the development of ichthyosis follicularis-alopecia-photophobia syndrome, keratosis follicularis spinulosa decalvans and Olmsted syndrome, characterized by mutilating palmoplantar keratoderma with periorificial keratotic plaques [30]. *SLC27A4* mutations lead to ichthyosis prematurity syndrome [31].

Disease-causing variants of genes implicated in trafficking can lead to severe multisystem disorders with overlapping clinical features: *AP1S1* mutations cause the mental retardation, enteropathy, deafness, neuropathy, ichthyosis, and palmoplantar keratoderma (MEDNIK) disorder, whereas *SNAP29* mutations are associated with cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma (CEDNIK) [34,35]. *VPS33B* mutations contribute to development of arthrogryposis-renal dysfunction-cholestasis syndrome [33].

Mutations of genes implicated in the formation and degradation of cell–cell junctions and, thus consequently epidermal barrier dysfunction, are associated with high clinical heterogeneity. *CDSN* mutations can lead to the development of epidermolysis bullosa simplex, hypotrichosis simplex and peeling skin syndrome [36]. *CTSA* mutations are also implicated in peeling skin syndrome [55]. *SPINK5* causative variants are associated with Netherton syndrome and atopy [38]. *ST14* disease-causing variants result in autosomal recessive congenital ichthyosis [37]. Since *GJB2, 3* and *4* genes are expressed in a wide range of organs and tissues, their mutations are implicated in numerous diseases, such as erythrokeratodermia variabilis, keratitis-ichthyosis-deafness syndrome, palmoplantar keratodermas, ectodermal dysplasia and deafness syndromes [40,42,43]. *CLDN1* mutations contribute to the development of neonatal sclerosing cholangitis associated with ichthyosis syndrome [39]. The functional classification of the diseases featuring epidermal barrier dysfunction gives insight into the functional relationships among

these entities and also highlights the significant clinical heterogeneity of these diseases.

DEVELOPMENT OF THE EPIDERMAL BARRIER

All of the anatomic elements of the skin are fully developed by weeks 22 to 24 of gestation, whereas functional and biochemical maturity requires a much longer time. At gestational week 24, the epidermis is immature, with the SC consisting of only one to two cell layers [56,57]. In preterm infants, the full thickness of the skin (0.9 mm) is much less than in term infants (1.2 mm), and this is also the case for the thickness of the epidermis and SC [56,57]. The uppermost layer of the epidermis, the SC, which consists of corneocytes, plays a considerable role in the barrier function of the skin [58-60]. The physical barrier of the skin represented by the SC is mainly determined by its thickness and integrity. By weeks 33 to 34 of gestation, the SC has attained structural and functional maturity, although the active adaptation and maturation processes continue after birth [56,57,59,60]. Changes in skin pH, development of the protecting acid envelope and continuous colonization by cutaneous microbiota play a crucial role in the adaptation process [56-59].

The basic structural differences between the skin of a preterm neonate, a term neonate and an adult are of considerable importance in clinical practice. The structure of the skin of a full-term neonate is similar to that of an adult, but it is much thinner and more vulnerable [60,61]. The skin of a term neonate is structurally and functionally more ready to adapt to an air environment than the skin of a premature infant, which is in homeostasis with a fluid environment. After delivery, premature skin matures rapidly over 2 to 8 weeks, but this process takes significantly longer for extremely premature neonates [56,57,60].

In premature infants, the structural and functional maturation of the epidermis accelerates significantly, taking approximately 2 weeks after birth. As a consequence of this accelerated maturation period, the epidermis of an extremely premature infant undergoes a dramatic development during these 2 weeks, resulting in markedly decreased transepidermal water loss (TEWL) and a reduced possibility to absorb various toxic agents [58,60]. Preterm neonates are obviously highly vulnerable during this 2-week period. Septic complications occur mainly in the first few days or the first 2 weeks of life and are the most common cause of mortality in this special population. The compromised epidermal barrier function results in an enhanced susceptibility to severe invasive infections, high rates of TEWL, thermal instability, electrolyte imbalance, increased percutaneous absorption of chemicals and drugs, and easily induced skin traumas; these clinical complications are relevant determinants of the high morbidity and mortality rates for preterm infants [56-58,61]. In extremely premature infants, the TEWL can be as much as 10–15 times higher than in full-term infants; a neonate born at 24 weeks of gestation can lose 13% body weight on the first day of life as a consequence of the high fluid loss due to the virtual absence of the epidermal barrier [57,58,60]. TEWL is influenced by the gestational age of the infant, body region, and humidity of the environment. TEWL and percutaneous absorption are inversely proportional to gestational age, and SC hydration increases with age [56,57,60].

Another anatomical-structural difference between premature and mature infants is that the dermoepidermal junction is flat and anchoring fibrils, anchoring filaments and hemidesmosomes are fewer and smaller in preterm neonates, which results in a decreased resistance to shear forces. Due to the immaturity of the dermoepidermal junction, the epidermis and dermis can easily separate from each other; moreover, bullae can develop much more easily after thermal or mechanical impact. The skin is therefore fragile and prone to inadvertent cutaneous injury [56,57,61]. The dermis is also thinner, less collagenized and more gelatinous, and this gives rise to an increased risk of oedema, resulting in the risk of ischaemic injury. Moreover, in consequence of the thin layer of subcutaneous fat and immature eccrine glands, premature infants have a compromised thermoregulatory capability [56,57].

IMPAIRED EPIDERMAL BARRIER IN INFLAMMATORY SKIN DISEASES

Defective epidermal barrier function is the main feature of the most common inflammatory skin disorders, such as atopic dermatitis and, to a lesser extent, psoriasis [62,63]. Atopic dermatitis is a chronic skin disease affecting up to 20% of the pediatric population in developed countries. The main pathogenetic factor of atopic dermatitis is skin barrier damage, where the crucial predisposing factors are mutations and intragenic variations in the copy number of *FLG* monomers [49,64]. However, recent data suggest that the inflammatory cytokines and chemokine milieu in atopic skin can also down-regulate *FLG* gene expression, resulting in filaggrin deficiency and impaired epidermal barrier. These data suggest that severe skin inflammation and filaggrin mutations similarly alter the skin barrier [65,66]. As inherited or acquired barrier defect is the major pathogenic factor of the disease, restoration of the impaired barrier is the key therapeutic approach in the treatment of atopic dermatitis (Fig. **1**).

Psoriasis is also a multifactorial inflammatory skin disease, affecting approximately 2–3% of the population. Genetic and environmental factors result in impaired skin barrier in psoriatic lesions, and this danger signal might contribute to the hyperproliferation and inflammatory cytokine production of keratinocytes [63]. As damage to the skin barrier results in further inflammatory cytokine production, barrier restoring therapy is an important strategy in the management of chronic inflammatory skin diseases.

BARRIER RESTORING THERAPIES

Moisturizers

Xerosis, or dry skin, is frequently the most apparent sign indicating impairment of the skin's barrier function. Dry skin can be caused by altered environmental factors (e.g., seasons, climate, excessive bathing, etc.) or endogenous factors (e.g., aging, deficiencies in the skin's natural moisturizing factor (NMF), barrier lipid content, etc.) [67-69]. Moisturizers are widely applied to treat dry skin caused by different factors and are based on several formulations which contribute to barrier repair, reduction of TEWL or aesthetic improvement of irritated skin [67]. Moisturizers, which can be prescription but are more often over-the-counter formulations, belong to the standard therapy of atopic dermatitis [70] and may also be useful in the treatment of irritant contact dermatitis. Three main classes of moisturizers can be distinguished: emollients, occlusives and humectants (Table **2**).

Emollients are oily substances which are designed to bring small lipid droplets into the cracks between desquamating corneocytes in dry skin, thereby increasing the softness, flexibility and smoothness of the skin [71]. Containing oils and lipids (e.g., different fatty acids from palm oil, coconut oil and wool fat) [72], emollients are designed to maintain healthy skin conditions rather than to repair damaged skin or have long-term effects [67].

Occlusives are also lipophilic preparations which provide a hydrophobic barrier on the skin surface to reduce TEWL. Moreover, this film protects from external irritants. Thus, these preparations are applied to dry or damaged skin because they promote barrier repair due to the mentioned mechanisms. Petrolatum is one of the most effective occlusives. Lanolin, mineral oil, beeswax, soybean oil, paraffin and propylene glycol are also used as occlusives. Silicones (e.g., dimethicone, cyclomethicone) are relatively new hypoallergenic occlusives. A disadvantage of occlusive preparations is that they can be aesthetically less pleasing than oil-in-water emulsions [67,68,71].

Humectant-based moisturizers are frequently used to treat dry skin. These preparations, which contain *inter alia* polyols (e.g.,

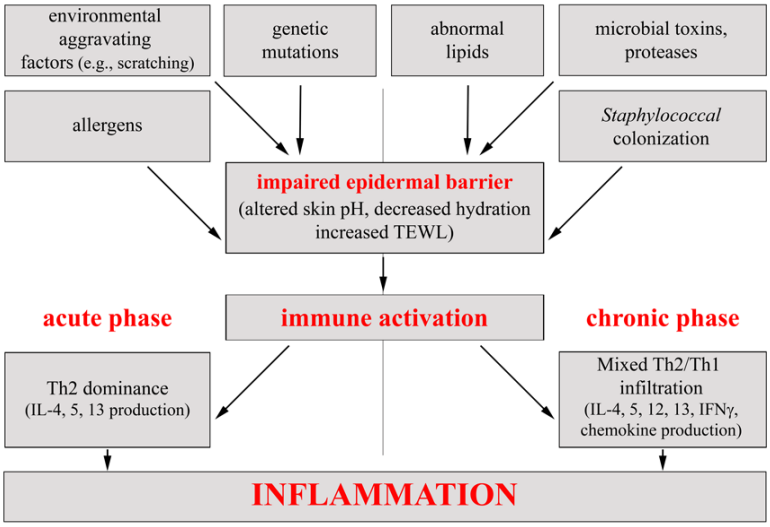

Fig. (1). The pathogenesis of atopic dermatitis. The interaction of genetic, environmental and immunologic factors create a permissive environment for the initiation and progression of the disease. Through the impared epidermal barrier penetration of external allergens, microbes and microbial compounds are enhanced, creating immune activation and subsequent inflammation.

Table 2. Main classes of moisturizers.

Type	Characteristics	Examples
Emollients	Smoothing and softening skin	Oils, lipids (e.g., stearic, linoleic, linolenic, oleic, lauryl acids)
Occlusives	Providing hydrophobic barrier which protects from loss of water and external irritants	Petrolatum, lanolin, mineral oil, beeswax, propylene glycol, silicones, etc.
Humectants	Attracting and binding water in SC	Glycerol, sorbitol, urea, lactic acid, amino acids, etc.

glycerol, sorbitol and mannitol), provide hydrating effects to the skin by attracting and binding water from the deep epidermis and the environment. Compared to other formulations, they are absorbed faster and therefore are aesthetically better, promoting patient compliance [67,73]. Polyols may provide protection against irritation via different pathways. Certain advantageous effects of polyols originate in their chemical structure. It is known that glycerol, which is the most frequent polyol in dermatological topical preparations, diffuses into the SC and retains water in the skin. Further, glycerol may interact with SC lipid structures and proteins, altering their water-binding and/or hydrophilic properties [74]. The hydrating effect of glycerol has been shown in a number of animal experiments and human studies [75]. Furthermore, glycerol promotes skin barrier function [76,77]. This effect may be explained by the moisturizing property of glycerol, since an inverse relationship between TEWL and SC hydration is known [1]. Moreover, glycerol reduces the density and the average radius of aqueous pores in SC, hereby decreasing the ability of irritant agents to penetrate the SC [78]. In addition to moisturizing and providing barrier restoring potential, glycerol improves mechanical properties of the skin [79]). There is a correlation between SC hydration and skin mechanical properties [80]. The skin friction coefficient also shows positive correlation with SC hydration [81]. Another study suggests that the effects of glycerol on mechanical parameters may be independent of its hydrating ability [79]. Effects of glycerol have been extensively studied; however less information is available for other polyols. Previously, we found that xylitol also suppresses skin irritation [65]. Mannitol alone had no effect on skin irritation [65], but combined application of mannitol and hyaluronic acid increased skin hydration and elasticity [82]. In contrast, sorbitol was found to improve barrier function and act as a moisturizer [83].

In addition to inducing physical or chemical alterations, polyols might also change gene expression. *In vitro* experiments have shown that glycerol decreases the expression of human leukocyte antigen DR (HLA-DR), thereby reducing inflammation, whereas xylitol increases the expression of filaggrin [84]. Filaggrin, as a source of NMF and in other ways, contributes to hydration and homeostasis of the skin [85]. Recent animal experiments have revealed that both glycerol and xylitol decrease the expression of tumor necrosis factor alpha (TNF-α) and interleukin 1-beta (IL-1ß) in sodium lauryl sulphate (SLS) induced acute irritation [86]. In this experimental setup, application of polyols inhibited the SLS-induced elevation of TEWL, moderated the irritant-induced increase in dermal blood flow and prevented accumulation of lymphocytes and neutrophil granulocytes. Further, it was found that both glycerol and xylitol hamper the penetration of irritant agents [86]. Thus, polyols exert anti-irritant effects via different pathways.

Other important factors are the antimicrobial effects of polyols. Barrier damage leads to changes in skin flora [87]; therefore, patients with irritant or atopic dermatitis may need protection against bacterial colonization. Glycerol in 85% concentration was found to have antimicrobial effects [88]. Additionally, glycerol possesses virucidal properties [89]. However, Gram-positive species are less susceptible to glycerol than Gram-negatives. Xylitol, combined with farnesol [90] or with glycerol [91] effectively eliminates Gram-positive bacteria, and its effective concentration was found to be 5% in both studies. Hence, application of polyols may prevent both irritation and bacterial colonization of the skin (Fig. **2**).

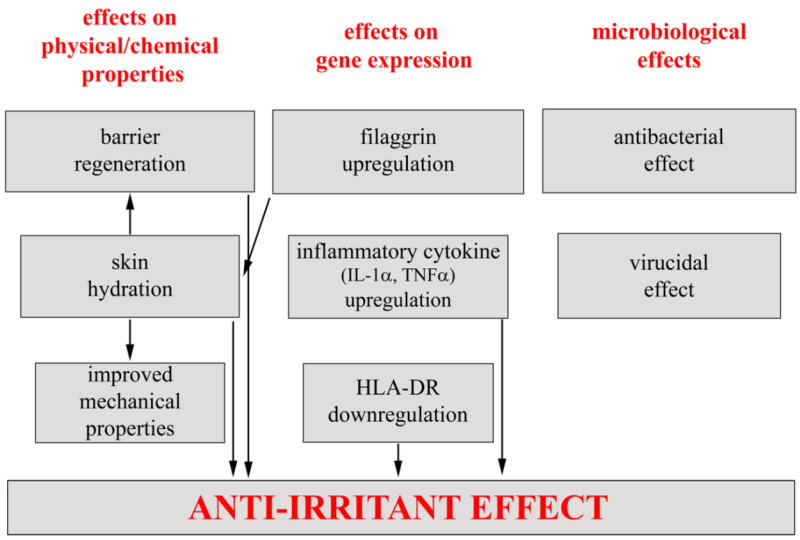

Fig. (2). Effects of polyols on the skin.

A new generation of moisturizers could contain special ingredients which supply normal skin components. Ceramides, with cholesterol and fatty acids, belong to the main lipid groups of SC and play a pivotal role in barrier function [92]. NMF, originating from the catabolism of filaggrin, maintains adequate SC hydration [85]. NMF is a mixture of free amino acids, inorganic salts, sugars, lactic acid and urea. It should be noted that that a combination of different types of moisturizers can be advantageous. Humectants can be combined with occlusive components when applied to skin with a defective barrier to attract water and to prevent its evaporation [93]. Ceramides, pseudoceramides and NMFs have been studied and added to commercial moisturizers to hydrate skin and improve barrier function [94].

Anti-Inflammatory Therapies

Inflammation in the skin results in epidermal barrier damage similar to that present in patients with filaggrin mutation, suggesting that anti-inflammatory treatment might affect the barrier functions of the skin.

Topical Glucocorticosteroids

Topical corticosteroids (TCS) have been widely used for the treatment of inflammatory skin disorders in dermatological practice for decades. Corticosteroids have numerous anti-inflammatory, antiproliferative and immunosuppressive effects, and topical medications are available in different formulations with a wide range of potency. TCS remain the mainstay and gold standard for the treatment of acute inflammatory symptoms in AD and can achieve excellent results in both short-term and proactive maintenance therapy [57,95-98]. Topical steroids result in rapid improvement of skin inflammation and pruritus, but have opposing effects on barrier functions [95,99,100]. Positive effects of topical corticosteroids on the skin barrier include the increase of SC hydration, decrease of TEWL, and normalization of filaggrin and loricrin expression [95,100,101]. However, human studies and animal models demonstrated that long-term application of local glucocorticoids resulted in significant impairment of epidermal barrier function and homeostasis. Morphological, physicochemical and functional alterations include decreased epidermal proliferation and differentiation, inhibition of the synthesis of epidermal barrier lipids and antimicrobial peptide formation, decrease of lamellar body formation, delay of barrier recovery, and reduction of the integrity and cohesion of SC; additionally, topical steroids also have negative effects on epidermal immune cells [95,99,100]. The application of topical steroids in special, advanced vehicle formulations and adequate adjunctive barrier repair therapy with emollients and moisturizers can significantly reduce these negative effects and promote the maintenance of epidermal barrier functions [95,99]. There are also several well known cutaneous side-effects of prolonged use of topical glucocorticosteroids, such as atrophy, striae, purpura, telangiectasias, hypertrichosis, alopecia, hyperpigmentation and impairment of wound healing; these effects are mostly preventable with rational application of the medications [57,95,102].

Topical Calcineurin Inhibitors

The introduction of topical calcineurin inhibitors (tacrolimus and pimecrolimus) resulted in a significant breakthrough in the anti-inflammatory treatment of atopic dermatitis. These macrolactam derivatives have more specific immunomodulatory and anti-inflammatory effects than glucocorticoids, acting by inhibition of proinflammatory cytokine expression in T-lymphocytes and other inflammatory cells. In contrast to topical steroids, pimecrolimus and tacrolimus do not induce skin atrophy, and thus calcineurin inhibitors are suitable for long-term maintenance therapy even on the head, neck and intertriginous areas [96-98]. Topical calcineurin inhibitors influence the epidermal barrier function in several ways, and a beneficial effect is observed by improving SC hydration and reducing TEWL [100,103]. However, local calcineurin inhibitors might also have negative impacts on epidermal barrier function by decreasing epidermal lipid synthesis, lamellar body secretion and antimicrobial peptide expression and production. The calcineurin inhibitor-induced impairment of the permeability and antimicrobial barrier could be prevented by emollient treatment [103].

TRANSDERMAL DRUG DELIVERY

Barrier function provided by the SC is indispensable to avoid the loss of water and to provide protection against irritant and causative agents. However, the same barrier often hampers transdermal drug delivery. This method is a useful alternative pathway for therapeutic agents that are prone to decompose in the gastrointestinal tract and permits the achievement of relatively high local drug concentrations without systemic side effects. Due to the epidermal barrier, different techniques are required for the enhancement of skin permeability. Methods modifying the barrier properties can be passive or active. Passive methods include the influencing of drug and vehicle interactions and optimization of formulation to modify SC structure [104]. A widely used passive method is the application of chemical penetration enhancers that facilitate drug

permeation across the skin. Several compounds are able to contribute to a better penetration, e.g., alcohols, amides, esters, ether alcohols, fatty acids, glycols, pyrrolidones, sulfoxides, surfactants and terpenes [105]. Penetration enhancers have different mechanisms of action, e.g., increasing the fluidity of the SC lipid bilayers, interaction with intercellular proteins, disruption or extraction of intercellular lipids, increasing the drug's thermodynamic activity or increasing SC hydration [106,107]. Their primary disadvantage is that chemical penetration enhancers often evoke skin irritation (local inflammation) and their efficacy is relatively low [108-110].

Another passive method is the use of special carrier systems to increase drug flux into and through the skin. These carrier systems can be nanoparticles and nanofibers which can be used to enhance solubility of highly hydrophobic drugs, provide controlled and sustained release of drugs, increase the stability of therapeutic agents, and deliver higher drug concentrations to target areas. Several types of nanoparticles are available: natural polymeric (e.g., chitosan and albumin), synthetic polymeric (e.g., tyrosine-derived polymeric nanospheres, poly(lactic-co-glycolic acid)), lipid-based (liposomes, solid-lipid), metallic and silica, as well as dendrimers. However, only a few of these techniques have been translated into clinically used products so far. Hence, further clinical studies are needed [111]. Furthermore, prodrugs can also be used. Prodrugs are synthesized by a chemical modification of a drug for more optimal physicochemical and/or pharmacokinetic properties. After delivery, the prodrug is cleaved by enzymes leading to the formation of the parent drug [112]. Hyaluronic acid is an effective moisturizer, but its penetration ability is poor. However, cross-linking the molecule results in better penetration through human epidermis and living animal skin [113].

Active, physical methods involve several different techniques (e.g., use of electrical forces, lasers, ultrasound and microneedles). Electrical force can be used as iontophoresis or electroporation. The latter is a promising method which temporarily creates aqueous pores in cell membranes using electric pulses of high voltage and short duration. Electroporation successfully enhances skin permeability for molecules with different lipophilicities and sizes, including high-molecular-weight biopharmaceuticals. Nevertheless, the relationship between electroporation and skin irritation should be clarified. Since high voltage pulses are used, it is important to ensure that there are no harmful effects in the skin [114].

Lasers can also be used to assist drug delivery. By means of laser, fractional photothermolysis can be performed: i.e., multiple vertical columns of tissue in the SC and underlying layer are thermally destroyed to create unimpeded channels. On treated skin, the channels and the surrounding thermally coagulated tissue enhance penetration while the untreated area serves as a reservoir for regeneration [115].

Ultrasound is able to affect skin permeability. Penetration of cationic, neutral and anionic particles and also those of gold nanoparticles and dendrimers are enabled by ultrasound [116].

Microneedle devices are composed of arrays of micron-size needles. When applied to the skin surface, they bypass the SC without stimulating dermal nerves. The holes created by the needles can be used to deliver drugs on the skin surface to the dermal microcirculation [117]. These techniques may also be combined to increase efficacy and to reduce side effects.

THE EPIDERMAL BARRIER: FUTURE PERSPECTIVES

Understanding the molecular mechanism of epidermal barrier function and the inherited factors leading to genetic diseases resulting in skin barrier defects might pave the way for better treatments not only for rare hereditary, but also for common multifactorial inflammatory skin diseases. Because of the importance of the intact barrier for the healthy state of our bodies, maintenance and restoration when it becomes compromised is vital.

Transdermal drug delivery represents an attractive opportunity for the administration of different drugs. Scientific and technological advances by targeted disruption of the epidermal barrier resulting in better transdermal penetration of various treatments will have a widespread impact in medicine.

ACKNOWLEDGEMENTS

This work was funded by The Hungarian Scientific Research Fund (OTKA NK105369) K. Szabó and Zs. Csoma are recipients of the János Bolyai Research Scholarship from the Hungarian Academy of Sciences.

REFERENCES

[1] Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol 2008; 17: 1063-72.
[2] Jensen JM, Proksch E. The skin's barrier. G Ital Dermatol Venereol 2009; 144: 689-700.
[3] Brandner JM. Importance of Tight Junctions in Relation to Skin Barrier Function. Curr Probl Dermatol 2016; 49: 27-37.
[4] Fuchs E. Epidermal differentiation: the bare essentials. J Cell Biol 1990; 111: 2807-14.
[5] Schurer NY, Elias PM. The biochemistry and function of stratum corneum lipids. Adv Lipid Res 1991; 24: 27-56.
[6] Wertz PH, Dowing DL. Epidermal lipids. In: Goldsmith LA (ed.), Physiology, Biochemistry and Molecluar Biology of the Skin Vol.2. New York: Oxford University Press; 1991: pp. 205-231.
[7] Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. Adv Lipid Res 1991; 24: 1-26.
[8] Visscher MO, Narendran V, Pickens WL, *et al.* Vernix caseosa in neonatal adaptation. J Perinatol 2005; 25: 440-6.
[9] Schmid-Wendtner MH, Burgdorf W. Ultrasound scanning in dermatology. Arch Dermatol 2005; 141: 217-24.
[10] Sanford JA, Gallo RL. Functions of the skin microbiota in health and disease. Semin Immunol 2013; 25: 370-7.
[11] Toulza E, Mattiuzzo NR, Galliano MF, *et al.* Large-scale identification of human genes implicated in epidermal barrier function. Genome Biol 2007; 8: R107.
[12] Wickett RR, Visscher MO. Structure and function of the epidermal barrier. Am J Infect Control 1996; 34: S98-S110.
[13] Samuelov L, Sprecher E. Peeling off the genetics of atopic dermatitis-like congenital disorders. J Allergy Clin Immunol 2014; 134: 808-15.
[14] Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005; 6: 328-40.
[15] Sumigray KD, Lechler T. Cell adhesion in epidermal development and barrier formation. Curr Top Dev Biol 2015; 112: 383-414.
[16] Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM. Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry 1990; 29: 9432-40.
[17] Takeichi T, Akiyama M. Inherited ichthyosis: Non-syndromic forms. J Dermatol 2016; 43: 242-51.
[18] Korge BP, Ishida-Yamamoto A, Punter C, *et al.* Loricrin mutation in Vohwinkel's keratoderma is unique to the variant with ichthyosis. J Invest Dermatol 1997; 109: 604-10.
[19] Dahlqvist J, Klar J, Tiwari N, *et al.* A single-nucleotide deletion in the POMP 5' UTR causes a transcriptional switch and altered epidermal proteasome distribution in KLICK genodermatosis. Am J Hum Genet 2010; 86: 596-603.
[20] Weeda G, Eveno E, Donker I, *et al.* A mutation in the XPB/ERCC3 DNA repair transcription gene, associated with trichothiodystrophy. Am J Hum Genet 1997; 60: 320-9.
[21] Nakabayashi K, Amann D, Ren Y, *et al.* Identification of C7orf11 (TTDN1) gene mutations and genetic heterogeneity in nonphotosensitive trichothiodystrophy. Am J Hum Genet 2005; 76: 510-6.
[22] De L, V, Rogers GR, Hamrock DJ, *et al.* Sjogren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. Nat Genet 1996; 12: 52-7.
[23] Schmuth M, Martinz V, Janecke AR, *et al.* Inherited ichthyoses/generalized Mendelian disorders of cornification. Eur J Hum Genet 2013; 21: 123-33.
[24] Yoneda K. Inherited ichthyosis: Syndromic forms. J Dermatol 2016; 43: 252-63.

[25] Yu Z, Schneider C, Boeglin WE, Marnett LJ, Brash AR. The lipoxygenase gene ALOXE3 implicated in skin differentiation encodes a hydroperoxide isomerase. Proc Natl Acad Sci USA 2003; 100: 9162-7.

[26] Lefevre C, Bouadjar B, Karaduman A, *et al.* Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. Hum Mol Genet 2004; 13: 2473-82.

[27] Lugassy J, Hennies HC, Indelman M, Khamaysi Z, Bergman R, Sprecher E. Rapid detection of homozygous mutations in congenital recessive ichthyosis. Arch Dermatol Res 2008; 300: 81-5.

[28] Basler E, Grompe M, Parenti G, Yates J, Ballabio A. Identification of point mutations in the steroid sulfatase gene of three patients with X-linked ichthyosis. Am J Hum Genet 1992; 50: 483-91.

[29] Derry JM, Gormally E, Means GD, *et al.* Mutations in a delta 8-delta 7 sterol isomerase in the tattered mouse and X-linked dominant chondrodysplasia punctata. jderry@immunex.com. Nat Genet 1999; 22: 286-90.

[30] Wang HJ, Tang ZL, Lin ZM, Dai LL, Chen Q, Yang Y. Recurrent splice-site mutation in MBTPS2 underlying IFAP syndrome with Olmsted syndrome-like features in a Chinese patient. Clin Exp Dermatol 2014; 39: 158-61.

[31] Klar J, Schweiger M, Zimmerman R, *et al.* Mutations in the fatty acid transport protein 4 gene cause the ichthyosis prematurity syndrome. Am J Hum Genet 2009; 85: 248-53.

[32] Akiyama M. ABCA12 mutations and autosomal recessive congenital ichthyosis: a review of genotype/phenotype correlations and of pathogenetic concepts. Hum Mutat 2010; 31: 1090-6.

[33] Di Rocco M, Callea F, Pollice B, Faraci M, Campiani F, Borrone C. Arthrogryposis, renal dysfunction and cholestasis syndrome: report of five patients from three Italian families. Eur J Pediatr 1995; 154: 835-9.

[34] Sprecher E, Ishida-Yamamoto A, Mizrahi-Koren M, *et al.* A mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma. Am J Hum Genet 2005; 77: 242-51.

[35] Montpetit A, Cote S, Brustein E, *et al.* Disruption of AP1S1, causing a novel neurocutaneous syndrome, perturbs development of the skin and spinal cord. PLoS Genet 2008; 4: e1000296.

[36] Oji V, Eckl KM, Aufenvenne K, *et al.* Loss of corneodesmosin leads to severe skin barrier defect, pruritus, and atopy: unraveling the peeling skin disease. Am J Hum Genet 2010; 87: 274-81.

[37] Alef T, Torres S, Hausser I, *et al.* Ichthyosis, follicular atrophoderma, and hypotrichosis caused by mutations in ST14 is associated with impaired profilaggrin processing. J Invest Dermatol 2009; 129: 862-9.

[38] Walley AJ, Chavanas S, Moffatt MF, *et al.* Gene polymorphism in Netherton and common atopic disease. Nat Genet 2001; 29: 175-8.

[39] Hadj-Rabia S, Baala L, Vabres P, *et al.* Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. Gastroenterology 2004; 127: 1386-90.

[40] Richard G, Smith LE, Bailey RA, *et al.* Mutations in the human connexin gene GJB3 cause erythrokeratodermia variabilis. Nat Genet 1998; 20: 366-9.

[41] Furuse M, Hata M, Furuse K, *et al.* Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 2002; 156: 1099-111.

[42] Common JE, Becker D, Di WL, Leigh IM, O'Toole EA, Kelsell DP. Functional studies of human skin disease- and deafness-associated connexin 30 mutations. Biochem Biophys Res Commun 2002; 298: 651-6.

[43] Djalilian AR, McGaughey D, Patel S, *et al.* Connexin 26 regulates epidermal barrier and wound remodeling and promotes psoriasiform response. J Clin Invest 2006; 116: 1243-53.

[44] Sugiura K, Akiyama M. Update on autosomal recessive congenital ichthyosis: mRNA analysis using hair samples is a powerful tool for genetic diagnosis. J Dermatol Sci 2015; 79: 4-9.

[45] Vahlquist A, Bygum A, Ganemo A, *et al.* Genotypic and clinical spectrum of self-improving collodion ichthyosis: ALOX12B, ALOXE3, and TGM1 mutations in Scandinavian patients. J Invest Dermatol 2010; 130: 438-43.

[46] Oji V, Hautier JM, Ahvazi B, *et al.* Bathing suit ichthyosis is caused by transglutaminase-1 deficiency: evidence for a temperature-sensitive phenotype. Hum Mol Genet 2006; 15: 3083-97.

[47] Fischer J. Autosomal recessive congenital ichthyosis. J Invest Dermatol 2009; 129: 1319-21.

[48] Smith FJ, Irvine AD, Terron-Kwiatkowski A, *et al.* Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006; 38: 337-42.

[49] Palmer CN, Irvine AD, Terron-Kwiatkowski A, *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006; 38: 441-6.

[50] Chipev CC, Korge BP, Markova N, *et al.* A leucine----proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis. Cell 1992; 70: 821-8.

[51] Chamcheu JC, Siddiqui IA, Syed DN, Adhami VM, Liovic M, Mukhtar H. Keratin gene mutations in disorders of human skin and its appendages. Arch Biochem Biophys 2011; 508: 123-37.

[52] Takizawa Y, Akiyama M, Nagashima M, Shimizu H. A novel asparagine-->aspartic acid mutation in the rod 1A domain in keratin 2e in a Japanese family with ichthyosis bullosa of Siemens. J Invest Dermatol 2000; 114: 193-5.

[53] Choate KA, Lu Y, Zhou J, *et al.* Mitotic recombination in patients with ichthyosis causes reversion of dominant mutations in KRT10. Science 2010; 330: 94-7.

[54] Furtado LV, Bayrak-Toydemir P, Hulinsky B, Damjanovich K, Carey JC, Rope AF. A novel X-linked multiple congenital anomaly syndrome associated with an EBP mutation. Am J Med Genet A 2010; 152A: 2838-44.

[55] Pavlovic S, Krunic AL, Bulj TK, *et al.* Acral peeling skin syndrome: a clinically and genetically heterogeneous disorder. Pediatr Dermatol 2012; 29: 258-63.

[56] Eichenfield LF, Frieden IJ, Zaenglein A, Mathes E. Neonatal and Infant Dermatology, 3rd ed. .Elsevier, 2015.

[57] Harper's Textbook of Pediatric Dermatology. Irvine AD, Hoeger PH, Yan AC, Eds, 3rd ed. Wiley-Blackwell; 2011.

[58] Rutter N. The immature skin. Eur J Pediatr 1996; 155 Suppl 2: S18-S20.

[59] Fluhr JW, Darlenski R, Lachmann N, *et al.* Infant epidermal skin physiology: adaptation after birth. Br J Dermatol 2012; 166: 483-90.

[60] Harpin VA, Rutter N. Barrier properties of the newborn infant's skin. J Pediatr 1983; 102: 419-25.

[61] Csoma Z, Meszes A, Mader K, Kemeny L, Talosi G. Overview of dermatologic disorders of neonates in a central regional intensive care unit in Hungary. Pediatr Dermatol 2015; 32: 201-7.

[62] Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. J Clin Invest 2004; 113: 651-7.

[63] Ghadially R, Reed JT, Elias PM. Stratum corneum structure and function correlates with phenotype in psoriasis. J Invest Dermatol 1996; 107: 558-64.

[64] Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med 2011; 365: 1315-27.

[65] Mocsai G, Gaspar K, Nagy G, *et al.* Severe skin inflammation and filaggrin mutation similarly alter the skin barrier in patients with atopic dermatitis. Br J Dermatol 2014; 170: 617-24.

[66] Dajnoki Z, Beke G, Mocsai G, *et al.* Immune-mediated Skin Inflammation is Similar in Severe Atopic Dermatitis Patients With or Without Filaggrin Mutation. Acta Derm Venereol 2015.

[67] Draelos ZD. Modern moisturizer myths, misconceptions, and truths. Cutis 2013; 91: 308-14.

[68] Weber TM, Schoelermann AM, Breitenbach U. Hand and foot moisturizers. In: Draelos ZD (ed.), Cosmetic Dermatology: Products and Procerures. Hoboken: Blackwell Plublishing Ltd.; 2010: 130-138.

[69] White-Chu EF, Reddy M. Dry skin in the elderly: complexities of a common problem. Clin Dermatol 2011; 29: 37-42.

[70] Saeki H, Furue M, Furukawa F, *et al.* Guidelines for management of atopic dermatitis. J Dermatol 2009; 36: 563-77.

[71] Varothai S, Nitayavardhana S, Kulthanan K. Moisturizers for patients with atopic dermatitis. Asian Pac J Allergy Immunol 2013; 31: 91-8.

[72] Anderson PC, Dinulos JG. Are the new moisturizers more effective? Curr Opin Pediatr 2009; 21: 486-90.

[73] Lodén M. Hydrating substances. In: Paye M, Barel AO, Maibach HI (eds.), Handbook of Cosmetic Science and Technology. Boca Raton: Taylor&Francis Group; 2006: 265-276.

[74] Batt MD, Davis WB, Fairhurst E, Gerrard A, Ridge BD. Changes in the physical properties of the stratum corneum following treatment with glycerol. J Soc Cosmet Chem 1988; 39: 367-81.

[75] Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. Br J Dermatol 2008; 159: 23-34.

[76] Fluhr JW, Gloor M, Lehmann L, Lazzerini S, Distante F, Berardesca E. Glycerol accelerates recovery of barrier function in vivo. Acta Derm Venereol 1999; 79: 418-21.

[77] Gloor M, Gehring W. Increase in hydration and protective function of horny layer by glycerol and a W/O emulsion: are these effects maintained during long-term use? Contact Dermatitis 2001; 44: 123-5.

[78] Ghosh S, Blankschtein D. The role of sodium dodecyl sulfate (SDS) micelles in inducing skin barrier perturbation in the presence of glycerol. J Cosmet Sci 2007; 58: 109-33.

[79] Bettinger J, Gloor M, Vollert A, Kleesz P, Fluhr J, Gehring W. Comparison of different non-invasive test methods with respect to the effect of different moisturizers on skin. Skin Res Technol 1999; 5: 21-7.

[80] Dobrev H. Use of Cutometer to assess epidermal hydration. Skin Res Technol 2000; 6: 239-44.

[81] Zhu YH, Song SP, Luo W, Elias PM, Man MQ. Characterization of skin friction coefficient, and relationship to stratum corneum hydration in a normal Chinese population. Skin Pharmacol Physiol 2011; 24: 81-6.

[82] Taieb M, Gay C, Sebban S, Secnazi P. Hyaluronic acid plus mannitol treatment for improved skin hydration and elasticity. J Cosmet Dermatol 2012; 11: 87-92.

[83] Muizzuddin N, Ingrassia M, Marenus KD, Maes DH, Mammone T. Effect of seasonal and geographical differences on skin and effect of treatment with an osmoprotectant: Sorbitol. J Cosmet Sci 2013; 64: 165-74.

[84] Szabó-Papp J, Sós K, Oláh A, Szöllősi AG, Tóth BI, Czifra G, Bíró T. Differential effects of common moisturizer polyols on normal human epidermal keratinocytes. J Invest Dermatol 132, S58. 2012.

[85] Harding CR, Aho S, Bosko CA. Filaggrin - revisited. Int J Cosmet Sci 2013; 35: 412-23.

[86] Szel E, Polyanka H, Szabo K, *et al.* Anti-irritant and anti-inflammatory effects of glycerol and xylitol in sodium lauryl sulphate-induced acute irritation. J Eur Acad Dermatol Venereol 2015; 29: 2333-41.

[87] de Almeida e Borges LF, Silva BL, Gontijo Filho PP. Hand washing: changes in the skin flora. Am J Infect Control 2007; 35: 417-20.

[88] Saegeman VS, De Vos R, Tebaldi ND, *et al.* Flow cytometric viability assessment and transmission electron microscopic morphological study of bacteria in glycerol. Microsc Microanal 2007; 13: 18-29.

[89] van Baare J, Buitenwerf J, Hoekstra MJ, du Pont JS. Virucidal effect of glycerol as used in donor skin preservation. Burns 1994; 20 Suppl 1: S77-S80.

[90] Katsuyama M, Kobayashi Y, Ichikawa H, *et al.* A novel method to control the balance of skin microflora Part 2. A study to assess the effect of a cream containing farnesol and xylitol on atopic dry skin. J Dermatol Sci 2005; 38: 207-13.

[91] Erős G, Korponyai Cs, Szabó K, Behány Z, Szél E, Kemény L. Antibacterial and skin hydrating effects of Xylinep® gel containing glycerol- and xylitol. Bőrgyógy Vener Szle 2014; **90**: 152-5.

[92] Jungersted JM, Hellgren LI, Jemec GB, Agner T. Lipids and skin barrier function--a clinical perspective. Contact Dermatitis 2008; 58: 255-62.

[93] Draelos ZD. Therapeutic moisturizers. Dermatol Clin 2000; 18: 597-607.

[94] Simpson E, Bohling A, Bielfeldt S, Bosc C, Kerrouche N. Improvement of skin barrier function in atopic dermatitis patients with a new moisturizer containing a ceramide precursor. J Dermatolog Treat 2013; 24: 122-5.

[95] Del Rosso JQ, Cash K. Topical corticosteroid application and the structural and functional integrity of the epidermal barrier. J Clin Aesthet Dermatol 2013; 6: 20-7.

[96] Ring J, Alomar A, Bieber T, *et al.* Guidelines for treatment of atopic eczema (atopic dermatitis) Part II. J Eur Acad Dermatol Venereol 2012; 26: 1176-93.

[97] Ring J, Alomar A, Bieber T, *et al.* Guidelines for treatment of atopic eczema (atopic dermatitis) part I. J Eur Acad Dermatol Venereol 2012; 26: 1045-60.

[98] Lee JH, Son SW, Cho SH. A Comprehensive Review of the Treatment of Atopic Eczema. Allergy Asthma Immunol Res 2016; 8: 181-90.

[99] Kao JS, Fluhr JW, Man MQ, *et al.* Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts for functional abnormalities. J Invest Dermatol 2003; 120: 456-64.

[100] Jensen JM, Pfeiffer S, Witt M, *et al.* Different effects of pimecrolimus and betamethasone on the skin barrier in patients with atopic dermatitis. J Allergy Clin Immunol 2009; 123: 1124-33.

[101] Jensen JM, Scherer A, Wanke C, *et al.* Gene expression is differently affected by pimecrolimus and betamethasone in lesional skin of atopic dermatitis. Allergy 2012; 67: 413-23.

[102] Bolognia J, Jorizzo J, Schaffer JV. Eds. Dermatology, 3rd ed. Elsevier Saunders; 2013.

[103] Kim M, Jung M, Hong SP, *et al.* Topical calcineurin inhibitors compromise stratum corneum integrity, epidermal permeability and antimicrobial barrier function. Exp Dermatol 2010; 19: 501-10.

[104] Alkilani AZ, McCrudden MT, Donnelly RF. Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the stratum corneum. Pharmaceutics 2015; 7: 438-70.

[105] Lane ME. Skin penetration enhancers. Int J Pharm 2013; 447: 12-21.

[106] Arora A, Prausnitz MR, Mitragotri S. Micro-scale devices for transdermal drug delivery. Int J Pharm 2008; 364: 227-36.

[107] Paudel KS, Milewski M, Swadley CL, Brogden NK, Ghosh P, Stinchcomb AL. Challenges and opportunities in dermal/transdermal delivery. Ther Deliv 2010; 1: 109-31.

[108] Basketter DA, Marriott M, Gilmour NJ, White IR. Strong irritants masquerading as skin allergens: the case of benzalkonium chloride. Contact Dermatitis 2004; 50: 213-7.

[109] Torma H, Lindberg M, Berne B. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo. J Invest Dermatol 2008; 128: 1212-9.

[110] Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta 2009; 1788: 2362-73.

[111] Goyal R, Macri LK, Kaplan HM, Kohn J. Nanoparticles and nanofibers for topical drug delivery. J Control Release 2015.

[112] Ita KB. Prodrugs for transdermal drug delivery - trends and challenges. J Drug Target 2016; 1-8.

[113] Berkó S, Maroda M, Bodnár M, *et al*. Advantages of cross-linked versus linear hyaluronic acid for semisolid skin delivery systems. Eur Polym J 2013; 49: 2511-7.

[114] Ita K. Perspectives on Transdermal Electroporation. Pharmaceutics 2016; 8.

[115] Ali FR, Al Niaimi F. Laser-assisted drug delivery in dermatology: from animal models to clinical practice. Lasers Med Sci 2016; 31: 373-81.

[116] Azagury A, Khoury L, Enden G, Kost J. Ultrasound mediated transdermal drug delivery. Adv Drug Deliv Rev 2014; 72: 127-43.

[117] Larraneta E, McCrudden MT, Courtenay AJ, Donnelly RF. Microneedles: A New Frontier in Nanomedicine Delivery. Pharm Res 2016 February 23 [Epub ahead of print].