Bicelles: New Lipid Nanosystems for Biomedical Applications

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ABSTRACT

Bicelles emerge as promising membrane models, and because of their attractive combination of lipid composition and physico chemical characteristics they become new nanostructures for biomedical research. Depending on the composition, temperature and other experimental factors, these nanosystems exhibit high structural and morphological versatility. Additionally, bicelles are able to modify skin biophysical parameters and modulate the skin barrier function. All this considered, these nanostructures appear as smart nanosystems with high potential in biomedicine and dermopharmacy.

Keywords: bicelles, lipid nanosystems, skin, dermal applications.

1 INTRODUCTION

Bicelles are a fascinating category of versatile and robust lipid assemblies that have been increasingly utilized in several research fields. They consist of nanostructures formed by long- and short-chain phospholipid molecules dispersed in aqueous solution [1,2]. These assemblies were described as discoidal nanostructures of approximately 15–50 nm in diameter. Although the definition of bicelle is commonly associated to the discoidal nanostructure, a wide range of morphologies have also associated with long- and short-chain phospholipids assemblies, including spherical and rod-like micelles, extended lamellae, perforated bilayers and vesicles. All this range of morphologies is refered in this context as bicellar systems. Some representative nanostructures are shown in Figure 1.

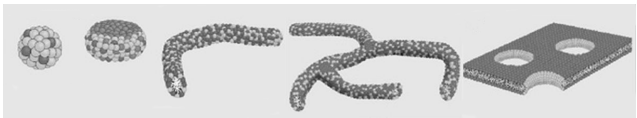

Figure 1: Different nanostructures adopted by long- and short- chain phospholipids in water.

The remarkable versatility of these bicellar system allows their use for different applications. The bicellar structure was developed by Sanders and coworkers to solve experimental problems associated with NMR studies of protein characterization [3]. From that time, techniques, such as electron microscopy, dynamic light scattering, small-angle X-ray scattering, electron paramagnetic resonance, electrokinetic chromatography, and neutron diffraction, have also been used to characterize bicelles [2,4-5]. The interesting combination of lipid composition, small size, and morphological versatility has made bicelles new targets for skin-related studies. These systems are well suited for such applications because of their structural resemblance to the lipid layers of the skin stratum corneum (SC), the absence of surfactants in their composition and the possibility of encapsulating different molecules in the their structure.

The SC is a bilayered lipid-rich matrix with embedded keratinocytes that forms the upper layer of skin. One of the SC key functions is to control skin permeability [6]. In this way, several systems have been designed as skin carriers, delivery systems, and penetration enhancers like micelles and liposomes. However, despite their beneficial effects, there are some limitations to their application. Liposomes are too large (usually 200–500 nm) to penetrate into the skin (thickness of SC intercellular spaces about 6–10 nm) and micelles normally contain surfactants that produce skin irritation. [7,8]. Discoidal bicelles present advantages over these two classical systems; they are small enough in size to pass through the narrow SC lipid lamellae, they contain a bilayer for molecular encapsulation, and they are composed entirely of lipids. These interesting features have been the basis for new bicelle research. In recent years, bicelle structure, function and interaction with skin tissue have been investigated [9-17]. This research includes the formation and characterization of bicelles with new compositions [14,16,17], the study of the interaction of these systems with skin both in vitro and in vivo [9-13,15], the encapsulation of pharmaceutical and cosmetic compounds in bicelle structures [16-17], and the development of strategies to stabilize these systems [18]. Some reports suggest that bicelles may permeabilize skin SC or reinforce the SC lipid structure [9-13,15]. As drug delivery systems, bicelles retard percutaneous absorption when encapsulating anti-inflammatory drugs and enhance percutaneous absorption when applied to the skin before drug application [14,16].

The potential of these nanostructures for skin research is outstanding; their applications could range from model membranes for the study of SC lipid behavior to their use as SC lipids regenerators, skin carriers, penetration enhancers or retardants, and drug delivery systems. These properties have led to the study of bicelles as nanostructured platforms for dermal applications.

2 MORPHOLOGICAL VERSATILITY OF BICELLAR SYSTEMS

Depending on their composition, lipid concentration (cL) and the long/short chain phospholipid molar ratio (q),

bicelles display various morphologies. In general, bicelle size increases with the value of q but decreases with increasing values of cL. Temperature also exerts significant effects on bicelles. At temperatures higher than the transition temperature (Tm) of the long chain phospholipid, discoidal bicelles are able to form vesicles.

Figure 2 shows three different bicellar systems formed by lipids with different Tm [19]. This fact involves that at room temperature lipids exhibit a different phase. Micrograph 2A and 2B show bicellar systems with lipids in a gel phase and panel 2C correspond to a system with lipids in a liquid crystalline phase. Different sized nanostructures are visualized.

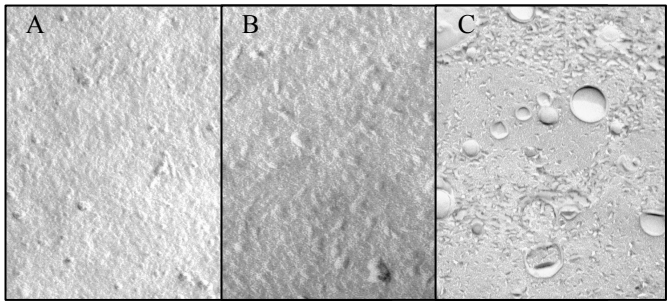

Figure 2: Different bicellar systems formed by lipids with different Tm.

Inclusion of lipids and/or other amphiphilic molecules forming bilayers increases diameter of the disks and induces formation of tubular, lamellar and vesicular structures. Bicellar systems with 5% Cer present small disk-like shaped bicelles but higher amounts of Cer promote a destabilization of the system, probably due to the effect of lateral separation, and other lipid structures as vesicles and rolled-up aggregates are formed (Figure 3) [17].

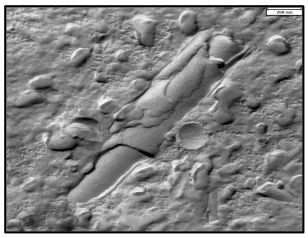

Figure 3: Bicellar system containing ceramides.

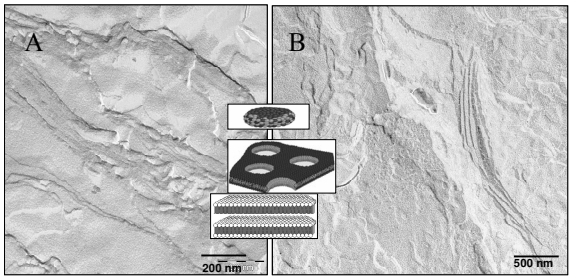

Figure 4: Bicellar systems containing flufenamic acid (A) and the antifungic nystatin (B).

Inclusion of drugs and other molecules promotes structural changes as a function of their hydrophilic lipophilic character. Then, flufenamic acid and nystatin, with poor solubility in water tend to form large stacks of lamellar structures [14,20] (Figure 4), whereas more hydrophilic compounts (dichlofenac diethylamine and caffeine) reduce the size of the disk forming spherical nanostructures [14,20] that are shown in Figure 5.

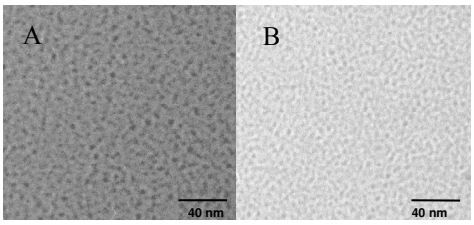

Figure 5: Bicellar systems containg dichlofenac (A) and caffeine (B).

Then, we can see that as a function of composition and temperature bicellar systems exhibit high structural and morphological diversity. Different factors affect structure and morphology, especifically the termotropic behaviour of the lipids forming the systems, the hydrophilic lipophilic character of the compounds included, the temperature and hydration degree of the environment and the different self-assembly properties of the molecules. These facts involve the distribution of the different molecules that form the systems in several nanoaggregates: small micelles, disks, rod-like structures, lamellar sheets, perforated lamellar sheet, vesicles.

3 APPLICATION OF BICELLES ON THE SKIN

Different bicellar systems involve different nanostructures, that induce different effects on the skin giving high applicability to these nanostructures.

In order to know the effect of bicellar systems on the skin, a number of in vitro and in vivo experiments have been carried out. SC samples from pig skin have been treated with different bicellar systems and SC structure was studied before and after treatment using small X ray scattering (SAXS), transmission electron microscopy (TEM) and attenuated transmission reflectance Fourier transformed infra red spectroscopy (ATR-FTIR) [9-13]. The effect in vivo is in general evaluated by the modification before and after treatment with bicelles of the biophysical parameters of the skin: transepidermal water loss (TEWL), lipid content, colour, elasticity hydration and pH [9-10].

Some bicellar systems (including dimyristoylphosphatidylcholine) promote changes in the SC inducing an increase in the intensity and in the sharpness of some reflections of SAXS patterns (Figure 6). This fact could indicate higher organization in the tissue by presence of additional lipids. TEM (Figure 6) shows that SC lipids

are well organized forming the typical lamellar structure after treatment with bicelles. The fluidity of SC lipids increases after treatment (ATR-FTIR) and in vivo experiments show that the TEWL of the skin increases after treatment [9,12]. This system reinforces the SC lipid structure but inducing a modification in the fluidity/movement of lipids that could change the permeability of the tissue.

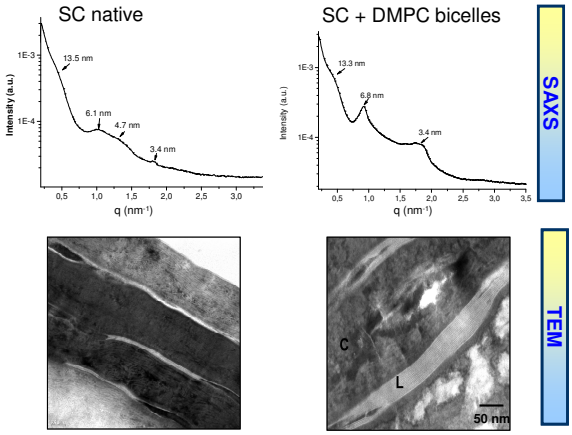

Figure 6: SAXS profiles and TEM images from SC before and after treatment with DMPC bicellar systems. C:corneocytes, L: lipid lamellae.

Other compositions induce differents effect on skin. Figure 7 shows SAXS in which reflections appear more intense and sharp, suggesting an increase in the order of the lipid structures. TEM shows formation of new lamellar/vesicular structures inside the SC after treatment. Aditionally, the fluidity of SC lipids was not modified after treatment, and this sytem does not modify skin biophysical parameters in vivo [10,13]. This bicellar system (including dipalmitoylphosphatidylcholine) reinforces the SC lipid structure forming new lamellar structures without modificating either fluidity/movement of lipids and problably permeability.

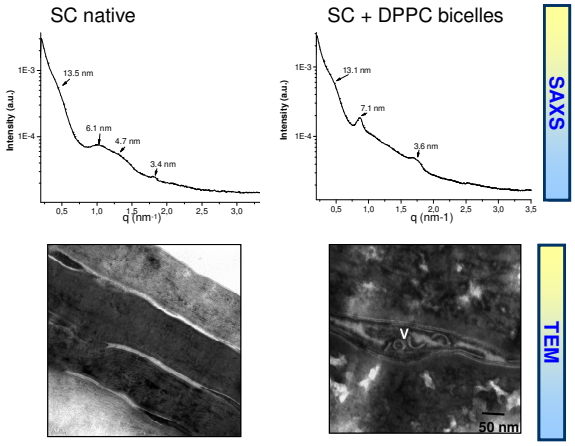

Figure 7: SAXS profiles and TEM images from SC before and after treatment with DPPC bicellar systems. V: vesicles.

To evaluate the potential effect of bicellar systems on the percutaneous absorption of a drug, percutaneous absorption studies have been performed using pig skin and Franz diffusion cells. On native skin, the percutaneous absorption of dichlofenac and flufenamic acid has been reported [14,20], and it is shown in Figure 8. Inclusion of these drugs in bicellar systems induces a retardant effect in the percutaneous absorption.

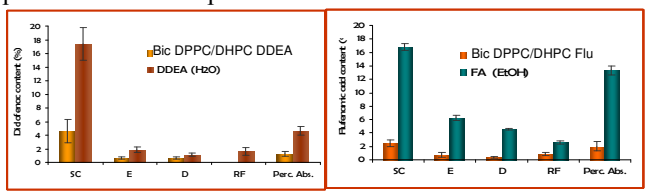

Figure 8: Percutaneous absorption profiles of dichlofenac (A) and flufenamic acid (B) included in bicellar systems and in other solvents.

However also have been demonstrated that when skin is pre treated with bicelles (without drug) and afterwards the drug is applied on treated skin and enhancer effect of the penetration is observed [14,20].
The different effects of bicellar systems on the skin and on the percutaneous absorption of drugs depend on the type of system used and on the conditions in which is used. These different effects are due to the great structural and morphological versatility of these systems. To be able to predict the behaviour of bicelles offer the possibility to modulate the cutaneous barrier without promote damaging.

4 STRATEGIES TO STABILIZE BICELLES IN HIGH-WATER-CONTENT ENVIRONMENTS

Bicellar systems constitute very promising systems for different applications. These nanostructures exhibit intelligent properties, since they respond to external stimuli. Its versatility, the ability to modulate its structure, organization and their lipid bilayer offer great advantages over other bilayer systems. Besides, as previous section showed, these systems are adequate to interact with the skin and to reinforce the structure lipid system. Structural characterization of bicelles has revealed interesting aspects about the behaviour of these systems and about the different effect induced by inclusion of skin lipids, such as ceramide. In this way, the utility of bicelles as membrane model to study skin lipids could be considered in future research.
Although the characteristic of bicellar systems to change structurally and morphologically is a key factor for their use in different areas, this property may limit its application in environments where these conditions are variable. Bicelles can be considered good carriers for the skin. However, their application as carriers for administration through the systemic route, where the water content is high, would represent a challenge, as well as its simple addition to

vehicles with higher water content. At diluted conditions discoidal bicelles become other nanostructures and their properties could be lost. In order to address this limitation, our research group has recently proposed a strategy to preserve the discoidal morphology of bicelles for its use in high water content environments. This strategy consists in the preparation of new structures also formed by phospholipids, the so-called bicosomes™ that would protect bicelles from dilution, preserving the aggregates until the target tissue is achieved [18]. Cryo-TEM images of bicosomes™ are shown in Figure 9.

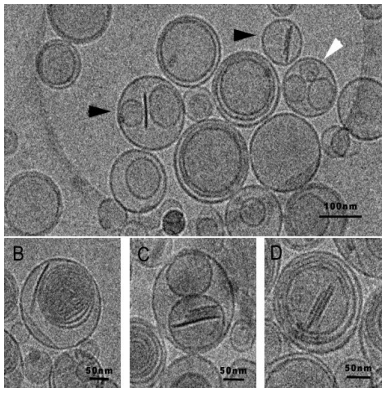

Figure 9: Cryo-TEM images of bicosomes™

Other methods have been used to stabilize the morphology of discoidal bilayers, such as using bicelles with charged amphiphiles [21] or disks formed by mixtures containing polyethylene glycol-lipid conjugates [22]. However, with the use of PEG-lipids, the properties of bicelles related to structural versatility, such as the enhancer effect of the permeability on some physiological barriers, could be lost.

All in all, the most recent research on bicellar systems point to depending on their composition, these nanostructures interact differently with the microstructure of the skin. Additionally, they are able to encapsulate drugs and their structure can be modify in order to be used at different environments. Then, these nanostructures emerge as smart nano-systems with the possibility to alter the skin lipid microstructure, to modulate skin barrier function and, possess promising applications for skin and other barriers.

REFERENCES

1. Sanders, C. R.; Prosser, R. S. Structure 1998, 6, (10), 1227-34.
2. Triba, M. N.; Devaux, P. F.; Warschawski, D. E. Biophys. J. 2006, 91, (4), 1357-67.
3. Sanders, C. R.; Hare, B. J.; Howard, K. P.; Prestegard, J. H. Prog. NMR Spectroscopy 1994, 26, 421-444
4. Van Dam, L.; Karlsson, G.; Edwards, K. Langmuir 2006, 22, (7), 3280-5.
5. Katsaras, J. H., T.A.; Pencer, J.; Nieh, M-P. Naturwissenschaften 2005, 92, 355-366
6. Elias, P. M. Arch Dermatol Res 1981, 270, (1), 95-117.
7. Bouwstra, J. A.; Honeywell-Nguyen, P. L.; Gooris, G. S.; Ponec, M. Progress in Lipid Research 2003, 42, 1-36.
8. Turkoglu, M.; Sakr, A. Int J Cosmet Sci 1999, 21, (6), 371-82
9. Barbosa-Barros, L.; Barba, C.; Cócera, M.; Coderch, L.; López-Iglesias, C.; de la Maza, A.; López, O. Int. J. Pharmaceut 2008, 352, 263-272.
10. Barbosa-Barros, L.; Barba, C.; Rodríguez, G.; Cócera, M.; Coderch, L.; López-Iglesias, C.; de la Maza, A.; López, O. Mol. Pharm. 2009, 6, (4), 1237–1245.
11. Barbosa-Barros, L.; de la Maza, A.; Estelrich, J.; Linares, A. M.; Feliz, M.; Walther, P.; Pons, R.; López, O. Langmuir 2008, 24, 5700-5706.
12. Rodriguez, G.; Barbosa-Barros, L.; Rubio, L.; Cocera, M.; Diez, A.; Estelrich, J.; Pons, R.; Caelles, J.; De la Maza, A.; Lopez, O. Langmuir 2009, 25, (18), 10595-603.
13. Rodriguez, G.; Rubio, L.; Cocera, M.; Estelrich, J.; Pons, R.; de la Maza, A.; Lopez, O. Langmuir 2010, 26, (13), 10578-84.
14. Rubio, L.; Alonso, C.; Rodriguez, G.; Barbosa-Barros, L.; Coderch, L.; De la Maza, A.; Parra, J. L.; Lopez, O. Int. J. Pharm. 2010, 386, 108-113.
15. Barbosa-Barros, L.; Rodriguez, G.; Barba, C.; Cocera, M.; Rubio, L.; Estelrich, J.; Lopez-Iglesias, C.; de la Maza, A.; Lopez, O. Small 2011, 8, (6), 807-18.
16. Rubio,L.;Rodríguez G; Alonso C.; López-Iglesias C.; Cócera M.; Coderch L.; De la Maza A.; Parra JL; López O. Soft Matter 2001, DOI: 10.1039/c1sm05692a.
17. Barbosa-Barros, L.; de la Maza, A.; Walther, P.; Estelrich, J.; López, O. J. Microsc. 2008, 230, 16-26.
18. Rodriguez, G.; Soria, G.; Coll, E.; Rubio, L.; Barbosa-Barros, L.; Lopez-Iglesias, C.; Planas, A. M.; Estelrich, J.; de la Maza, A.; Lopez, O. Biophys. J. 2010, 99, (2), 480-8.
19. Rodriguez, G.; Cocera, M.; Rubio, L.; Lopez-Iglesias, C.; Pons, R.; de la Maza, A.; Lopez, O. Mol Pharm 2012, 9, (3), 482-91.
20. Rubio, L.; Rodriguez, G.; Barbosa-Barros, L.; Alonso, C.; Cocera, M.; de la Maza, A.; Parra, J. L.; Lopez, O. Colloids Surf B Biointerfaces 2012, 92, 322-6.
21. Marcotte, I.; Dufourc, E. J.; Quellet, M.; Auger, M. Biophys. J. 2003, 85 328–339.
22. Soong, R.; Macdonald, P. M. Biophys. J. 2005, 88, (1), 255-68.