Breathable Shield Against Irritants – Effective Protection for Stressed Skin with Functional Silk Polypeptides
Skin is the interface between the inside of the human body and the environment. With its surface area of 1.5 to 2.0 m², it is the largest human organ. It comprises the epidermis (several single layers of different cells, closely linked to corium), the corium (feeds the epidermis and transports metabolites to and from the epidermis) and the subcutis (connective tissue and fat). The top layer of the epidermis, which is a squamous epithelium without connective tissue or blood vessels, is the stratum corneum. Although its thickness is only 7 to 35 microns, it plays a vital role in forming the indispensable protective physical barrier, as it prevents percutaneous entry of harmful substances into the body (1). The stratum corneum consists of interlocked corneocytes as well as long chain ceramides which are responsible for the cohesion of the corneocytes. Figuratively, the corneal layer is constructed like a brick wall (cells) with mortar (hydrolipidic layer) consisting mainly of ceramides, cholesterol, fatty acids and squalenes. Additionally to acting as physical barrier, it is also a chemical barrier. In particular, it prevents the penetration of hydrophilic substances and small solid particles. Several lipophilic substances can enter the body through the stratum corneum due to the intercellular lipid bilayers (2). The degree of the permeability of lipophilic as well as hydrophilic substances is varying and depends on molecular size (< 500 Da) and hydrophobicity of the molecules as well as on the condition of the skin. The stability and compact construction of the stratum corneum is very important for a functional and resistant barrier. The slightly acidic pH of the stratum corneum provides supplementary protection against pathogens (3).

In addition to serve as a physico-chemical barrier, the stratum corneum has other important functions, including thermoregulation, gas exchange and maintenance of proper moisture management. Due to the huge water uptake capacity of keratinocytes the normal water content of the stratum corneum is around 20%. A small percentage decrease of water content already leads to a dry and rough skin surface.

Healthy skin prevents penetration and permeation of molecules into the subcutis and the subsequent resorption into the circulatory system. The maintenance of a healthy skin is of major importance as the number of individuals consulting dermatologists with subjective enhanced skin sensitivity is increasing steadily. It is still unclear, whether this is a result of changing environmental conditions, increased awareness or both.
parameters (pollution, sun rays, occupational leisure-time activities, air conditioned rooms, radiant-heated homes in winter, skin care and cosmetic products) or merely an altered overall skin perception of modern individuals (4).

Breaches of the skin barrier are common events in our daily lives due to frequent use of detergents, surface active substances such as emulsifiers as well as various environmental factors. The exposure to surfactants erodes lipids and lipophilic compounds from the hydro lipid layer and natural moisturizing factors (NMF) from the stratum corneum. As a result, the stability of the corneal layer is decreased, the transepidermal water loss (TEWL) rises, the skin dries out, becomes itchy and various kinds of molecules, including harmful substances, attain easier epidermal access. Scratching or mechanical stress can further remove the upper layers of the skin which results in an increased local breach of the skin barrier. The scratching can severely compromise the skin barrier, enhancing inflammatory reactions that subsequently result in the itch-scratch cycle (5) (Fig. 1). Chronic contact dermatitis due to repeated exposure to low doses of irritants (e.g. bases or detergents and allergens) is a major clinical problem. Predominant features are skin dryness, scaling and roughness. It was shown, that more women than men suffer from irritant contact dermatitis as they are working more often in jobs using irritants (e.g. nurses, hair-dressers) (6).

Besides environmental factors, several skin diseases are also accompanied by an impaired barrier function of skin. Atopic dermatitis is an inflammatory skin condition which often is accompanied by dry, scaly skin. Alterations in skin barrier properties observed in atopic dermatitis include increased TEWL, changes in skin surface pH, compromised skin permeability and barrier integrity. Once the skin barrier is impaired, allergens, irritants and other unwanted agents can penetrate into the skin, leading to aggravation of symptoms associated with atopic dermatitis. Like the permeability barrier, the barrier against bacteria and other microbes is also compromised. Colonization by for instance Staphylococcus aureus on skin is found in up to 90% of atopic dermatitis patients. Moreover, Staphylococcus aureus can produce ceramidase, an enzyme capable of destroying the ceramides of the hydro lipid layer, thereby additionally undermining the barrier function (5, 7, 8).

Skin with a disturbed barrier function requires special protection from environmental influences as soon and as efficiently as possible. Either aiding barrier reestablishment or dampening the epidermal stress response may improve the treatment and accelerate recovery. Additionally, it is important to control itching, suppress inflammation and to restore the skin barrier. Zinc oxide pastes, hydrocolloid dressings, formulations containing bilayer components such as cholesterol, ceramides and squalenes and film-forming liquid acrylates are commonly used to support the skin barrier. Though usually effective in a short term, most of emollient moisturizers contain non-physiologic lipids such as petrolatum, lanolin, mineral oil and silicone. These substances may impede, rather than correct the underlying biochemical response of the skin barrier’s flawed structure, as they cause effects such as heat-accumulation or enhanced bacterial proliferation and sometimes even induce allergic reactions (9, 10, 11). Innovative functional silk polypeptides form a breathable film on the skin, thus serving as a physical, protective barrier aiding and improving the health status of stressed skin.

Silkgel – Breathable Protection for Stressed Skin

The innovative functional silk polypeptides are produced via an established and standardized biotechnological approach leading to a consistent high-quality material. Human gastrointestinal derived bacteria (Escherichia coli) are the standard production platform in biotechnology. These bacteria are cultivated in a special growth medium, containing sugars, salts and nitrogen sources. During this process the functional silk polypeptides are produced. The polypeptides are purified to homogeneity and are transformed into Silkbil (pure protein powder with spherical sub-structures) or Silkgel (hydrogel) subsequently. No animal derived sources are used in the whole manufacturing process rendering the proteins vegan. Unlike commonly used protein

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**Fig. 1** Compromised skin is characterized by an increased transepidermal water loss (TEWL) and a diminished content of the components of the hydrolipidic layer such as ceramides. The impairment of skin barrier results in dry and itchy skin that is not only prone to irritation by alkali, chemicals and harmful substances. These substances can also attain easier access to deeper epidermal layers, potentially leading to inflammation and immune reactions. Skin lesions that are aggravated by scratching can be infected by microbes.
ingredients, the silk polypeptides are fully functional and not hydrolyzed. Thus, the proteins can unfold their full efficiency on top of the skin. Due to their rather large molecular weight (47.7 kDa) they do not penetrate into the skin. Silkbeads as well as Silkgel are free of parabens, silicones and/or mineral oils and are produced in accordance with the quality management system of ISO 9001:2008. Both are sustainable and biodegradable.

Silkgel is a hydrogel containing functional, non-hydrolyzed silk polypeptides and water. It is miscible with water as well as oil based formulations and spreads easily. Due to its shear-thinning properties, even Silkgel with high viscosity/consistency is sprayable and dispensable. When applied on skin, Silkgel generates a thin and transparent film. The silk proteins form a network of stable, three dimensional structures with small pores for air and water vapor exchange. Although in principle any kind of surface can be coated with Silkgel, it is especially suited for human skin. Silkgel forms the protective and breathable film on the skin without the use of conventional chemicals or glues such as acrylates. The exclusively physical barrier becomes immediately functional after application. It supports the skin in its regeneration process. The silk film is long lasting, robust, not sticky and smooth to the touch. An enforced and even stronger barrier function is conceivable when using barrier supporting products containing parts of hydrolipidic layer accompanied with Silkgel.

Methods and Results

In-Vitro Experiments Elucidating the Protective Potential

To determine the efficacy of Silkgel to serve as protective barrier (i.e. shielding the skin from percutaneous absorption of small molecules and bacteria) an in-vitro setup using a Franz-type two-chambered diffusion cell was chosen. The diffusion cell consists of two chambers in which the membrane (or skin model) is positioned above a receptor chamber. Both sides of the membrane are exposed to solutions. The upper »donor« chamber contains a solution of the penetrating chemical in excess dose to maintain constant concentration while the lower chamber contains the receptor fluid for collecting the penetrating chemical. This chamber is stirred continually to avoid concentration gradients of the diffusing substance in the receptor fluid. A coated cellulose filter was used as carrier membrane for the thin polypeptide film. It was verified that the whole filter was completely coated with the silk polypeptide using laser scanning microscopy. The filter itself is no barrier for the investigated substances which hence can diffuse unhindered. A great range of hydrophilic (histamine, caffeine, methylene blue) as well as hydrophobic substances (testosterone, β-carotene) was investigated, mimicking various environmental influences on the skin. These small molecules are commonly used as model substances in diffusion experiments, as they are skin penetrating due to their molecular character and their size distribution.

The assay revealed that the silk film significantly decreases the diffusion rate of all tested hydrophilic substances (Fig.2) and lipophilic substances (Fig.3). The extent of the effect depends on the applied amount of silk polypeptide resulting in thinner or thicker film layers. The diffusion can be reduced to more than 50%. This retention is comparable to the value achieved when applying polyacrylate based barrier products. Due to limitations of the assay the lowest applicable protein amount tested was 0.3 mg/cm². A thin silk polypeptide film is more fragile in this setup and can be easily de-
destroyed by the mechanical forces affecting the silk film as the substrate filter has very large pores. On skin even lower amounts of Silkgel can be applied to achieve good and significant effects. This was demonstrated in the in vivo experimental setup described below. Microbes were also used as model substances. For this setup the Franz-type diffusion cell was used under sterile conditions. LB (Lysogeny broth) was used as donor, as well as receptor fluid. An overnight culture of bacteria (E. coli) was added to the upper chamber. Plate count was used to determine the amount of bacteria through an uncoated and a Silkgel-coated filter. It was shown, that up to 97% of bacteria were retained. The silk-coated filter membrane is thus an excellent barrier against microbes, since the diffusion is significantly lower compared to the control. Data reveal that the silk film is capable of retaining and delaying agent penetration. The barely visible film provides an efficient barrier function for small hydrophilic as well as hydrophobic molecules and microorganisms. The application of Silkgel additionally offers a soft and silky feeling. Taken together Silkgel is suitable for dry, sensitive and irritated skin as it significantly improves the skin’s barrier function as well as the overall skin appearance.

In-Vivo Experiments Show Significant Skin Preservation

A customized and newly developed in-vivo irritation test to show the protective properties of Silkgel was carried out by Dematest GmbH, Münster. Four common irritants (0.5% sodium hydroxide (NaOH), pH 13.21; 0.5% sodium dodecyl sulfate (SDS), pH 8.7; 20% acetic acid, pH 2.2 and 20% lactic acid) were individually applied once a day for five consecutive days on the volar forearm of five healthy test persons for 60 minutes using 12 mm Finn chambers. One side served as unprotected reference, the other side was treated with two puffs of the test item before irritation (spray volume: 100 µL; equates to approx. 0.2 mg/cm² silk polypeptide; composition of the test item: Aqua, Alcohol denat., Pentylene Glycol, Glycerin, Silk polypeptide (Silkgel), Sodium Hyaluronate, Citric acid). Endpoints of this test were optical appearance of the skin as well as transepidermal water loss (TEWL) and the formation of erythema (Chromametry) before and after irritation.

TEWL is the passive diffusion of water through the stratum corneum and is a good and frequently used indicator for the integrity of the stratum corneum. Structural changes of the stratum corneum induced by exposure to chemicals in most cases affect water diffusion. Healthy skin is characterized by TEWL-values up to 25 g/hm². The TEWL blank value was determined on the skin area directly next to the irritated segment (12).

Due to a strong irritation one test person was excluded from NaOH and SDS treatment on day five, one test person was excluded on day four in case of acetic acid and three were excluded on day four in case of lactic acid. The test persons included in the presented study had very healthy skin with an intermediate TEWL-value of around 10 g/hm². The TEWL value was determined on the skin area directly next to the irritated segment (12).

Fig. 4 Shown are the in vivo-results of the irritation test of unprotected vs. Silk protected skin. The transepidermal water loss (TEWL) is a commonly used indicator for the extent of skin barrier impairment. Data indicate that the applied Silk film protects the skin from damage.

Fig. 5 Chromametry is a method that can be used to assess the erythema formation indicating a very strong damage to the skin. The silk protected skin shows significantly lower values, indicating a strong protective potential of the silk polypeptide.
The unprotected skin of all test persons showed a very strong barrier impairment from day three on which reached a maximum (mean value of all test persons per day) of 102 g/hm² (NaOH), 54.5 g/hm² (SDS) and 13.3 g/hm² (acetic acid) on day five. The erythema formation of the sites irritated with lactic acid varied very much between the test persons, leading to the exclusion of two test persons after day three. The mean TEWL value of these test persons reached a value of 77.2 g/hm² and a mean redness of 13.8 (A-value) on day three. Other studies also irritating unprotected skin for five days attributed these observations to changes in the intercellular space. They showed, however, that the lipid bilayer of the upper stratum corneum seemed to stay intact at least in the short term so that the apparent barrier disruption has been attributed to changes in the amino acids and/or to protein denaturation within the corneocytes (13, 14, 15).

The application of the test item containing 1 % (w/w) Silkgel before irritation significantly reduced the impact of the irritants in all cases. Referred to the TEWL value (equates to 100 % damage), the damage to skin is reduced by 27 % (NaOH), 17 % (SDS), 32 % (lactic acid) and 11 % (acetic acid). Thus it can be concluded that the applied Silk film shields the skin from the negative influences of the investigated irritants significantly. The photographic documentation (Fig. 6) illustrates the reduced damage to skin impressively. Especially the area irritated with sodium hydroxide is significantly less harmed.

**Summary**

AMSilk’s novel and innovative silk polypeptides show new and unique properties. All data indicate that the breathable natural protein film formed after the application of Silkgel resembles a very efficient barrier against small molecules, microbes and irritants (Fig. 7). This renders this unique material highly interesting for various applications in the field of protection of sensitive or stressed skin, work safety and even as protective film forming ingredient in formulations targeting the treatment of diseased skin. The breathability of the silk polypeptide film decreases negative side effects such as heat accumulation or occlusion. The absence of tackiness, a soft feeling and a very good compatibility are only some of the great advantages of Silkgel.

**Referenzen**

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