Lipid model membrane systems as a tool for unraveling the underlying factors for skin barrier dysfunction

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Introduction, aim, and outline of this thesis

INTRODUCTION

The skin structure and barrier function

The skin is the largest organ of the body, weighing 12 to 15 percent of the total body mass, and covering 1.5-2 m^2 of the surface area [1-3]. Its primary function is the protection of the body against invasion by exogenous substances including dust, pathogens, chemicals, and ultraviolet radiation [4-10]. In addition to barring indiscriminate permeation of substances, the skin also prevents uncontrolled loss of water from inside the body to the external environment, referred to as transepidermal water loss (TEWL) [11, 12]. In effect, the skin is a two-way barrier, determining both the inward and outward movement of substances across the human body. Other important biological functions of the skin include thermoregulation, vitamin D synthesis, excretion of urea and salts as well as serving as a sensory organ for transmitting external environmental information, such as pain and heat [10, 13].

The skin is composed of three main morphological layers, from the innermost layer to the surface: hypodermis, dermis, and epidermis (figure 1A). The hypodermis or subcutaneous tissue attaches the skin to the underlying bones and muscles.

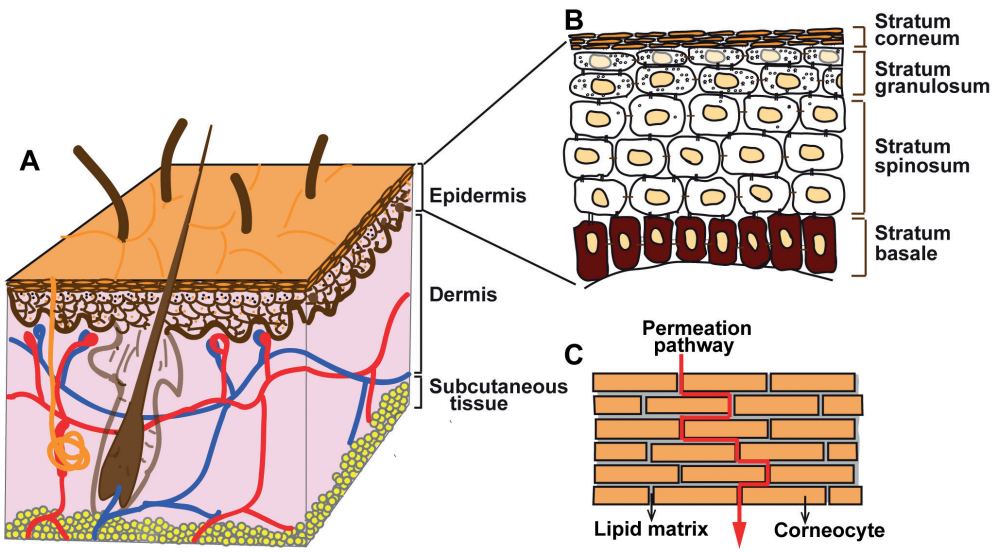

Figure 1. Schematic structure of the skin.

A) The skin consists of three major layers: Epidermis, dermis, and subcutaneous tissue. B) The epidermis is subdivided into four layers stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and stratum corneum (SC). C) In SC the corneocytes are embedded in a lipid matrix often referred to as a brick-and-mortar structure. The intercellular lipid matrix is the main permeation pathway for substances into the body.

The next layer is the dermis, which is primarily made up of connective tissue, collagen, and elastin, responsible for the skin's resilience, flexibility, and elasticity. The skin appendages including the sweat gland and hair follicle units cut across the dermis to the skin surface. The outermost layer of the skin is the epidermis and varies in thickness from 50 to 150 μm. It is composed of four layers characterized by different stages of keratinocyte (major cell type in the epidermis) differentiation. Moving from the lowest layer upwards to the skin surface, these layers are stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and stratum corneum (SC) (figure 1B). The SB, SS, and SG constitute the viable epidermis while the SC is the non-viable epidermis. The keratinocytes are generated by stem cells in the basal layer. After proliferation through mitosis, the keratinocytes migrate from the SB upwards, towards the surface of the epidermis, undergoing several stages of cell differentiation, changing composition and shape. During this process, the keratinocytes synthesize the SC structural proteins and lipids. At the SS, the keratinocytes are spherical, contain a nucleus and cell organelles, and are tightly connected by protein bridges referred to as desmosomes. Also, the initial formation of the membrane coating granules (lamellar bodies) occurs [14]. These granules contain the precursors of the barrier lipids and a series of hydrolytic enzymes and function as lipid-transporters.

At the SG, the keratinocytes flattened, while the keratohyalin granules (mainly containing proteins) and lamellar bodies are present at high concentrations [15-18]. The keratohyalin granules contain primarily the epidermal proteins: profilaggrin, loricrin, involucrin, and keratin [19-22]. The lamellar bodies contain polar lipids including glucosylceramides, sphingomyelin, phospholipids, (as well as the respective catabolic enzymes: β-glucocerebrosidase, sphingomyelinase, and phospholipase A2), and free sterols. During the terminal differentiation of the keratinocytes to form their final product, the corneocytes, cellular organelles are destroyed by the action of enzymes and the keratinocytes are flattened, highly differentiated, the content of the keratohyalin granules is released. Profillagrin is enzymatically converted into filaggrin, which aggregates keratin through the formation of disulfide bonds between keratin fibers. The corneocytes are then filled with the water-retaining keratin. The precursor proteins loricrin and involucrin, are cross-linked by the action of transglutaminase to form the stable, rigid cornified cell envelope. Filaggrin was shown to be a component of the cell envelope [23]. Simultaneously, the lamellar bodies migrate to the apical periphery of the uppermost granular cells and fuse with the membrane of the keratinocytes. The lipids and lipid processing enzymes are extruded via

exocytosis into the intercellular space at the SG-SC interface [15, 16, 18, 24-27]. The lipids are processed to form a highly organized intercellular lamellar matrix. Finally, the cell envelope surrounds the corneocytes and is embedded in the intercellular lipid matrix [4-6, 12]. The cell envelope has a layer of covalently bounded polar lipids. A major lipid class bound to the cornified envelope consists of an ultra-long carbon chain length (C30-C34) omega-hydroxy fatty acids in amide linkage with sphingoid base and attached mainly to the glutamate residues of involucrin [28-30]. This lipid monolayer referred to as cornified lipid envelope (CLE) provides a hydrophobic interface between the hydrophilic protein cell envelope and the highly hydrophobic intercellular lipid matrix. The lipid monolayer is postulated to act as a template for the subsequent addition of the intercellular lipids forming the lipid matrix, orienting the intercellular lipid lamellae parallel to the corneocyte surface [31, 32].

SC structure

The SC is 10-15 micron thick and composed of 10-20 layers of corneocytes arranged approximately parallel to the skin surface and linked by corneodesmosomes [4-6, 12, 33]. The structure of the SC has been described as being comparable to brick and mortar (figure 1C), with the corneocytes as the bricks and intercellular lipids as the mortar [34]. The highly impermeable nature of the cornified envelopes encapsulating the corneocyte redirects penetrating substances mainly through the tortuous pathway of intercellular lipids, which is the only continuous pathway for diffusion through the SC [35-38]. The intercellular lipids are thus key in maintaining the skin barrier function and alterations in lipid composition and/or organization may play a role in skin barrier dysfunction [34]. This is seen by lipid extraction of the epidermis increasing the recorded water permeation [11, 39-41]. Keratinocytes from the SC are eventually shed from the surface (desquamation). The desquamation process is due to the activity of proteolytic enzymes and lipolytic enzymes acting respectively on the corneodesmosomes and intercellular lipids.

SC lipid composition

The major lipid classes generated at the SG-SC interface are ceramides (CERs), cholesterol (CHOL), and free fatty acids (FFAs) [6, 34, 42-44]. These lipids are in an approximately equimolar ratio in the human SC [45]. A low concentration of CHOL sulphate is also present in the SC and plays a key role in the regulation of desquamation [46]. The FFAs are mostly saturated and exhibit a chain length distribution between 12 and 30 carbon atoms, with the most prevalent chain

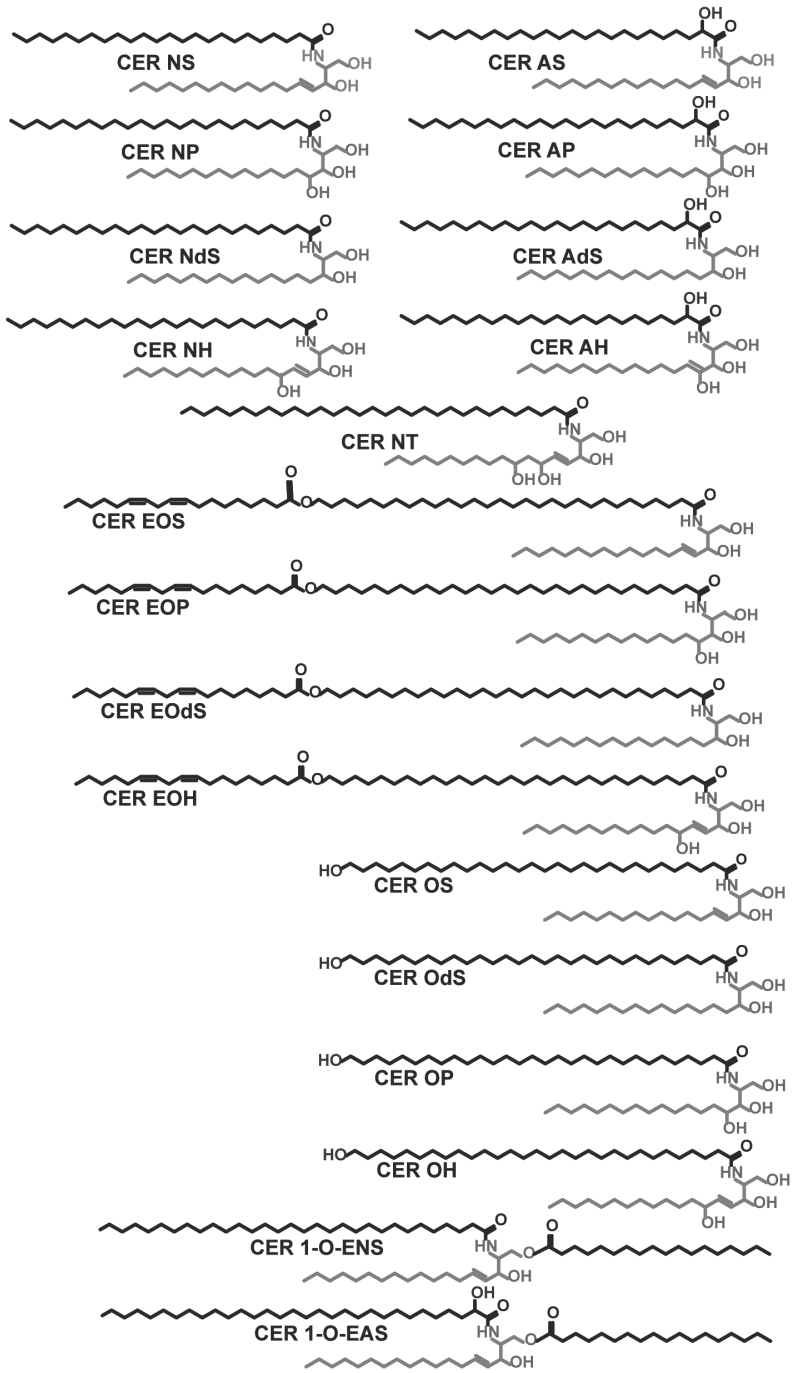

Figure 2. CER subclasses in the SC matrix.

CERs consist of a sphingoid base linked to a FA chain. The acyl chain can either be non-hydroxylated (N), α-hydroxylated (A), ω-hydroxylated (O), or esterified ω-hydroxylated (EO), while the sphingoid base is either sphingosine (S), dihydrosphingosine (dS), phytosphingosine (P), 6-hydroxysphingosine (H), or dihydroxy dihydrosphingosine (T).

lengths being 22, 24, and 26 carbon atoms [47, 48]. The CERs are made up of fatty acid linked to a sphingoid base via an amide bond between the carboxyl group of the fatty acid and the amino group of the sphingoid base (figure 2). The most abundant sphingoid base chain length is 18 carbon atoms [49, 50]. The sphingoid base head group is either sphingosine (S), phytosphingosine (P), 6-hydroxy sphingosine (HS), dihydrosphingosine (DS), or dihydroxy dihydrosphingosine (T). The fatty acid acyl chain is either non hydroxylated (N), α-hydroxylated (A), or omega hydroxylated (O). The most abundant chain lengths of the non-hydroxylated and the α-hydroxylated fatty acids are 24 to 26 carbon atoms. The omega hydroxylated fatty acid is of an ultra-long carbon chain length varying between 30 to 34 carbon atoms (although shorter chain lengths are also present) and subsequently esterified to an unsaturated fatty acid, usually, linoleic acid, esterified omega hydroxyl fatty acid (EO) also referred to as acylCER. Two recently identified CERs contain an additional acyl chain at position 1 of the sphingosine base chain (1-O-acylCER) [51]. The different combinations of the sphingoid bases linked to the variety of acyl chains result in at least 18 CER subclasses currently identified in the human SC (figure 2) [50, 52-60]. The generally adopted nomenclature for CER is based on Motta et al, [61] in which the CERs are denoted with 2-3 letters, defining the type of sphingoid base and linked fatty acid acyl chain.

Synthesis of the SC lipids

Some lipids are taken up by the epidermis from extracutaneous sources, such as the dietary lipids (e.g. essential FFAs and cholesterol esters), but most lipids are generated in the viable keratinocytes (de novo synthesis). It is a complex network of synthetic pathways involving many enzymes.

FFAs with a chain length of up to 16 carbon atoms are synthesized by fatty acid synthase using acetyl-coenzyme A and malonyl-coenzyme. Synthesized FFA C16 or FA supplied from dietary sources are then elongated in chain lengths by a series of 7 elongases (ELOVL1-7) in the endoplasmic reticulum and occurs with two carbons per cycle, hence synthesis occurs with the preference of even-numbered carbon chains [62]. The elongases are specific for a particular fatty acid chain length [41, 62]. ELOVL1 and ELOVL4 elongate FFAs with a carbon chain length of C20-C26 and ≥C26 respectively. ELOVL3 elongates FFA C18-C20 whereas ELOVL6 elongates FFA C16 to generate FFA C18. Besides elongation, the FAs can be converted to monounsaturated fatty acids (MUFAs) by stearoyl-coenzyme A desaturases. The elongation of MUFAs is catalyzed by ELOVL3, 7, and 1, whereas ELOVL5 is involved in the elongation of polyunsaturated fatty

acids (PUFAs). Subsequently, the synthesized FFAs can be used for CER synthesis or transformed into phospholipids and then stored in the lamellar bodies (LBs). During lamellar body extrusion at the SG-SC interface, secretory phospholipase A2 converts phospholipids back to FFAs.

CER synthesis occurs in the endoplasmatic reticulum of the keratinocytes. The variety in the CER subclasses arises from the final stages in the CER synthesis pathway in the body. In the first step, the enzyme serine palmitoyl catalyzes the condensation of L-serine and palmitoyl-CoA into 3-keto-dihydrosphingosine, which is consecutively reduced to dihydrosphingosine. Dihydrosphingosine is then acetylated by one of the 6 CER synthases ($CERS_{1-6}$) to form dihydro-CER using acyl-CoAs as FA donors [63]. Each CER synthase has specificity for fatty acid chain length and saturation. ($CERS_1$, C18; $CERS_2$, C22, and C24; $CERS_4$, C20, C22 and C24; and $CERS_5$ and $CERS_6$, C16), except for $CERS_3$, which exhibits broad substrate specificity toward medium- to long-chain fatty acyl-CoAs (short-chain having ≤C18; medium-chain, C18–C22; and long-chain, ≥C26) [64]. $CERS_3$ specifically synthesizes the acylCERs and together with $CERS_4$ are the major CERSs in the skin. Finally, the dihydro-CERs are desaturated to sphingosine CER or hydroxylated to phytosphingosine CER subclasses by dihydro-CER desaturase 1 and 2, respectively. In addition to hydroxylase activity, dihydro-CER desaturase 2 also has weak desaturase activity that can create sphingosine CER. CERs can also be produced by the salvage pathway, in which sphingosine generated by the deacylation of CER is re-acylated by as yet undetermined factors [63].

After synthesis, the primary hydroxy group of the CER is linked to either a glucose or phosphocholine group by the action of the enzymes glucosylceramide synthase and sphingomyelin synthase, respectively. The products glucosylceramides and sphingomyelin are packed in lamellar bodies, and released together with their catabolic enzymes at the SG-SC interface during the terminal differentiation of keratinocytes into corneocytes.

SC lipid organization

The SC intercellular lipids are present in stacks of lamellae arranged parallel to the skin surface. The presence of the lamellar layers in the SC was first observed using freeze-fracture electron microscopy [65]. Thereafter, the broad-narrow-broad sequence of electron translucent bands was visualized after ruthenium tetroxide fixation of the SC [66-68]. The lipids adopt a highly ordered, 3-dimensional structure of stacked densely packed lipid layers. X-ray diffraction studies revealed the coexistence of two crystalline lamellar phases of 13 nm repeat distance known as the long periodicity phase (LPP) and 6 nm

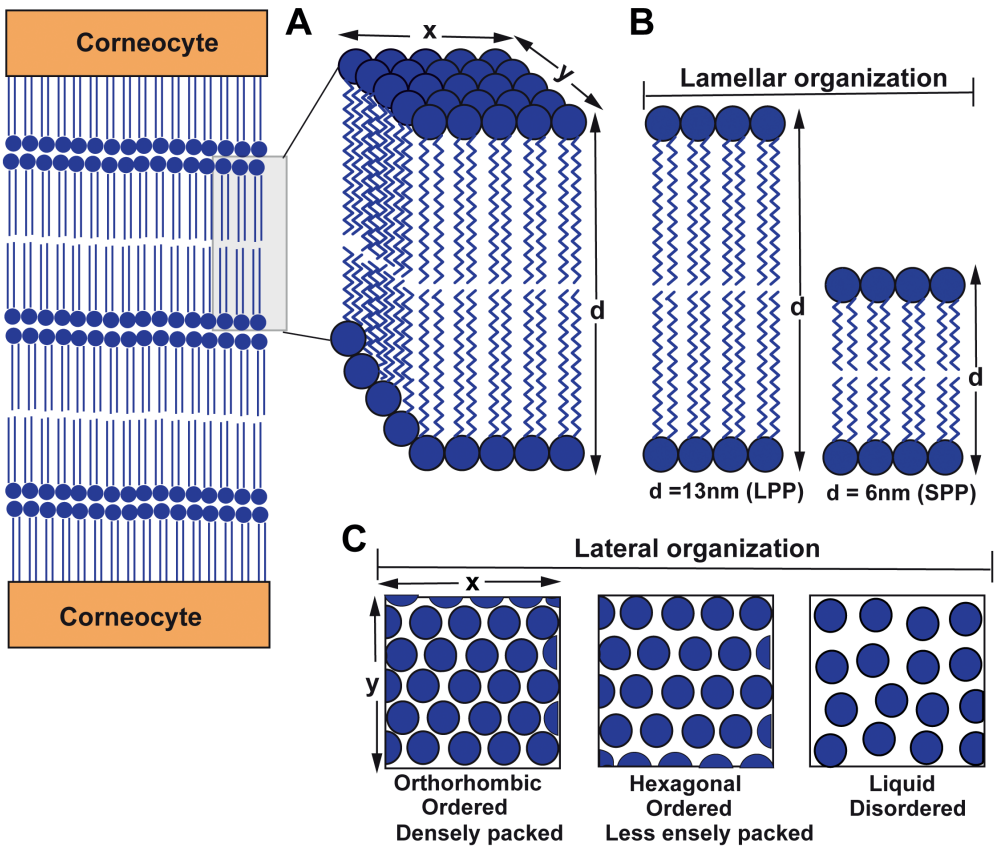

Figure 3. Organization of the lipids within the SC.

A) The lipids are stacked in layers (lamellae) in between the corneocytes. B) Two lamellar phases coexist with a repeat distance of either 13 nm (LPP) or 6 nm (SPP). C) Within the lamellae (lateral organization) three arrangements are possible: either an orthorhombic, hexagonal, or liquid packing.

repeat distance known as the short periodicity phase (SPP) [69-72]. The LPP is a trilayer structure that is unique in the SC and considered to be important for the skin barrier function [73, 74]. Perpendicular to the basal layer of the lamellae, the lipid organization is referred to as the lateral organization. In this organization the lipids can be ordered, adopting either the dense orthorhombic or less dense hexagonal packing or lipids can be disordered (liquid) [75-77]. Infrared spectroscopic studies showed that the majority of the SC lipids in human skin adopt the densely packed phase, which is necessary to limit the permeability through the skin [78, 79].

SC in skin diseases with an impaired barrier

Skin barrier dysfunction indicated by increased trans-epidermal water loss is habitually observed in inflammatory skin diseases including autosomal recessive congenital ichthyosis (ARCI), Netherton syndrome, psoriasis, and atopic dermatitis (AD) [32, 80-84]. The SC lipid composition of diseased skin differs from that of healthy skin and is considered to play a role in the impaired skin barrier function [85]. Netherton syndrome is a severe, rare genetic skin disorder characterized by congenital erythroderma, hair shaft defects, and severe atopic manifestations [83, 86-88]. In Netherton syndrome patients' skin, the level of acylCERs is reduced while there is an increased level of short-chain FFAs and CERs (particularly CER NS and CER AS), unsaturated CERs, and MUFAs [83]. Consequently, an altered lamellar organization and increased disordering of the lipids were observed.

ARCI is a rare skin disease characterized by abnormal desquamation over the whole body [84, 89]. In ARCI patients, the impaired $CERS_3$ via genetic defects results in alterations of the sphingolipid metabolism. This includes reduced levels of ultra-long chain CERs, and a disturbed epidermal permeability barrier function [57, 84, 90, 91]. Likewise, deficiency of $CERS_3$ in mice resulted in complete loss of ultra-long chain CERs (≥C26), altered lamellar organization, and a non-functional CLE [92-94]. Enzymatic oxidation of the linoleate ester in CER EOS by lipoxygenases (LOXs) is required for the formation of the CLE. In ARCI patients, Loss-of-function of 12*R*-lipoxygenase (12*R*-LOX) or epidermal lipoxygenase-3 (eLOX3) by gene mutations resulted in the absence of CLE on the surface of the cornified cell envelope [32]. Consequently, lamellar structures were defective and barrier function was severely impaired, indicating the importance of the CLE for a proper lamellar organization [95]. Psoriasis is another chronic inflammatory skin disease that is characterized by both increased desquamation and keratinocyte hyperproliferation in lesional skin leading to incomplete differentiation in distinct regions [82, 96]. In the lesional areas, the SC is thickened to about 10 times the situation in healthy skin [82]. Analysis of the lipid composition of psoriatic skin showed a reduction in CER subclasses: EOS, CER NP, and CER AP, whereas CER NS, and CER AS increased [61, 82]. Regarding the other lipid classes, psoriasis scales have been shown to contain an increased level of CHOL and a decreased level of FFA in comparison to SC of normal skin [96].

AD is a chronic, multifactorial, inflammatory skin disease characterized by redness, itching, dryness, and lesions [97]. In industrialized nations, the incidence of AD has increased to about 15-30% in children, and 2-10% in adults making it the most prevalent skin disease [98]. Due to the combined facts of its increasing

prevalence, its devastating effect on the quality of life, and consequential financial burden, AD remains of vital research interest [98-102]. Initially, AD was thought to be solely an allergic response [103, 104]. However, the loss-of-function mutations in the filaggrin gene appeared to be a major predisposing risk factor for developing AD [81, 105-107]. Filaggrin is an important protein for the maintenance of structural integrity and the breakdown products are part of the natural moisturizing factor (NMF), necessary for proper SC hydration [105, 108-110]. Reduced barrier function was subsequently demonstrated in certain AD patients unrelated to loss-of-function mutation of the filaggrin gene suggesting the involvement of factors other than filaggrin genotype in barrier dysfunction of those patients [57]. In AD patients' skin, the expression of certain enzymes involved in lipid metabolism differs when compared with control skin [57]. The activity of Sphingomyelinase and β-glucocerebrosidase, which catalyzes the conversion of their respective lipid precursors, sphingomyelin and glucosylceramide to CERs were shown to be reduced in lesional and non-lesional AD skin. [111, 112]. This correlated with reduced SC CER content and barrier dysfunction. Conversion by sphingomyelinase generates only CER subclasses NS and AS, while CER formation via glucocerebrosidase may result in various subclass [112].

It has been reported that the enzymes sphingomyelin deacylase and glucosylceramide deacylase that stimulates the cleavage of the acyl chain from the glucosylceramide and sphingomyelin are increased in activity, resulting in a decreased CER level [113-115]. It has been proposed that in AD, the activity of Ceramidases, which catalyzes the breakdown of CERs to a sphingoid base and a fatty acid chain is increased [57]. This resulted in accumulation of the CER metabolite, sphingosine-1-phosphate, which stimulated the release of the inflammatory mediators (tumor necrosis factor alpha (TNF-α) and interleukin (IL)-8) from the keratinocytes [116].

A strong correlation has been observed between changes in SC lipid composition and barrier function impairment in AD patients [117-119]. The lipid composition of AD patients' skin shows a reduction in the level of CER NP, CER NH, and the acylCERs, while there is an increase in levels of CER NS and AS [117, 118, 120, 121]. With regards to chain length variation, increased levels of short-chain CER particularly in the CER subclasses CER NS and CER AS was reported in diseased skin. CERs with a total chain length (sphingoid base chain + fatty acid chain) of 34 carbon atoms increased significantly in AD patients [112, 117, 118] when compared with controls. An increase in the level of lipid adopting the hexagonal lateral packing was observed. Apart from the increase in the level

of short-chain CERs, an increased level of short-chain FFAs was also observed. Reduced levels of ELOVL1 and ELOVL4 reported in the AD murine model suggests that a reduced average chain length of CERs and FFAs in SC of AD patients may be caused by a reduced expression and/or activity of these elongases [57, 122].

Alterations in lipid composition observed in diseased skin result in changes in lipid organization and possibly contribute to the barrier function impairment. Studying the effect of the changes in the lipid composition on the organization of the SC lipids is therefore an important aspect for investigating the factors for barrier dysfunction in diseased skin. However, the complexity of the SC structure makes it difficult to delineate the link between lipid organization and lipid composition in healthy and diseased human skin. As several alterations in the lipid composition of diseased skin occur simultaneously, it is not possible to determine the effect of the individual alterations in the lipid composition on lipid organization and barrier function in clinical studies. Lipid models in which the lipid composition can be varied at will and as desired, including mimicking that of diseased skin, offers an attractive option to achieve this feat.

SC lipid models.

SC lipid models range from simple ternary systems containing a CER, CHOL, and a FFA to more complex models incorporating a large number of lipid subclasses and chain lengths. The unique SC lipid organization has been mimicked by lipid model mixtures prepared with either isolated [72, 123-127] or synthetic [128-131] CERs together with CHOL, and FFAs. Previous X-ray diffraction studies carried out with mixtures prepared from isolated pig CER [123] or human CER [124, 127] and CHOL revealed the formation of two lamellar phases, the SPP and LPP mimicking the lipid organization in native human SC in which the SPP and LPP with a repeat distance of approximately 6 and 13 nm are present. The repeat distances of the lamellar phases formed by the pig CER: CHOL mixture were slightly shorter, being 5.2 and 12.2 nm, while the human CER: CHOL mixture mainly formed an LPP. Analysis of the WAXD data of both lipid mixtures showed that the lipids adopted a hexagonal lateral packing. The addition of long-chain FFA in both mixtures induced a phase transition from a hexagonal to an orthorhombic packing but also promoted the SPP formation in the human CER: CHOL mixture. Absence of CER EOS in the isolated human CER: CHOL mixture resulted in the predominance of the SPP [124] indicating the importance of CER EOS for the LPP formation.

Studies were performed in which the natural CER EOS in a human CER:CHOL:FFA mixture was replaced by synthetic CER EOS linoleate (double unsaturation) or CER EOS oleate (single unsaturation), or CER EOS stearate

(saturated) [132]. The liquid phase was more prominently formed and the LPP less prominent in presence of CER EOS oleate compared to the linoleate, while both the liquid phase and the LPP were not detected in presence of the stearate [132, 133] indicating that a certain level of unsaturation in the C18 chain of EO CER is required for LPP formation. X-ray and infrared spectroscopy studies showed that CER EOS linoleate and CER EOS oleate have a similar influence on lipid phase behavior in SC models [133].

The use of isolated CERs is limited by the laborious and lengthy procedure required for the isolation of CERs from human skin. Moreover, human skin is not as readily available. Synthetic CERs are used as an alternative and offer the additional advantage of having a well-defined chain length favorable for reproducibility of experimental data and interpretation of results. Equimolar mixtures of CHOL, FFAs, and synthetic CERs (mimicking the composition of pig CERs) formed the SPP and LPP and adopted the orthorhombic packing, closely resembling the lamellar and lateral native SC lipid organization [129]. As the synthetic CER composition can be altered as desired, SC lipid models can be employed to obtain detailed insight regarding the role of the individual lipid classes and subclasses in SC lipid organization. For instance, CER EOS was shown to be crucial for the formation of the LPP [129, 134]. Substitution of CER EOS by CER EOP reduced the formation of the long periodicity lamellar phase [129]. Though complex lipid mixtures prepared with synthetic CERs offer an attractive tool to unravel the importance of the molecular structure of individual CER for proper lipid organization, detailed interpretation of the interaction of the CER subclasses is complicated by the presence of diverse CER headgroups.

The interpretation of the interaction and lipid phase behavior in simple systems can be more detailed than in complex systems as interference from multiple lipid subclasses does not arise. Furthermore, it is easier to include deuterated lipids to obtain additional information. Previous studies of a simple SC model comprising an equimolar ratio of CHOL, palmitic acid (FA C16), and a CER reported differences in the physical properties such as phase transition temperature when the CER headgroup was varied [135-137]. However, chain length mismatch between the CER and FFA chains in these simple systems often resulted in the formation of separate domains of the lipid species. In other simple systems, FA C16 was replaced with lignoceric acid (FA C24), which is the most abundant FA chain length in the native SC [138, 139]. Despite the important role of the LPP in the characteristic SC lipid organization, these systems did not contain CER EOS and thus did not form the LPP. Examining the effect of lipid composition on the lipid organization and molecular interaction within the LPP

is important for a comprehensive and detailed understanding of the processes, factors, and mechanisms underlying skin barrier function.

Model membrane system for assessment of barrier function

To obtain detailed insight regarding the role the individual lipid classes and subclasses play in the skin barrier function, SC lipid model membranes are developed on a porous membrane. This system can be clamped in a diffusion cell to be able to perform permeation studies [73]. As the lipid composition in the model can easily be modified, the lipid model membranes enable to study of the relationship between lipid composition, molecular organization, and barrier function. The model membranes offer the opportunity to mimic the lipid composition and organization in healthy as well as in diseased skin. Barrier function properties of the SC lipid model membranes can be evaluated with various techniques. The inside-out barrier can be determined by measuring trans-epidermal water loss (TEWL). The outside-in barrier function can be examined by the measurement of the amount of a model drug permeating a membrane per unit area per unit time (flux) in diffusion experiments.

A previous study evaluated the barrier integrity of the SC model membrane that mimicked pigCER composition using diffusion studies of three model compounds with different lipophilicities: benzoic acid, para-aminobenzoic acid (PABA), ethyl PABA and butyl PABA [73]. Isolated human SC was used as a control sample. The permeation profile of all these compounds across the model membrane revealed a similar flux as observed in human SC, demonstrating the suitability of the model membrane as a substitute for the native SC. In that study, modification of the lipid composition of the pigCER model, generating a model membrane that lacks CER EOS, was accompanied by a two-fold increase in permeability indicating the importance of CER EOS not only in the lamellar organization but also for proper skin lipid barrier function. The pigCER model membrane as control was subsequently modified to mimic several alterations in the lipid composition observed in diseased skin [140]. When the pigCER model was altered to mimic the composition in recessive X-linked ichthyosis by incorporating high levels of CHOL sulphate, the model membrane displayed a two-fold increase in permeability compared to the pigCER control model. The barrier impairment was attributed to the formation of separate phases and the reduction in packing density. To mimic aspects of the lipid composition of psoriasis patients' skin, the level of CHOL in the model mixture was increased and the level of FFA was reduced. The permeability and lamellar organization were similar to those of the control though psoriasis skin is characterized by

impaired barrier function. Thus, the reported changes may not be responsible for the observed impaired barrier function in vivo [140]. A reduction in CER chain length and FFA chain length which may have a profound effect on permeability were not investigated as no information was available on the CER and FFA chain length distribution in psoriasis SC at that time [140]. The psoriatic skin model was modified to incorporate the reported CER composition of psoriatic scales thereby mimicking the psoriasis patients' skin more closely [141]. Lower permeability was observed across the psoriatic skin model than across the pigCER model and SC, which was suggested to be probably due to the higher level of phase-separated crystalline CHOL. In another study, the pigCER model composition was altered by the incorporation of increased levels of monounsaturated fatty acids, as observed in the skin of Netherton syndrome patients [131]. The permeability of the model membrane increased with the degree of unsaturation and was attributed to the phase transition of the lipid chains from orthorhombic to the less dense hexagonal phase.

To determine whether the reported changes in lipid composition contribute to the impaired barrier function of atopic dermatitis skin, it is important to design a model membrane that can mimic the lipid composition of atopic skin as closely as possible. PigCER model has been used as control in the studies with the diseased skin models described above. However, the pigCER mixture contained short-chain CER and high levels of CER NS which are not present in healthy human skin. There is therefore a need for a SC model that will mimic human CER composition and lipid organization as the result obtained with such a model can be better extrapolated directly to the in-vivo situation.

AIM OF THIS THESIS

The studies described in this thesis aimed to unravel the underlying factors and mechanisms for the impaired skin barrier function in AD using complex model membrane systems mimicking the human SC lipid composition and simple model membranes to obtain more detailed information.

OUTLINE OF THIS THESIS

Studies in **Chapter 2** describe the development of a lipid model membrane mimicking the lipid composition and lipid organization of human SC by replacing the pigCER composition with a CER composition that mimics closely that in human SC. To establish the resemblance of the skin model to the native skin, the lipid organization and permeability of the lipid model membrane were compared with those in SC of the native skin. In subsequent studies, the lipid composition was altered to mimic several aspects of the lipid composition in AD skin. The changes in lipid composition investigated in this study include i) incorporation of short-chain length CERs with a total chain length of 34 C atoms. ii) Increased levels of CER NS and CER AS and reduction in the level of CER NP. iii) Reduction in CER EOS concentration and iv) Increased level of short-chain FFAs. As the penetration through these membranes can also be measured, the lipid membrane models enabled a systematic examination of the effect of the various changes reported in the lipid composition of AD patients' skin on the permeability and lipid organization of the model membrane. Similar deviations in lipid composition are observed in some other inflammatory skin diseases including Netherton syndrome and psoriasis [82, 83].

In the subsequent chapters, simple model systems mimicking important aspects of the native skin lipid organization were prepared for a more detailed study based on the changes in CER composition of AD patients' skin investigated in chapter 2.

Chapter 3 describes studies involving varying levels of CER EOS in the SC lipid model. The level is altered in AD, but also in other inflammatory skin diseases [32, 80-84]. As CER EOS concentration affects the formation of the LPP, SC models with gradually increasing levels of CER EOS (10/30/50/70/90) mol% were employed for a detailed investigation of the influence of CER EOS concentration on lamellar organization, LPP unit cell structure, lateral organization, and permeability of the model membrane. The LPP intensity distribution in the models was compared to that previously observed for the complex SC model and native SC [144] to determine the similarity of their LPP structures. The mechanism underlying the changes in permeability with varying concentrations of CER EOS was investigated.

The simple models used in the subsequent studies were prepared as an equimolar mixture of CERs, CHOL, and FFA with CER EOS constituting 40% of the CER fraction. This high CER EOS concentration opposed to the physiological 10% (approximate) [57, 145] was necessary to prepare lipid models forming only

the LPP [136, 146, 147] with a similar structure to that of the native skin (hereafter referred to as LPP model).

In **Chapter 4**, a detailed study is presented that describes the effect of CER headgroup variation on the lipid organization and barrier function of LPP models focusing on the sphingosine- and phytosphingosine-based CERs as their levels are altered in AD and psoriasis. Biophysical techniques were employed to determine the mechanisms for the differences in the lipid phase behavior and permeability of the models containing the different CER head groups.

In **Chapter 5**, studies are reported in which the level of the long acyl chain length CER NS(C24) in the simple LPP model was gradually substituted with short-chain CER NS(C16) as observed in AD but also in Netherton syndrome patients' skin to systematically evaluate the effect of the acyl chain length of CERs on the permeability and phase behavior of model membranes. Finally, the overall findings are summarized and the future perspectives are presented in **Chapter 6**.

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