

Sorption–desorption test for functional assessment of skin treated with a lipid system that mimics epidermal lamellar bodies

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Summary

Background. Many skin diseases are associated with either increases or decreases in lamellar body secretion, or dysfunctional lamellar bodies. Consequently, diseased skin is characterized by reduced barrier function and altered lipid composition and organization. Human skin is commonly evaluated *in vivo* with non-invasive biophysical techniques. The dynamic functions of the skin are evaluated with repeat measurements such as the sorption–desorption test (SDT).

Objectives. The aim of this study was to evaluate *in vivo* skin hydration–dehydration kinetics after treatment with a lipid system that mimics the morphology, structure and composition of lamellar bodies in both healthy and irritated human skin.

Methods. A patch with an aqueous solution of 2% sodium lauryl sulfate (SLS) was used to irritate the skin of the volunteers. The SDT was performed with the CM 820 corneometer.

Results. After treatment with this system, both healthy and SLS-irritated skin increased their ability to retain water and to release water slowly during the desorption phase.

Conclusions. Treatment with this system seems to reinforce the barrier function in both healthy and SLS-irritated human skin. Therefore, the present study provides evidence that this system could be of interest for developing future treatments for protecting and repairing the skin.

Key words: accumulated water decay (AWD); corneometer; skin lipids; sorption–desorption test; water-holding capacity (WHC); water sorption capacity (WSC).

Skin barrier function depends on the composition and lipid organization of the outermost layer of the epidermis, the stratum corneum, whose main functions are to prevent excessive water loss and the penetration of xenobiotics and pathogens. The stratum corneum

(10–20 μm in thickness) contains approximately 15–20 layers of corneocytes, which are embedded in a lipid matrix organized in a lamellar structure (1). The whole stratum corneum contains ~15% lipids, mainly ceramides (45–50% of the total lipid mass), cholesterol (25%), and free fatty acids (10–15%), and smaller amounts of cholesterol sulfate (5%) and cholesterol ester (2%) (2).

Epidermal lamellar bodies are produced in the stratum granulosum by keratinocytes, and secrete their contents – precursors of the stratum corneum lipids, hydrolases, protease inhibitors, and antimicrobial peptides – into the intercellular space at the stratum

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granulosum–stratum corneum interface to form the lamellar structure of this tissue (3). Two models have been proposed for skin barrier formation: the Landmann model, which proposes that lamellar bodies are discrete vesicles (4); and the membrane-folding model, which proposes that lamellar bodies form part of a tubuloreticular membrane network (5, 6).

A large number of skin diseases are associated with either increases or decreases in lamellar body secretion or dysfunctional lamellar bodies (7–11). Consequently, diseased skin is characterized by reduced barrier function and alterations in lipid composition and organization. Atopic dermatitis is a very common skin disease in which there are deficiencies in certain lipids, especially ceramides and free fatty acids (12, 13). Moreover, the synthesis of antimicrobial peptides, which play a role in protecting the skin from microbial infection, is reduced in atopic dermatitis (14). In addition, an alkaline pH of the skin surface is another cause of increased skin susceptibility to bacteria, mainly *Staphylococcus aureus*, in atopic dermatitis (15).

Human skin is commonly evaluated *in vivo* with non-invasive biophysical techniques such as skin hydration, which gives information about the water content, and transepidermal water loss for study of skin barrier function. The dynamic functions of the skin are evaluated with repeat measurements, such as the sorption–desorption test (SDT). This test assesses the behaviour of the skin surface when it is in contact with applied water. Therefore, the SDT provides information about the ability of the skin to take up water (water sorption capacity) and to retain water, counteracting a dehydration process (water-holding capacity) (16). In this way, the SDT is used for the functional assessment of different skin diseases, such as psoriasis (17) and atopic dermatitis (18), and the results are compared with those obtained with healthy skin. In addition, this test is useful for evaluating the efficacy of moisturizers (19, 20).

In previous work, we designed a lipid system based on bicosomes that mimic the morphology, structure and composition of epidermal lamellar bodies. In addition, we performed *in vitro* studies to evaluate the effect of this system on delipidized stratum corneum (21). We concluded that treatment with the lamellar body mimetic system (LBms) re-establishes part of the lipid lamellar structure of delipidized stratum corneum, mainly the short periodicity phase described by Bouwstra et al. (22).

In the present study, we sought to evaluate the effect of the application of the LBms on the skin *in vivo*. Accordingly, we evaluated hydration–dehydration skin kinetics in both healthy and irritated human skin, and how

treatment with this system affected water sorption and desorption.

Materials and Methods

Chemicals

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (CAS no. 63-89-8) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) (CAS no. 34506-67-7) were from Avanti Polar Lipids (Alabaster, AL, USA).

Cholesterol from lanolin (chol) (CAS no. 57-88-5), cholesteryl sulfate sodium salt (Schol) (CAS no. 2864-50-8), cholesteryl palmitate (Echol) (CAS no. 601-34-3) and stearic acid (SA) (CAS no. 57-11-4) were from Sigma-Aldrich (Steinheim, Germany). Ceramide IIIb, *N*-oleyl phytosphingosine (Cer) was from Evonik (Essen, Germany). Lipoid S100, whose main component (>94%) is soybean phosphatidylcholine (PC), and Lipoid S LPC 80, whose main component (80%) is soybean lysophosphatidylcholine (LysoPC) (CAS no. 92128-87-5), were from Lipoid GmbH (Ludwigshafen, Germany).

Chloroform (CAS no. 67-66-3), methanol (CAS no. 67-56-1) and sodium lauryl sulfate (SLS) (CAS no. 151-21-3) were from Merck (Darmstadt, Germany).

Preparation and characterization of the lipid systems

Discoidal structure preparation. Four types of discoidal structure were prepared: (i) DPPC/DHPC, $q = 3.5$ ($q = \text{DPPC/DHPC molar ratio}$), including 10 mol% Cer; (ii) DPPC/DHPC, $q = 3.5$, including 5 mol% chol; (iii) DPPC/DHPC, $q = 3.5$, including 5 mol% Schol; and (iv) DPPC/DHPC, $q = 3.5$, including 5 mol% Echol. For each of the discoidal systems, appropriate amounts of lipids were mixed in a chloroform/methanol (2:1) solution and evaporated to dryness with a rotary evaporator. The systems were hydrated with distilled water to obtain a 10% (wt/vol) total lipid concentration, and then subjected to several cycles of sonication, freezing and heating until the samples became transparent.

Next, different volumes of these samples were selected to obtain a mixture in which the molar ratio of Cer, chol, Schol and Echol was similar to that found in the skin. To obtain 1 ml, 440 μl of (i), 440 μl of (ii), 88 μl of (iii) and 32 μl of (iv) were mixed. This mixture was used to prepare the LBms.

LBms preparation. A chloroform solution containing 95 mg/ml PC, 5 mg/ml LysoPC and 1.6 mg/ml SA was rota-evaporated to remove the chloroform and

thus form a lipid film. The film was hydrated with the previously prepared mixture of discoidal structures approximating the stratum corneum lipid composition. The total lipid concentration in the final system was 20% (wt/vol), with a Cer:SA:chol:Schol:Echol molar ratio of 1:1:0.5:0.1:0.04. The LBms was analysed 24 h after preparation by dynamic light scattering (DLS) and cryogenic transmission electron microscopy (Cryo-TEM). The sample was maintained at 4°C during the 10 days of the experiment.

Dynamic light scattering. The hydrodynamic diameter (HD) was determined by DLS with a Zetasizer nano ZS (Malvern Instruments, Malvern, UK). The DLS technique measures the diffusion coefficient (D) of the particles corresponding to Brownian motion, and converts this to size by using the Stokes–Einstein equation,

$$HD = kT/3\pi\eta D$$

where HD is the hydrodynamic diameter, D is the translational diffusion coefficient (m^2/s), k is the Boltzmann constant ($1.3806503 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$), T is the absolute temperature (298 K), and η is the viscosity of the dispersant at 25°C (water, 0.8872 mPa s). The particle sizes were determined from the scattered light that was detected at an angle of 173° when a laser beam of wavelength 633 nm crossed the sample. Measurements were performed in triplicate.

Cryogenic transmission electron microscopy. The LBms was visualized by the use of Cryo-TEM. A thin aqueous film was formed by dipping a glow-discharged holey carbon grid in the suspension and then blotting the grid against a filter paper. The resulting thin sample film spanning the grid holes was vitrified by plunging the grid into ethane, which was maintained at its melting point with liquid nitrogen, by use of a Vitrobot (FEI Company, Eindhoven, The Netherlands). The vitreous sample film was transferred to a Tecnai F20 transmission electron microscope (FEI Company) by use of a cryotransfer holder (Gatan, Barcelona, Spain), and the sample was observed in low-dose mode. Images were acquired at 200 kV at a temperature between −170°C and −175°C, under low-dose imaging conditions. Ten overviews and ~50 detailed electron micrographs were taken.

In vivo study

Five females aged 29–51 years with healthy skin participated in this study. Written informed consent was obtained from all participants. Four different skin areas

(4 cm²) on the lower leg corresponding to untreated (control), treated with the LBms (treated), irritated with SLS (SLS) and irritated with SLS and treated with the LBms (SLS-treated) were delimited. These areas were randomized on each participant. The participants were not allowed to use moisturizers on the lower leg for 1 week prior to, and during, the period of the experiment. To obtain reliable measurements, the participants were acclimatized for 15 min in a conditioned room at $23 \pm 1^\circ\text{C}$ and $50 \pm 3\%$ relative humidity before the measurements.

Study design. SDTs on two non-irritated skin areas were performed on days 0, 4, and 7. One of these areas was treated daily with 10 µl of the LBms for 7 days, and the other was not treated.

On irritated skin, SDTs on two skin areas were performed on day 0 (before and 30 min after SLS irritation), day 1, and day 4. Both skin areas were irritated as described: 10 µl of SLS 2% aq. was applied on a filter paper placed in each of two aluminium chambers (diameter = 8 mm; Finn Chambers®; SmartPractice, Phoenix, AZ, USA). The chambers were fixed to the skin for 2 h with adhesive tape. Upon removal of the patches, the skin was gently rinsed with water and dried with a paper towel. One of these areas was treated daily with 10 µl of the LBms for 4 days, and the other was not treated.

Sorption–desorption test. A CM 820 corneometer (Courage & Khazaka, Cologne Germany), which measures skin capacitance in arbitrary units, was used to record the different measurements obtained with the SDT. The SDT was performed as described by Tagami et al. (16), with slight modifications, such as a longer water application time (1 min instead of 10 s). The first capacitance measurement represented the prehydration value. Soaked cotton wool with 1 ml of distilled water was placed onto the skin, left for 1 min, and wiped with a paper towel, and the hydration was immediately measured (60 s). The capacitance was subsequently measured every 30 s for a maximum of 2.5 min. A curve was then constructed, with the value of maximum hydration as 100%, and the other values were calculated accordingly. This test provides information about the ability of the skin to take up water [water sorption capacity (WSC)] and to retain water, counteracting a dehydration process [water-holding capacity (WHC)]. Also, it provides information about the amount of water released from the stratum corneum through the desorption phase [accumulated water decay (AWD)]. These parameters were calculated from the curve as described by Pellacani and Seidenari (18). Figure 1 shows an example of an SDT curve and the different parameters.

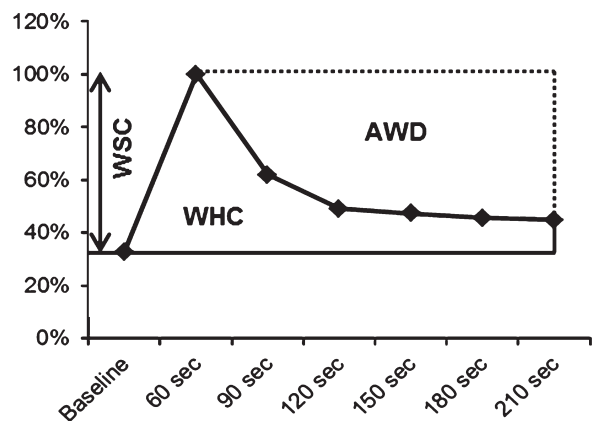


Fig. 1. Example of sorption–desorption test curve indicating the different parameters. AWD, accumulated water decay; WHC, water-holding capacity; WSC, water sorption capacity.

Table 1. Changes in water-holding capacity (WHC), water sorption capacity (WSC) and accumulated water decay (AWD) of healthy skin after 4 and 7 days of treatment with the lamellar body mimetic system

	WHC (%)	WSC (%)	AWD (%)
Day 4	28	–1	–25
Day 7	77	19	–31

The results are expressed as percentage increase or decrease with respect to the initial value at day 0 and the untreated control.

In order to be able to make interpretable statements on the effects of the treatment on WHC, WSC and AWD parameters, the results were calculated by use of the following equation (23):

$$\text{Change in the parameters (\%)} = [(P_t - P_0) / P_0] \times 100$$

In Table 1, P_t is the mean of the quotients of the calculated parameters of treated and control areas after the application time t in all participants, and P_0 is the mean of the quotients of the calculated parameters of treated and control areas before application in all participants. In Table 2, P_t is the mean of the calculated parameters of the irritated area after the application time t in all participants, and P_0 is the mean of the calculated parameters before irritation in all participants.

Statistical analysis. Means and standard deviations were calculated. Statistical analysis of the experimental data was performed by use of the Wilcoxon rank-sum test with STATA/SE 12.0 software. Differences were considered to be statistically significant at $p < 0.05$.

Table 2. Changes in water-holding capacity (WHC), water sorption capacity (WSC) and accumulated water decay (AWD) of irritated skin and irritated skin after treatment with the lamellar body mimetic system at day 0 (after irritation), day 1, and day 4

	Irritated skin			Irritated and treated skin		
	WHC (%)	WSC (%)	AWD (%)	WHC (%)	WSC (%)	AWD (%)
Day 0 (SLS)	47	23	–33	57	14	–27
Day 1	4	3	–3	19	–4	–15
Day 4	–6	8	14	43	12	–18

SLS, sodium lauryl sulfate.

On day 0, the two skin areas are equivalent. The results are expressed as percentage increase or decrease with respect to the initial value (before irritation) at day 0 for each skin area.

Results

Characterization of the system

DLS was used to determine the average size of the LBms. The size distribution curve of the system, which was obtained by analysis of the scattered light intensity, showed two peaks corresponding to a population of small particles with an HD of 27 ± 2 nm that scattered $26 \pm 1\%$ of the light, and a population of large particles of 345 ± 17 nm that scattered $74 \pm 1\%$ of the light.

Cryo-TEM allows us to directly visualize the sample and to characterize the dimensions and morphology of the different structures present in the system. Figure 2 shows a micrograph of the LBms. This image shows two types of structures, which is in agreement with the data obtained by DLS, corresponding to non-encapsulated disks (white arrows) with diameters of ~ 35 nm, and disks encapsulated in vesicles (black arrows) with a size of ~ 320 nm. This image also shows the disks in edge-on (white open arrows) and face-on (white closed arrows) configuration.

Effect of the treatment on healthy skin

SDTs were performed on healthy skin before treatment (day 0) and after 4 and 7 days of treatment with the LBms. The sorption–desorption curves and the different parameters calculated from the curves are shown in Fig. 3. After the application of water, the relative increase in capacitance values was similar before (black curve) and after (orange and brown curves) treatment with the LBms. The second parts of the sorption–desorption curves show that all of the relative capacitance values were higher after treatment (orange and brown curves), although the differences were not statistically significant. However, the parameter WHC calculated from the curves increased

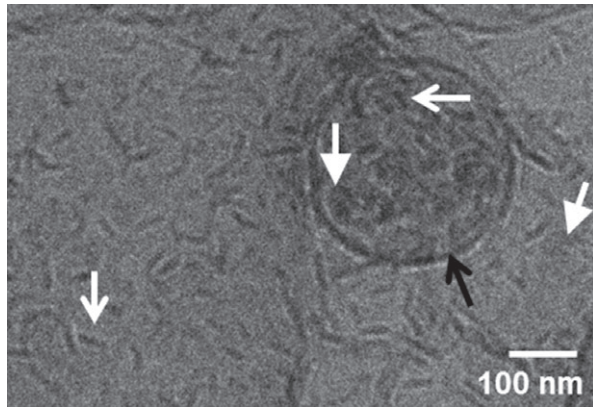
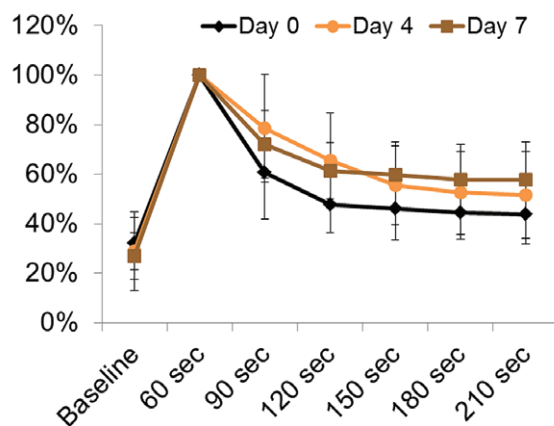


Fig. 2. Cryogenic transmission electron microscopy micrograph of the lamellar body mimetic system. The system is formed by non-encapsulated disks with diameters of ~ 35 nm, and vesicles encapsulating disks (black arrow) with sizes of ~ 320 nm. The disks are shown in all projections: edge-on (white open arrows) and face-on (white closed arrows).



	WHC	WSC	AWD
Day 0	132.6 $\pm$ 27.1	68.2 $\pm$ 10.2	64.5 $\pm$ 15.2
Day 4	181.9 $\pm$ 31.8*	71.2 $\pm$ 16.0	49.3 $\pm$ 20.0
Day 7	185.1 $\pm$ 28.4*	73.2 $\pm$ 9.4	47.9 $\pm$ 16.7

Fig. 3. Sorption–desorption test performed on healthy skin before treatment (day 0) and after 4 and 7 days of treatment with the lamellar body mimetic system. AWD, accumulated water decay; WHC, water-holding capacity; WSC, water sorption capacity. *Statistically significant differences ($p < 0.05$) as compared with day 0.

significantly after treatment with the LBms, and no differences were detected between days 4 and 7.

In order to be able to make valid statements on the effect of the LBms in healthy skin, the results were doubly evaluated as a percentage of modification with respect

to the initial value on day 0, and as a percentage of modification with respect to the untreated control. The results are shown in Table 1. When healthy skin was treated for 4 and 7 days, WHC increased by 28% and 77%, respectively. WSC remained unaltered after 4 days of treatment, but increased by 19% after 7 days. Finally, AWD decreased by 25% and 31% after 4 and 7 days of treatment, respectively.

Therefore, after treatment with the LBms, healthy skin increased its capacity to retain water, and also showed a decrease in the rate of water loss. Additionally, visual inspection of the treated area did not identify any irritant effect or colour change, and no participant of this study perceived irritation of the test areas.

Effect of the treatment on irritated skin

Two skin areas were exposed to SLS on day 0, and one of these areas was then left untreated, whereas the other was treated daily with the LBms for 4 days. The present study aimed to evaluate the effect of the lipid system on the skin. The lipid system is formed in water. However, water is not appropriate as a control, as the effect of water alone could be different from the effect of the water part of the system. Also, in a previous study, we showed that hydration values after treatment with water are almost the same as those in untreated skin (24).

SDTs were performed on day 0 (before and after SLS exposure), day 1 and day 4 on the two areas, and the curves are shown in Fig. 4a and b. Figure 4a shows the different sorption–desorption curves and the corresponding parameters calculated from the curves for the irritated untreated skin. After the application of water, the relative increase in capacitance was higher just after irritation of the skin (grey curve) than before irritation (black curve). In contrast, the amount of water absorbed by the skin 1 day after irritation (green curve) was similar to that before irritation (black curve). Moreover, no differences were detected between days 1 and 4 (green and orange curves). The second parts of the sorption–desorption curves show that all relative capacitance values were higher just after SLS exposure (grey curve) than before irritation (black curve). This indicates that, just after SLS irritation, the skin response is to release water slowly, although the tissue loses this ability in 1 day, as shown by the descending part of the curves at days 1 and day 4 (green and orange curves).

Figure 4b shows the different sorption–desorption curves and the corresponding parameters calculated from the curves for skin irritated and treated with the LBms. The behaviour of the skin after SLS irritation was the same as shown in Fig. 4a. Only after 4 days of treatment did the irritated and treated skin (orange

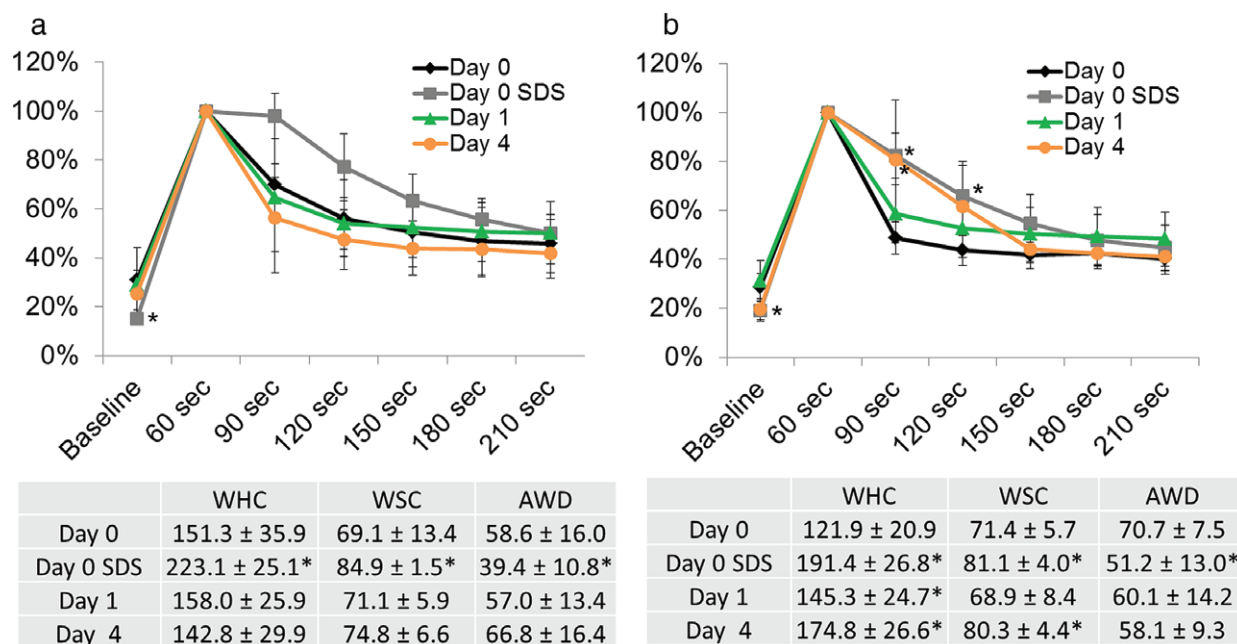


Fig. 4. Sorption-desorption test (SDT) performed on irritated skin (a) and irritated skin treated with the lamellar body mimetic system (b). AWD, accumulated water decay; WHC, water-holding capacity; WSC, water sorption capacity. *Statistically significant differences ($p < 0.05$) as compared with day 0.

curve) show a higher amount of water absorbed than before treatment (black curve). The second parts of the sorption-desorption curves show that all of the relative capacitance values were higher after treatment of the skin (green and orange curves) than before treatment (black curve). This indicates that the treatment causes skin to release water slowly. This response was not observed in the irritated untreated skin.

In both skin areas just after SLS exposure, WHC and WSC increased and AWD decreased, with significant differences. As shown in Fig. 4a, if the irritated skin was maintained untreated, these parameters were restored in 1 day and remained unaltered after 4 days. However, after 1 day of treatment, the skin showed a WHC that was higher than on day 0, and both WSC and AWD remained unaltered. After 4 days of treatment, both WHC and WSC increased significantly in comparison with day 0, and AWD decreased, although the differences were not statistically significant.

These results were also evaluated as percentage increase and decrease with respect to the initial values (before SLS exposure) at day 0, as shown in Table 2. Just after skin irritation, WHC and WSC increased by approximately 50% and 20%, respectively, and AWD decreased by approximately 30%. If the skin was left untreated, these parameters were restored in 1 day, and no differences were detected after 4 days. However, the irritated

skin showed an increase in WHC after treatment, namely, 19% after one application and 43% after four applications. WSC decreased by 4% after 1 day of treatment, but increased by 12% after 4 days of treatment. Moreover, AWD decreased by 15% and 18% after 1 day and 4 days of treatment, respectively. Therefore, if the irritated skin was treated with the LBms, a notable increase in WHC and a decrease in AWD were observed in comparison with irritated untreated skin.

Therefore, after treatment with the LBms, irritated skin increases its capacity to absorb and retain water, and also shows a decrease in the rate of water loss, in comparison with irritated untreated skin.

Discussion

The LBms

On the basis of extensive electron microscopy studies, Landmann described epidermal lamellar bodies as ovoid, membrane-delimited and lamellate organelles measuring ~200 nm in diameter (4). However, recent studies conducted by Norlen's group suggested that the lamellar body system is composed of a single and coherent tuboreticular membrane network (5). In both models, the presence of stacked lamellar structures seems to be relevant, although these lamellar structures are packed into discrete vesicles

in the Landmann model and into a tubuloreticular membrane system in the Norlen model.

The LBms proposed in this work is also based on lamellar structures, some of them stacked as planar discoidal assemblies that resemble those described in the Landman and Norlen models. Furthermore, these discoidal structures are encapsulated in vesicles. The morphology and structure of the LBms observed in our Cryo-TEM images are quite similar to those of the epidermal lamellar bodies observed by other authors in electron micrographs (25, 26). The LBms, similarly to epidermal lamellar bodies, is organized in a lipid bilayer that contains discoidal lamellar structures and is also similar in structure to bicosomes previously described in other studies (27, 28). Bicosomes formed exclusively by phospholipids have been shown to constitute a good vehicle for incorporating different molecules into the skin, owing to the specific interaction of these structures with the stratum corneum (28).

The LBms proposed here approximates the stratum corneum lipid composition and, in addition, includes phospholipids, among them LysoPC, which has bactericidal activity (29). Unlike epidermal lamellar bodies, which contain the precursors of stratum corneum intercellular lipids (30), the LBms contains lipids that approximate the stratum corneum lipid composition.

In a previous study, the LBms was shown to undergo a specific interaction with the skin *in vitro* (21). Treatment with this system re-established part of the lipid lamellar structure of delipidized stratum corneum, mainly the short-periodicity phase described by Bouwstra et al. (31). This fact could be related to the effect of this system *in vivo* described here.

As in our previous work, the LBms was designed so that the disks would contain the lipids that form the stratum corneum lamellar structure, and the external vesicle would include the compounds that should remain on the skin surface. For disk formation, both the DPPC constituting the flat bilayer and the DHPC stabilizing the rim of the structure are needed (32). Only one of the stratum corneum lipids, stearic acid, was included in the membrane of the vesicle. The rationale for this approach was that this lipid, in addition to participating in the formation of the stratum corneum lamellar structure, acts as a pH regulator (33). The normal pH on the skin surface is in the range of 5.4–5.9, and it is known that changes in skin pH may play a significant role in the pathogenesis, prevention and treatment of dermatitis and other cutaneous conditions. The vesicle also includes PC, which is necessary to form the vesicular structure and LysoPC. Following the mechanism of bicosome–skin interaction previously described (34), when external vesicles contact the skin, the bilayer of the vesicle bursts, and the encapsulated

disks are released from the inside. Once incorporated into the stratum corneum, the discoidal structures increase in size as a result of the hydration gradient of the skin, and lipids from the LBms mix with the stratum corneum lipids, supposedly reinforcing the skin barrier. The composition of the LBms, like that of skin lipids, plays an important role in the effect of this system on the skin. It is known that liposomes approximating the stratum corneum lipid composition, despite the limitations of these structures, interact with the epidermal barrier to a greater extent than other liposomes with different compositions (35). In our case, mimicking the composition, and the combination of discoidal and vesicular assemblies forming the LBms, could potentiate the effects of both nanostructures on the cutaneous tissue.

Dynamic hydration tests

In order to study the *in vivo* hydration dynamics of the skin, three tests have been developed: the SDT (16, 36), the moisture accumulation test (37), and the plastic occlusion stress test (38). In the present study, the SDT was used to perform a functional analysis of stratum corneum hydration–dehydration kinetics. This test is simple and quick, and provides information about WHC, WSC, and AWD. With this test, some skin diseases, such as atopic dermatitis and psoriasis, have been investigated (17, 18). This test has also been applied to assess the effects of different moisturizers under different experimental conditions of impairment of skin barrier function (19, 20), and to study the dynamic hydration changes occurring in the skin surface after application of irritants such as SLS (19). Therefore, the SDT appears to be appropriate for studying the effect of treatment with the LBms in healthy and irritated skin.

Effect of the treatment on healthy skin

In the present study, we found an increase in WHC of healthy skin after treatment with the LBms. This result could be explained by the specific structure and composition of the LBms and its interaction with the skin. In order to investigate the role of lipids in WHC of the stratum corneum, some authors removed the lipids from the stratum corneum and studied which lipid is primarily responsible for WHC (39). It is known that lipids themselves have little or no affinity for water molecules (40). However, after application of mixtures of stratum corneum lipids to a depleted stratum corneum, the skin recovered its WHC, suggesting that the lipids that form the lamellar structure of this tissue could be specific modulators of water-retaining properties (41–44). Among these lipids,

ceramides show the highest capacity to recover diminished WHC (45).

In a previous study, we evaluated the effect of the LBms *in vitro* in porcine delipidized stratum corneum. By using grazing-incidence small-angle scattering and freeze substitution transmission electron microscopy, we showed that this system re-established part of the stratum corneum lamellar structure of delipidized stratum corneum (21). We can assume that, in the present study, this system incorporates lipids into the stratum corneum lamellar structure in a similar way as in the *in vitro* assay. Therefore, the incorporation of these lipids into the lamellar structure of the stratum corneum seems to reinforce the skin barrier, increasing its WHC. Both the composition and structure of the LBms would work synergistically to improve the skin condition.

Effect of the treatment on irritated skin

Anionic surfactants such as SLS are used as model irritants in experimentally induced irritant contact dermatitis (46). SLS has been reported to induce epidermal cell proliferation or accelerated stratum corneum turnover, thickening of both the stratum corneum and viable epidermis, and dryness of the outer layers of the former (47).

In general, it has been reported that surfactants partially remove lipids from the stratum corneum, but this depends on the concentration and exposure time. Froebe et al. found that no ceramide and only 8–15% of free fatty acids and cholesterol were removed by a surfactant at a concentration of 2% (48). Studies by Fartasch point in the same direction, as they found that the surfactant at a low concentration of 0.5–1% did not seem to alter the existing lipid structure, but rather the synthesis of new lipids (49). Therefore, in our study, after exposure to 2% of surfactant, we expected a small amount of lipid to be removed but a perturbation of the skin barrier owing to the interaction of SLS with the lipids of the stratum corneum.

In the present study, we found increases in WHC and WSC and a decrease in AWD just after SLS exposure (Fig. 4a and b, day 0 SLS). This is possibly attributable to the action of this surfactant on the cutaneous tissue. SLS could interact with the stratum corneum lipids, increasing lipid fluidity and thereby enhancing skin permeability and allowing greater penetration of the water applied on the skin surface (50). Also, it has been reported that the hydrophobic alkyl chain of SLS interacts with the skin lipid structure, leaving the end sulfate group of the surfactant exposed, and creating additional sites in the membrane. This would result in forces that separate the protein matrix, uncoil the filaments, and expose

more water-binding sites, hence increasing the hydration level of skin (51). It is also known that, in healthy skin, perturbation of the barrier function (in this case induced by the surfactant) initiates a sequence of events that rapidly results in the return of lipids to the stratum corneum and the restoration of its barrier function, owing to an increase in lamellar body secretion (52). Furthermore, surfactants also induce changes in the concentrations of several natural moisturizing factor components immediately following exposure. It has been reported that the concentrations of most of these components are decreased, although those of a small group, including ornithine, lactate, and urea, are increased (53). In addition, some authors have reported that creams containing urea increase WHC (20). Therefore, the increases in WHC and WSC and the decrease in AWD detected just after SLS exposure could result from a combination of these mechanisms, re-establishing the normal barrier function.

Regarding the irritated and LBms-treated skin, we found increases in WHC and WSC after 4 days of treatment. This could be attributable to the incorporation of lipids into the stratum corneum lamellar structure in a similar way as in healthy skin, as discussed above. The importance of the intercellular lipids in regulating the water content is well known. Lipid lamellar organization in the stratum corneum are essential for maintaining the WHC of the skin and for regulating the water permeability of the stratum corneum (44).

Probably the most striking finding in skin irritated and then treated with the LBms was the skin behaviour after 4 days of treatment. As described before, just after irritation with SLS, all of the biophysical and biochemical mechanisms of the skin attempt to restore normal conditions (Fig. 4a and b, day 0 SLS) (52). Thereby, skin parameters related to sorption and desorption of water are re-established 1 day after irritation with SLS, regardless of treatment with the LBms (Fig. 4a and b, day 1). However, if the irritated skin is treated with the LBms for 4 days, the parameters point to an extra improvement of the skin condition (Fig. 4b, day 4). Some of the natural repair mechanisms of the skin that had been activated by the effect of the SLS damage and were normalized 1 day after irritation could be supported by the effect of treatment with the LBms.

Conclusions

Treatment with the LBms seems to reinforce the barrier function in both healthy and SLS-irritated human skin. Therefore, the present study provides evidence that this system could be of interest for the development of future treatments for protecting and repairing the skin.

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