

Pharmacological targeting of the epidermal barrier

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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.

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 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 lorincrin 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-Hünemann-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: *APIS1* 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 erythrokeratoderma 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 (**Figure 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., 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-1 β) 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 (**Figure 2**).

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 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.

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Tables

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

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. |

Figure 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 impaired epidermal barrier penetration of external allergens, microbes and microbial compounds are enhanced, creating immune activation and subsequent inflammation.

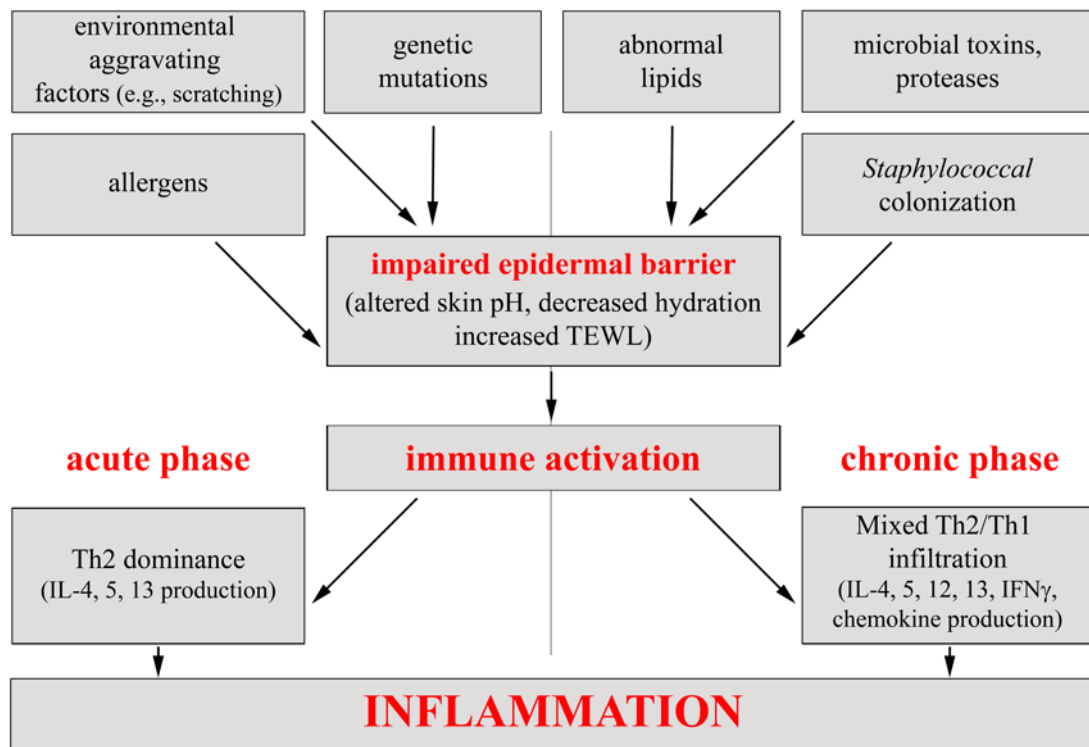
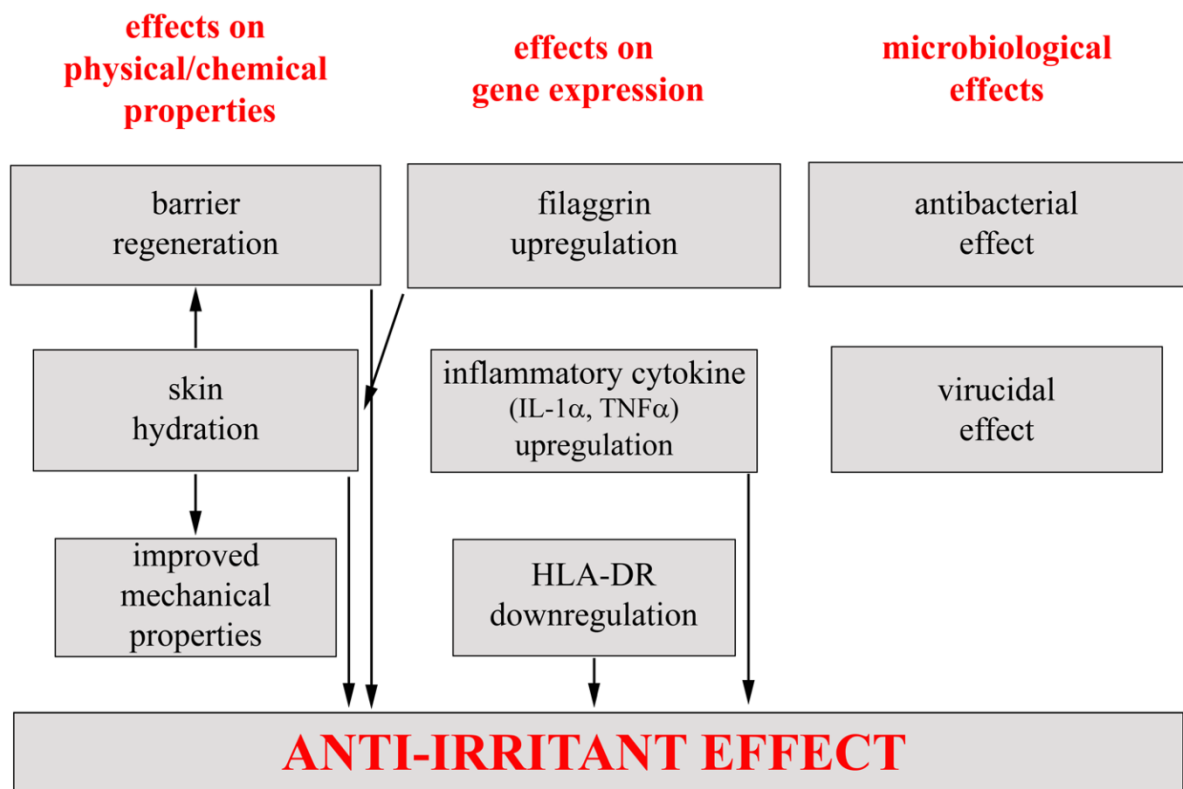


Figure 2. Effects of polyols on the skin.



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