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8. Bicelles: New nano systems for skin applications

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Abstract. Bicellar systems are lipid nanostructures formed by long- and short-chained phospholipids dispersed in aqueous solution. Because of their attractive combination of lipid composition, small size and morphological versatility, bicelles became new targets for skin research. Bicelles modify the skin biophysical parameters and modulate the skin barrier function acting as enhancers for drug penetration. Moreover, these aggregates have the ability to penetrate through the narrow intercellular spaces of the skin stratum corneum and to reinforce its lipid lamellae. Their structures allows for the incorporation of different molecules that can be carried through the skin layers. The remarkable versatility of bicelles is their most important characteristic, which makes it possible their use in different fields. These aggregates represent new nanosystems for skin applications. In this work we provide an overview of the main properties of bicelles and their effects on the skin.

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Introduction

Bicelles consist in nanostructures formed by long and short chain phospholipid molecules dispersed in aqueous solution [1, 2]. These structures represent a fascinating category of versatile and robust lipid assemblies whose uses are expanding in several research fields. Bicelles have been described as discoidal nanostructures in which a long chain phospholipid, usually dimyristoyl-phosphatidylcholine (DMPC), forms a flat bilayer and a short chain phospholipid, normally dihexanoyl-phosphatidylcholine (DHPC), stabilizes the rim of the structure (Fig. 1). Although this description has been observed in several bicellar systems, currently it is known that bicelles may display different morphologies depending on the sample preparation and experimental conditions.

The bicellar structure was developed by Sanders and Schwonek in 1992 to solve experimental problems of lipid vesicles and micelles as membrane models for NMR studies of protein characterization [3]. However, the interesting combination of lipid composition, small size and morphological versatility made bicelles new targets for skin related studies. The lipid bilayered structure of this system, with diameters in the range of 15–50 nm and thickness about 4–6 nm, presents optimal conditions as potential platforms for applications related to skin research. This is because of the structural resemblance of bicelles and lipid layers of the skin stratum corneum (SC), the absence of surfactants in the composition of bicelles and the possibility of encapsulating different molecules in the their structures.

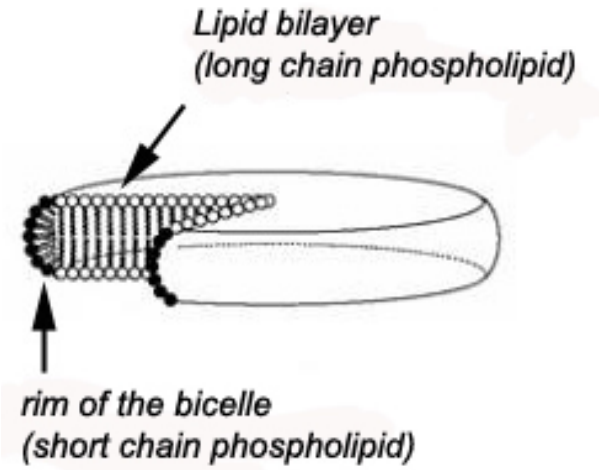

Figure 1. Schematic representation of a bicelle showing the flat bilayer region composed of long chain phospholipid, surrounded by a rim of short chain phospholipid.

The SC is a bilayered lipid-rich matrix structure with embedded keratinocytes that builds the upper strata of skin [4]. One of the SC key functions is to control permeability being the main target and the main barrier for transdermal drug delivery [5-7]. Perturbations in the structure and lipid composition of SC are associated to different diseases [8-10]. In order to replace the SC lipids or to provide drugs and other substances needed to restore skin functionality, several systems have been designed as skin carriers, delivery systems, penetration enhancers, etc. Micelles and liposomes are perhaps some of the most used systems for these purposes. However, despite their demonstrated beneficial effects, their use has some limitations [11, 12]. In fact, the large size of liposomes (usually in the range of 200-500 nm) makes their penetration in the skin improbable since the approximated thickness of the SC intercellular spaces are around 6 - 10 nm [13, 14]. In case of micelles, their surfactant components normally produce skin irritation [15, 16]. The use of bicelles presents advantages over these two classical systems because bicelles exhibit dimensions small enough to pass through the narrow SC lipid lamellae, they contain a bilayer for molecules encapsulation and they are formed exclusively by lipids [17, 18].

These interesting features of bicelles have been the basis for the new research line that is being developed with bicelles. In the last years bicelles have been investigated as regard to their structure, function and interaction with the skin tissue. The potential of this nanostructures for skin research is outstanding since their applications would range from model membranes to study the SC lipid behavior to applications such as SC lipids regenerators, skin carriers, penetration enhancers or retarders and drug delivery systems [19]. These studies gave rise to a consistent basis for considering bicelles as new nanosystems for skin applications.

In this work, we review the main properties of bicelles and the effects of their interaction with the skin *in vitro* and *in vivo*. To this aim we focus on two systems: the classic one formed by dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC) and another more compatible with the skin characteristics formed by dipalmitoylphosphatidylcholine (DPPC) and dihexanoylphosphatidylcholine (DHPC).

1. Preparation and morphological characteristics of bicelles

A typical preparation of bicelles involves hydrating the desired mixture of long- and short-chain phospholipids, followed by a series of cycles of freezing, thawing, and gentle vortexing until a clear solution is obtained. The archetypal model of bicelles is formed by dimyristoyl-phosphatidylcholine (DMPC) as large chain phospholipid, and dihexanoyl-phosphatidylcholine (DHPC) as short-chain

one. However, in order to imitate better the environment of biological membranes or to adapt the systems for an specific effect, bicelles with different lipid compositions have been prepared. In this way, DMPC can be doped with phospholipids that have identical chain lengths but different headgroups (e.g., dimyristoyl-phosphatidylglycerol (DMPG), dimyristoyl-phosphatidylserine (DMPS), dimyristoylphosphati-dylethanolamine (DMPE) [20, 21], palmitoyl-stearoyl-phosphatidylcholine (PSPC) [22] or with cellular lipids such as cholesterol and cardiolipin) [23]. Bicelles can also be prepared with dipalmitoyl-phosphatidylcholine (DPPC) or dilauryl-phosphatidylcholine (DLPC) to vary the total bilayer thickness, or with an unsaturated lipid, palmitoyl-oleoyl-phosphatidylcholine (POPC) to obtain bicelles capable of forming micrometre-scale lipid domains [24]. Apart from DHPC, the rim of bicelles can be formed by a bile-salt derivative such as 3-(cholamidopropyl) dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPSO) [25]. The so-called ideal bicelle model supposes a strict segregation between the two main phospholipids [26].

Depending on the composition, lipid concentration (c_L) and the long/short chain phospholipid molar ratio (q), bicelles display different morphologies. In general, the bigger is the q value, the bigger is the size. Inversely, the bigger is c_L, the smaller is the size. Temperature also exerts important effects on bicelles. In temperatures higher than the melting or transition temperature (T_m) of the long chain phospholipid, bicelles undergo some morphological transitions and may align in magnetic fields.

The spontaneous alignment of bicelles is their most important characteristic regarding protein characterization by NMR techniques. It is generally accepted that bicelles with q values between 2.8 and 6.5 and lipid concentration in the range of 3 – 40% w/w spontaneously align in magnetic fields in such a way that the bilayer plane is parallel to the magnetic field, giving rise to a ^{31}P NMR spectrum showing two well-resolved resonances. The high field resonance corresponds to the long chain phospholipid localized in the planar surface of the aggregate, whereas the low field resonance is attributed to the short chain phospholipid distributed in the torus [3, 27, 28]. The ^{31}P NMR spectra of bicelles are therefore used to diagnose the formation of bicelles and to verify the quality of the sample orientation. Also these spectra give information on the morphology of the aggregates.

Although the classic description of DMPC/DHPC bicelles as disk-shaped objects formed by a DMPC bilayer and closed on the edges by DHPC molecules is well accepted for bicelles with $q < 2.8$ at temperatures below the T_m of the DMPC, in the last decade, this characterization has been extensively debated for bicelles with $q > 2.8$ and temperatures above DMPC T_m [27-29].

The disk-shaped model is not consistent with the mechanism of bicellar alignment as a function of temperature in the magnetic field. According to

Ottiger and Bax, disk fusion would be necessary to reach an appropriate size for cooperative alignment [30]. The changes in viscosity and sample transparency that occur under temperature variations corroborate this hypothesis. Rowe and Neal added that the classical model does not explain the increase of viscosity at temperatures where alignment begins, which suggests the formation of large aggregates [31].

Based on their observations, Nieh et al. proposed a model for bicelles with q = 3.2, both with and without lanthanide cations (Ln^{3+}) [29, 32, 33]. At temperatures below the T_m of DMPC, the bicelles were disk shaped. As the temperature rose and the systems changed from the gel to the liquid crystalline phase in the presence of Ln^{3+}, the bicelles fused together in an end-to-end manner to form lamellar sheets with perforated holes that were lined with DHPC. Further increases in temperature caused phase separation, with the formation of DHPC-rich mixed micelles and DMPC-rich oriented lamellae that incorporated the DHPC-rich mixed micelles, even at higher temperatures [34]. In the absence of Ln^{3+}, bicelles were disk-shaped in the gel phase and chiral nematic or "wormlike micelles" in the liquid crystalline phase. These bicelles became multilamellar vesicles at higher temperatures.

While some authors have proposed that the aligned samples correspond to elongated aggregates, which are present when samples become viscous [35], others claim that the aligned bicelles are perforated lamellar sheets, which are present when the sample viscosity drops and its appearance becomes milky [28, 30, 36]. This latter model is known as the "Swiss cheese model" (Fig. 2). In a comprehensive study, Triba et al. [27] found that both elongated and perforated lamellar structures are compatible with the NMR aligned spectra, although they did not disprove the possibility of discoidal structure in the aligned phase.

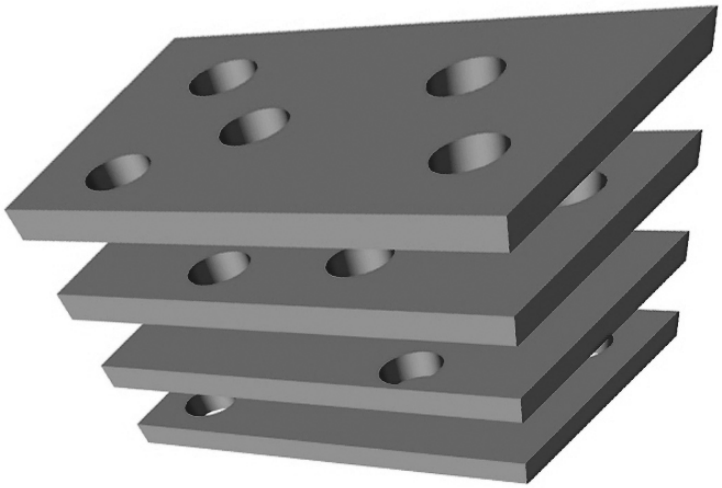

Figure 2. Representation of the Swiss cheese model consisting of flat bilayer sheets of long chain phospholipid perforated with surrounding short chain phospholipid.

Recently, a comparative study related the morphologies of alignable (q = 3.5) and non-alignable (q = 2) bicellar systems, c_L =20%, with temperature changes [37]. ^{31}P-NMR spectra indicated that the q = 2 bicelles exhibited isotropic behavior at all temperatures (20-60 ºC), while the q = 3.5 bicelles varied considerably with temperature. Even at 20 ºC, a low field resonance peak and a broad higher field signal suggested partial orientation of the sample with the magnetic field. Just above the DMPC T_m, at 25 ºC, the spectrum showed two broad resonances near the positions of those observed at 20 ºC. At higher temperatures, the lines became more intense, indicating increased magnetic alignment that reached a maximum at 40 ºC. These peaks disappeared at higher temperatures and were replaced by a broad signal at higher field resonances, which are characteristic of a phase transition from bicellar aggregates to larger, slow-moving structures, such as vesicles. There was also a gradual up field shift of both peaks with increasing temperature, which represents an increase in DMPC bilayer order and the gradual incorporation of DHPC molecules into this bilayer. This study suggested that increasing temperatures promote the migration of DHPC molecules from the edges of the structures to the bilayer area of the alignable bicelles. Depletion of DHPC molecules at the edges leads to bilayer fusion, which increases the bicellar diameters and improves the alignment up to a certain point at which bigger and/or non-flat structures are formed and the alignment is lost.

Electron microscopy techniques have proven to be very useful as complementary techniques to determine samples' morphology. The study of these samples by Cryo-SEM and TEM found round aggregates of approximately 20 nm for bicelles with q = 2 below T_m and elongated aggregates of approximately 2000 nm above T_m. These larger aggregates do not align in magnetic fields, although their size and morphology explain the increase in viscosity with temperature. At q = 3.5, discoidal bicelles of approximately 40 nm were observed below T_m, and extended areas of stacked lamellar sheets were observed above T_m. The authors considered these aggregates to be the most ordered phase because the best alignments in the ^{31}P-NMR experiments were obtained at the same temperatures.

Van Dam et al. applied Cryo-TEM technique to characterize DMPC/DHPC q = 3.2 and c_L=5% w/w doped with DMPG and CTAB [28]. Through this technique they observed disk shaped structures viewed face-on and edge on, threadlike cylindrical micelles and branched toroidal structures at 20 ºC, 25 ºC and 30 ºC, respectively. In another study, these authors used this technique to investigate the morphology of DMPC/DHPC samples over a wide range of c_L and q, covering isotropically tumbling bicelles and larger aggregates [38]. They concluded that temperature and the ratio q are the dominating variables for changing sample morphology, while c_L to a lesser

extent affects the aggregate structure. They observed at q = 0.5, small, possibly disc-shaped aggregates with a diameter of approximately 6 nm. At higher q-values, they observed distorted discoidal micelles that tend to sort cylindrical micelles similar to those reported by Barbosa-Barros et al. [37].

Extensive and comprehensive studies have been reported on bicelle morphology and phase behavior [34, 39-42]. Throughout this work, researchers have reported that (a) bicelles are disk-shaped nanoaggregates at temperatures below the T_m of the long-chain phospholipid; (b) increases in temperature cause an initial increase and subsequent drop in viscosity; and (c) when the temperature is raised above the long-chain phospholipid T_m, bicelles with $q > 2.8$ undergo morphological transitions from disks to elongated micelles, perforated lamellar sheets and mixed multilamellar vesicles.

However, the studies disagree on the exact morphology of the structures when $T \geq T_m$ and the sample exhibit magnetic alignment. Works describing the morphology of bicelles with $q < 2.8$ at temperatures above the long-chain phospholipid T_m are scarce. For these reasons, most recent reports use the term “bicelle” to refer only to the sample composition and not the disk-shaped morphology.

2. Effects of bicelles on the skin

The motivation for using bicelles in the skin arises from the resemblance of these structures to micelles and liposomes. Bicelles combine some of the most attractive characteristics of these systems and therefore present several advantages for skin applications compared to either micelles or liposomes. Bicelle structures contain a bilayer that allows for the incorporation of different molecules but are much smaller (approximately 15-40 nm) than a regular liposome (≅200 nm). This is due the presence of DHPC molecules on the edges of the structures, which control the diameter of the assembly. These molecules are responsible for discoidal-shaped bicelles and the formation of other structures, such as small vesicles. In fact, systems formed by long alkyl chain phospholipids and DHPC have been reported to produce reasonably monodisperse unilamellar vesicles that are thermodynamically stable, with radii ranging between 10 and 40 nm [43-46]. Several kinetic studies have shown that discoidal bicelle morphology is a precursor to small vesicle morphology [46, 47]. DHPC molecules solubilize the DMPC bilayer similar to a surfactant; however, DHPC is a phospholipid with the same polar head group as DMPC. These two lipids differ only in the lengths of their hydrophobic chains. The use of systems composed only of lipids avoids damage to the skin barrier function caused by surfactants, which is

characterized by breaking of the corneocyte envelopes and disorganization of the intercellular lipid structures.

As already mentioned, above the long chain phospholipid T_m (approximately 23 °C for DMPC), DMPC/DHPC bicelles undergo phase transitions, changing from small aggregates of approximately 15-20 nm to structures bigger than 500 nm. Because skin intercellular spaces lie in the range of 6-10 nm and physiological skin temperatures are near 37 ºC, this phase transition would present a handicap for skin penetration. DMPC/DHPC bicelles are likely not able to penetrate into SC intercellular spaces, and their effects are limited to the skin's surface.

A different scenario is obtained if the long chain phospholipid DMPC is substituted by another phospholipid with higher T_m, such as DPPC. This phospholipid has two additional carbons in its hydrophobic chain and a T_m of 41 °C. At physiological temperatures, bicelles composed of DPPC and DHPC are still small aggregates. Some studies have found that at 37 °C, DMPC/DHPC bicelles these structures have dimensions of approximately 15 nm in diameter and 5.4 nm in thickness. These structures have been shown to penetrate through the intercellular spaces of the skin SC and achieve deeper internal layers [48].

In vitro studies

Experiments using human and pig SC samples treated with bicelles to study the effects of these systems on the SC microstructure have been reported. In one of these studies, fresh human SC samples were incubated with DMPC/DHPC bicelles with c_L = 20% and q = 2 for 18 h at 25 °C. Treated and untreated SC samples were cryofixed, cryosubstituted and visualized by TEM. No differences, including microstructural alterations and/or apparent damage, were observed in images of the treated SC compared with untreated samples (Fig. 3A and 3B).

Given the small sizes of bicelles (20 nm in diameter and 4.5 nm in thickness) and their bilayered structures, the lipid dispersion through the SC lamellae and the reinforcement of the SC bilayer area would be expected. However, this was not observed, suggesting that these bicelles were not able to penetrate or disperse through the SC lipid area [19]. This result was clarified by Rodriguez et al. [49] using ATR-FTIR. Th authors reported that the application of DMPC/DHPC q = 2 bicelles caused phase transitions in the SC lipid conformation from the gel state to the liquid crystalline state. This transition would be promoted by the incorporation of phospholipids from bicelles in the SC lipid lamellar structure. This process involves an increase in the fluidity and/or disorder of the lipids. An analysis of phosphate vibrations only detected effects from DMPC/DHPC bicelles on lipids at the

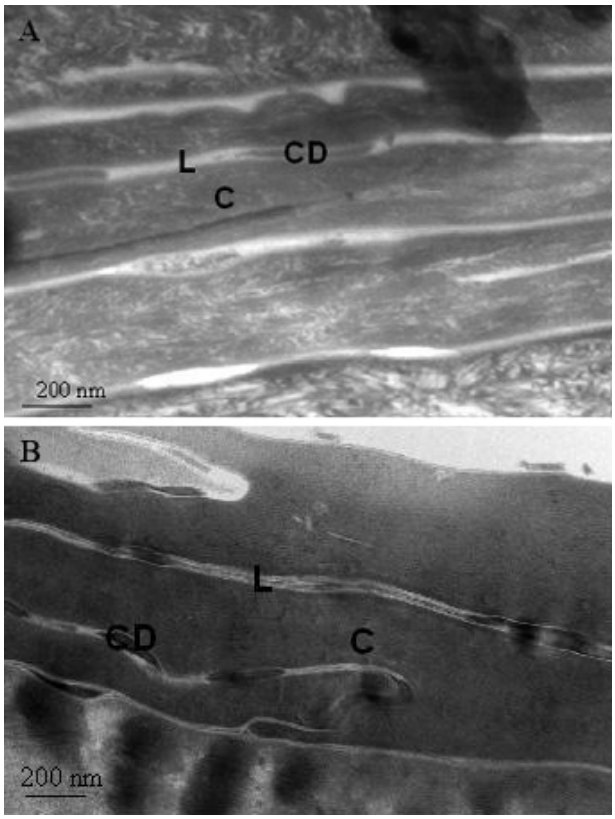

Figure 3. FSTEM micrograph of (A) native SC and (B) SC treated with DMPC/DHPC bicelles. Both images show regular areas of corneocytes, (C), lipid intercellular spaces (L) and corneodesmosomes (CD) [19].

outer layer of the SC, suggesting that the majority of DMPC/DHPC bicelles remained in the outermost part of the tissue. This is likely why Barbosa-Barros et al. [19] did not visualize microstructural differences between untreated and treated SC; transitions occurring mainly on the SC surface do not imply structural modifications that can be visualized in EM experiments.

In another study, DPPC/DHPC bicelles with c_L = 10% and q = 3.5 were incubated with fresh pig skin SC samples for 18 h at 37 ºC. This DPPC/DHPC system was specially developed to obtain improved effects on the SC microstructure and better skin penetration at physiological conditions. Because the DPPC T_m is 41 ºC, the structures do not undergo phase transitions at physiological temperatures (approximately 37 ºC), and small bicelle structures are favored during the incubation process. After incubation, both treated and untreated SC samples were high pressure frozen (HPF) from an initial temperature of 37 ºC, freeze fractured and observed using Cryo-SEM [48]. These bicelles produced quite different results in the SC than the DMPC/DHPC q = 2 system. The DPPC/DHPC system could penetrate and interact with the SC, forming lipid vesicles and new lamellar-like structures observed by Cryo-SEM (Fig. 4). To study this phenomenon, Rodriguez et al.

[50] studied the effects of DPPC/DHPC on SC lipids using ATR-FTIR spectroscopy coupled to a tape-stripping methodology. Analysis of the lipid organization in terms of chain conformational order and lateral packing showed that bicelles hampered the temperature-dependent fluidization of SC lipids in the most superficial layers of the SC and led to a lateral packing corresponding to a stable hexagonal phase. CH_2 stretching and phosphate vibrations in the ATR-FTIR spectra of subsequent stripping indicated that DPPC/DHPC bicelles penetrated into and were widely distributed in deep layers of the SC. These results corroborate the presence of different DPPC/DHPC structures inside the SC layers, and bicellar reinforcement of the SC structures was observed.

Various authors have reported studies of bicellar state-transitions from disks to vesicles that were induced by dilution or temperature changes. There is a general consensus that these transitions occur in progressive steps, implying the coexistence of different aggregates in the medium [28, 39, 51]. To study the bicelle-to-vesicle transition, DLS studies were performed by Barbosa-Barros et al. 2008 [48]. A DPPC/DHPC sample with q = 3.5 and c_L = 10% was sequentially diluted with water in seven steps, and each diluted sample was measured using DLS at 37 °C to imitate physiological conditions. The DLS curves indicated that the hydrodynamic diameter (HD) of the structures increased upon dilution from 11.3 nm (assigned to bicelle disks) to aggregates larger than 1 μm. The morphologies of the aggregates were analyzed using EM, which confirmed the DLS results, indicating a dilution-induced transition from disks to vesicles.

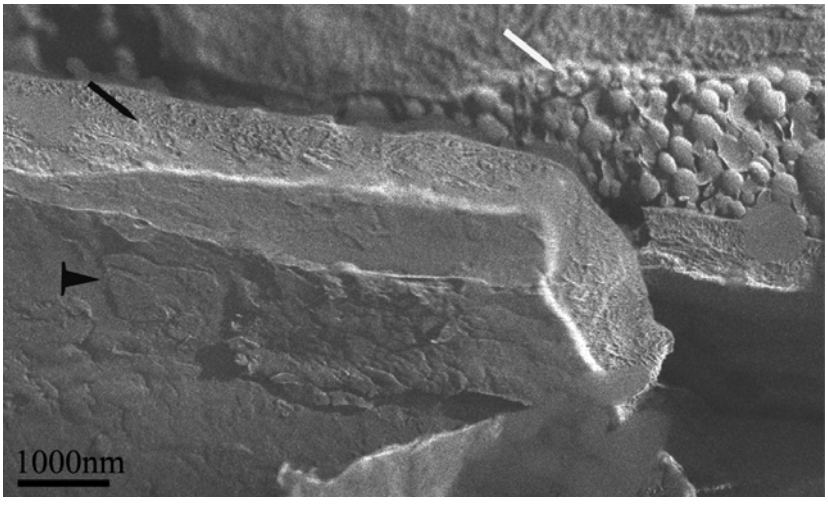

Figure 4. Cryo-SEM micrograph of SC treated with DPPC/DHPC bicelles. The white arrow shows vesicle structures with sizes around 200 nm and the arrowhead points to the lamellar-like structures in the intercellular lipid areas. The black arrow shows a corneocytes area [48].

In bicellar systems, the DHPC molecules are found mainly on the edges of the disk structures and in the water (as monomers). With increasing dilutions, the DHPC concentration in water decreases, and DHPC is transferred from the bicelle edges into solution, maintaining monomeric equilibrium. Hence, disk diameters increase, and high dilutions lead to the fusion and closure of large bilayered disks, forming vesicles. This bicelle-to-vesicle transition explains the presence of vesicles in the SC intercellular spaces treated with DPPC/DHPC [48]. This process would have been promoted by the dilution of aggregates because the SC pieces were washed with water after incubation. Bicelles that have been transformed into vesicles presumably follow a process similar to that observed in the DLS observations of diluted bicellar solutions outside of the skin.

Regarding the ability of bicelles to encapsulate drugs, Rubio et al. [52] performed percutaneous penetration studies with DMPC/DHPC and DPPC/DHPC systems with c_L = 10% and q = 2 to evaluate their effects on the skin penetration of dichlofenac diethylamide (DDEA). The authors reported that the incorporation of DDEA in the bicelles led to markedly decreased bicelle sizes, indicating that DDEA tends to be located at the bicelle edges, similar to DHPC. Fig. 5 shows Cryo-TEM images of DPPC/DHPC bicelles with and without DDEA. Both systems decreased the percutaneous absorption of the DDEA compared to an aqueous solution of DDEA, suggesting a retarded effect after treatment with bicelles. This effect was more marked for the DMPC/DHPC bicelles. This could be related to the different T_m values for the two systems. At 37 ºC, DMPC/DHPC ($T_m \approx$ 23 ºC) bicelles were in the liquid crystalline phase, in contrast to the gel phase SC lipids ($T_m \approx$ 60 ºC). However, DPPC/DHPC bicelles ($T_m \approx$ 41 ºC), were in gel phase, similar to the SC lipids. Different mixing behaviour of lipids from bicelles with SC lipids could induce different effect on the retention of DDEA in the upper layers of the skin. This retarder effect was ascribed in part to rigidity in the head groups of bicelle phospholipids caused by the carboxyl groups of DDEA. This rigidity would hinder the penetration of DDEA through the skin. However, DDEA was likely unable to diffuse out of the bicellar systems because of its high affinity for its vehicle.

A second percutaneous penetration assay was also performed by these authors to evaluate the potential of bicelles as penetration enhancers. In this experiment, the skin was pretreated with the bicellar systems (DMPC/DHPC and DPPC/DHPC systems with c_L = 10% and q = 2) before the application of an aqueous DDEA solution. The global results obtained showed that pretreatment of the skin with bicelles promotes an increase in the percutaneous absorption of DDEA, with no significant differences between DMPC/DHPC or DPPC/DHPC systems. These studies suggest that treatment

with these bicelle systems prior to drug treatment can enhance drug absorption. This enhancement is ascribed to an initial interaction of bicelles with the SC that causes some disorganization of the intercellular lipids, which are responsible for the SC barrier functionality. These results suggest a route to aid the absorption of DDEA through the skin.

In vivo studies

Non-invasive biophysical studies performed with healthy volunteers have evaluated the effects of bicelles on skin *in vivo*. These studies report mainly on measurements of skin hydration, elasticity, erythema and transepidermal water loss (TEWL). Skin hydration and elasticity are useful measures of skin water content, distensibility, extensibility and tonicity [53, 54]. Erythema indicates skin tolerance [55], while TEWL indicates barrier function integrity [56, 57].

The application of DMPC/DHPC bicelles with c_L = 20% and q = 2 to the skin of healthy volunteers has been reported [19]. In this experiment, intra-individual comparisons of three test areas on the volar forearms of 6 healthy Caucasian female (ages 25–38) volunteers with no visible skin abnormalities were performed. In the test areas, bicelles, deionized water and control (non-treated) areas were randomized regarding the test sites on each subject. The solutions were applied daily over a period of 10 days, and the skin properties were measured each day before application.

Successive bicelle applications led to an increase in TEWL from days 0 to 11. This increase was moderate and did not reach pathological levels, which are from 25 to 40 g/m^2/h [58, 59]. Decreasing of the skin hydration was also observed. Elasticity, in turn, showed improvement with the application of bicelles. The changes in the erythema of the skin, considering inter- and intra-individual variability, were not indicative of an irritation process [60]. The bicelles were found to promote increases in TEWL and skin elasticity and harmless decreases in skin hydration.

Bicelles act to enhance penetration, causing phase transformations in lipid domains that may be relevant to skin permeation [61]. As reported by Rodriguez et al. [49], the phase transition of the SC lipid conformation from the gel state to the liquid crystalline state, which causes the fluidity of these lipids, explains the increase of TEWL *in vivo*. Compared to other enhancers, bicelles would have the additional advantage of not causing skin irritation [62].

Another study reported on the effects of the application of DPPC/DHPC bicelles with c_L = 20% and q = 2 on the skin of healthy volunteers. The results were similar to those obtained with the DMPC/DHPC system, although they were more discrete. In a similar way, this system led to an

increase in TEWL and a decrease in skin hydration. However, the effects were approximately 75% and 50% less intense, respectively, than those obtained with the DMPC/DHPC system [63]. This result is not unexpected because the DPPC/DHPC system contains less DHPC, so the aggregates formed are slightly bigger and have longer bilayer areas, which would exert a protective effect on the SC. In addition, as observed in the *in vitro* studies, this system penetrates into the skin SC and reconstitutes its lipids in lipid vesicles inside of the skin lamellae, which reinforce the lipid structure of the tissue.

3. Conclusion

The use of bicelles for skin applications is a scientific novelty. This new lipid system represents a unique versatile structure that has different effects on the skin depending on the self-assembly adopted. Bicelles are effective skin carriers due to their size, structure and composition. Although bicelles have no aqueous internal compartment for encapsulating drugs, their bilayered structure allows for the encapsulation of lipophilic and amphiphilic compounds. Additionally, because of their ability to increase the permeability of the SC, these structures enhance the penetration of hydrophilic components dissolved in aqueous medium. Further, the conversion of bicelles into vesicles inside the SC hinders their migration outside of the tissue and allows a lipid reinforcement effect on the skin. By modulating their physical and chemical characteristics, bicelles may be useful for a wide range of applications. Bicelles are therefore promising nanostructures with the potential to become new multifunctional, skin-compatible delivery system.

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