Introduction

Skin is a highly complex tissue acting as a protector against physical, chemical and biological attack. It plays a crucial role in the protection against dehydration and the control of body temperature (1). This barricade is provided by the "horny layer" (stratum corneum, SC), representing the outermost layer of the epidermis. The horny layer is a thin inert, water-retaining barrier which both regulates the moisture content of the skin and protects it against external influences. Due to its structure it is often compared to a brick wall in which the nonviable corneocytes are embedded like bricks in a matrix of lipids ("Mortar") (2). The lipid mixture is assembled into densely packed lamellar structures consisting of ceramides, cholesterol and fatty acids. In the literature different compositions of skin lipids are given depending on extraction and analytical methods exerted and the origin of the skin used for analysis. On a weight basis, these lipids constitute of approximately 47 % ceramides, 24 % cholesterol, 11 % free fatty acids and 18 % cholesterol esters (3). The physical organization of the membrane bilayer structure is crucial for an effective skin barrier and this is provided by a lipid lamellar assembly in a tightly packed orthorhombic configuration. The lipid environment of the stratum corneum is an essential factor for maintaining the skin's equilibrium.

As a result of age, health or environmental conditions, changes in the lipid composition occur, leading to a weakening of the barrier function (4, 5, 6).

Ceramides as the major epidermal lipid components are attributed to play an important role in the physical organization of the extracellular matrix. These findings lead to the concept that they are valuable components of Skin Care products, since the topical application of ceramide-containing products can replenish any low levels of stratum corneum lipids.

The Importance of the Skin-Identical Ceramide 3 Configuration for the SC's Lipid Barrier

Changes in the SC lipid composition have been linked directly to barrier function impairments such as dry and rough skin showing an increased transepidermal water loss and augmented penetration of harmful compounds from the environment finally resulting in inflammation.

Furthermore, during aging the functions of the skin underlie severe changes in both structure and chemistry. For instance, the amount of ceramides present in the stratum corneum decreases drastically with age (7) because keratinocytes have a reduced ability to synthesize certain classes especially the quantity of phytosphingosine-based ceramides like Ceramide 3 (8, 9, 10).

Additionally, in certain skin diseases accompanied by elevated water loss rates such as atopic dermatitis, psoriasis and
Ichthyosis abnormal intercellular membrane structures for barrier function are observed pointing out the importance of a correctly packed lipid matrix (5, 6).

So, a balanced application of SC-identical lipids such as pure ceramides or a combination thereof with cholesterol and fatty acids should replenish any low levels of stratum corneum lipids.

Starting the development of sphingolipids efficient for the treatment of skin and/or skin diseases lead finally to the production of phytosphingosine-based ceramides and identification of their key role in the barrier function of the skin (5, 6, 11). The production technology of Degussa combines the biotechnological production of the sphingoid base with a final chemical coupling of a fatty acid to achieve the exact chemical configuration present in the skin (Fig. 1).

Therefore, the phytosphingosine precursor tetra acetyl phytosphingosine (TAPS) is obtained by yeast fermentation with the strain Pichia ciferii followed by a deacetylation to get the free sphingoid base with the (2S, 3S, 4R) configuration. A subsequent acylation of the sphingoid base with a fatty acid finally leads to ceramides with the correct configuration of the skin, e.g. Ceramide 3 shown in Fig. 2.

Detailed research on the assembly of SC lipids has been performed by Bouwstra et al., in conclusion establishing the so-called "Sandwich model" (12): By electron microscopy and x-ray diffraction the SC lipids are observed as alternating broad/narrow/broad sequences of bilayers representing two broad lipid layers with crystalline structure separated by a narrow central lipid layer with fluid domain. X-ray diffraction analysis demonstrates that the adjacent crystalline sublattices are based on long saturated hydrocarbon chains of ceramides. Both lamellar phases incorporated in the sandwich model, the "Long Periodicity Phase" (LPP) of 12-13 nm, and the "Short Periodicity Phase" (SPP) of 5-6 nm, are crucial for the permeability barrier of the skin.

Based on studies with mixtures of isolated SC lipids, Bouwstra et al. proved the importance of the singular lipids, especially the ceramides (13). The need for the right ceramide stereochemistry is exemplified by more recent studies. Ceramide 3 (N-stearoyl phytosphingosine; s. Fig. 2) offered by Degussa is a human skin-identical ceramide supporting the protective layer of the skin. Due to its correct three-dimensional structure of the phytosphingosine backbone, Ceramide 3 is completely integrated into mixtures of human lipids as shown in Fig. 3.b. Unlike to that, the addition of a chemically synthesized racemic sphinganine-based Ceramide 2 (N-stearoyl sphinganine) to the human SC lipids leads to a disruption of the lipid matrix resulting in a completely different x-ray diffraction profile and particularly a loss of the LPP (Fig. 3.c). These findings were further supported by in vitro experiments where cultured human primary keratinocytes were cultivated either in the presence of 25 µM Ceramide 3, in the presence of 25 µM of the racemic Ceramide 2 or without any additions. The cells incubated with racemic Ceramide 2 showed significantly enlarged flattened cell morphology which is a sign of senescence. This induced aging-process can be explained by observed incompatibility of non skin-identical ceramides with subcellular structures. The
cells that were incubated with Ceramide 3 showed normal behaviour.

Considering the latest understanding of dry skin, a correction of the generally lowered ceramide levels, particularly of the phytosphingosine-based ceramides, is needed (14). The efficacy of the skin-identical ceramides such as Ceramide 3 distributed by Degussa has been approved by several short term and long term in vivo studies, with a significant efficacy in reduction of transepidermal water loss (TEWL) and skin irritation.

**Efficacy of Ceramide 3 as a Crucial Component of the SC’s Lipid Barrier**

To estimate the lipid barrier restoring effect of Ceramide 3 the forearms of 15 female volunteers (age: 20-41 years) were exposed for two hours with an occlusive patch to a 5 % aqueous solution of Sodium Dodecyl Sulphate (SDS). After removing of the patches, washing and air-drying of the damaged skin areas the degree of skin irritation was measured with a Tewameter (Courage & Khazaka, Cologne) determining the increase of the water evaporation.

Subsequent, the volunteers applied two times daily over 14 consecutive days a vehicle formulation on the one arm and a formulation containing 0.2 % Ceramide 3 on the other arm. The dose of application was about 2 mg/cm². Measurements were evaluated before application, after SDS challenge and at day 3, 7 and 14.

The results summarized in Fig. 4 show the TEWL obtained in % related to the pretreatment values. It demonstrates the highly significant efficiency of Ceramide 3 in reduction of transepidermal water loss.

The protecting effect of Ceramide 3 against surfactant-induced dermatitis was additionally estimated in an in vivo screening test with five volunteers (19-35 years). To achieve skin protection they applied two times daily over 7 days 2 mg/cm² of a vehicle formulation and a formulation containing 0.2 % Ceramide 3, respectively, to their forearms whereas one area remained untreated.

Subsequently, the skin was damaged with a 5 % SDS solution to induce skin irritation (See test design of the TEWL study described above). After 2 hours exposure of the SDS soaked patches to the skin’s test areas the patches were removed and the skin areas were washed with clear water and air-dried.

Afterwards, the degree of skin irritation was determined with a chromameter measuring the redness of the skin. The values for skin redness (%) were expressed relative to the pretreatment values (See Fig. 5).

![Fig. 4: TEWL reducing effect of Ceramide 3 on surfactant-induced dermatitis](image)

**Fig. 4: TEWL reducing effect of Ceramide 3 on surfactant-induced dermatitis**

![Fig. 5: Protecting effect of Ceramide 3 against surfactant-induced dermatitis](image)

**Fig. 5: Protecting effect of Ceramide 3 against surfactant-induced dermatitis**

The results demonstrate in an impressive way that the skin irritation represented by the redness could be reduced to an amount of only 10 % when the Ceramide 3 containing formulation was applied.

Recently the knowledge about Ceramide 3 efficacy on the skin performance was enlarged by finding that a 2 week topical application of Ceramide 3 effectively increases hydration and additionally smoothes the skin.

Therefore, 20 female volunteers were recruited (age 30-69 years) who applied O/W test formulations (Vehicle and a cream containing 0.2 % Ceramide 3) two times daily on their inner forearms for a period of 14 days. To help differentiate changes...
The measurement of the skin profile was carried out with the FOITS equipment. The "Fast optical in vivo topometry of human skin" (FOITS) is a contactless method to measure the three-dimensional profile of skin areas and therefore to estimate the influence of active ingredients on skin roughness or wrinkles.

The FOITS measuring system consists of a projection unit projecting a grid on the skin areas. A fixed camera records and visualizes the surface curvatures of the detected skin sections. Changes in skin profile can be quantified with the parameters Rz and Ra via the measurement of approx. 50 singular lines perpendicular to the mainstream of wrinkles.

Changes in skin profile can be displayed by the Rz value which is defined as the mean value thereof. A smoothing effect related to the macro structure of the skin is expressed by decreased Rz values. The Ra parameter stands for the fine structure of the skin due to its definition as the arithmetic mean value of all deviations of the roughness profile beyond the axis. A smoothing efficacy and an improvement of the fine structure are represented by decreased Ra values.

Fig. 7 illustrates that Ceramide 3 shows good efficacy in reducing the parameters Ra and Rz when taken into account that cosmetic formulations generally decrease the parameters up to 2-3 % whereas skin smoothing active ingredients boost the decrease of the parameters typically to at least 5 %.

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Fig. 6 illustrates the statistically significant increase of skin hydration after a two weeks application period of test formulations. From these studies it could be demonstrated that topical application of Ceramide 3 effectively increases the moisture content in the epidermis.

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Both studies, corneometry and the measurement of the skin profile, were carried out under standardized conditions in a climatic room (22 °C, 50 % relative humidity) before and after the application period.

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With the help of the FOITS equipment the skin smoothing influence of Ceramide 3 before and after a 14 days application period could be measured. It has been confirmed that Ceramide 3 shows excellent efficacy in reducing the parameters Ra and Rz. That indicates that Ceramide 3 improves fine lines and the macro structure of the skin.

**Conclusion**

The outermost layer of the skin - and hence of the body - is represented by the "horny layer" (stratum corneum, SC) consisting of corneocytes and lipids which are assembled into densely packed lamellar structures. These lipids constitute of ceramides, cholesterol, esters thereof and free fatty acids.

The physical assembly of the stratum corneum is crucial for an effective skin barrier and therefore finally to maintain the skin's equilibrium.

As a result of age or health, changes in the lipid composition occur, leading to a weakening of the barrier function. Lots of
Efforts in skin research pointed out to the fact that ceramides, as the major epidermal lipid component, are key players in maintaining the epidermal integrity and health. These findings lead to the concept that ceramides are high-performance active ingredients for Skin Care products, since their topical application can replenish low levels of stratum corneum lipids.

Especially the skin-identical Ceramide 3 described in detail in the present article shows complete biocompatibility with the supramolecular biophysical structures of the natural occurring mixture of human skin lipids. In contrast to this, a chemically synthesized non skin-identical ceramide, based on racemic sphinganine, leads to a disassembly of the lipid barrier matrix and senescence when applied to cultured keratinocytes. Therefore body identical stereochemistry of ceramides is the key to maximum activity of this molecule class.

This article summarizes formerly obtained results showing the superior efficacy of Ceramide 3 on restoring the skin barrier and completes these findings with recently performed studies on concomitant improvement in skin hydration and firmness. After an application period of 14 days the moisture content in the epidermis increased effectively in that *in vivo* study. Additionally, the skin soothing properties of Ceramide 3 to reduce fine lines and to improve the macro structure of the skin has been proven. These findings once again proof the integration of Ceramide 3 into the skin’s own lipid lamellar system finally contributing to an improved barrier function and skin performance.

**References**