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A rhenium tris-carbonyl derivative as a model molecule for incorporation into phospholipid assemblies for skin applications

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ABSTRACT

A rhenium tris-carbonyl derivative (fac-[$Re(CO)_3Cl$(2-(1-dodecyl-1H-1,2,3,triazol-4-yl)-pyridine)]) was incorporated into phospholipid assemblies, called bicosomes, and the penetration of this molecule into skin was monitored using Fourier-transform infrared microspectroscopy (FTIR). To evaluate the capacity of bicosomes to promote the penetration of this derivative, the skin penetration of the $Re(CO)_3$ derivative dissolved in dimethyl sulfoxide (DMSO), a typical enhancer, was also studied.

Dynamic light scattering results (DLS) showed an increase in the size of the bicosomes with the incorporation of the $Re(CO)_3$ derivative, and the FTIR microspectroscopy showed that the $Re(CO)_3$ derivative incorporated in bicosomes penetrated deeper into the skin than when dissolved in DMSO. When this molecule was applied on the skin using the bicosomes, 60% of the $Re(CO)_3$ derivative was retained in the stratum corneum (SC) and 40% reached the epidermis (Epi). Otherwise, the application of this molecule via DMSO resulted in 95% of the $Re(CO)_3$ derivative being in the SC and only 5% reaching the Epi.

Using a $Re(CO)_3$ derivative with a dodecyl-chain as a model molecule, it was possible to determine the distribution of molecules with similar physicochemical characteristics in the skin using bicosomes. This fact makes these nanostructures promising vehicles for the application of lipophilic molecules inside the skin.

1. Introduction

The topical application of different drugs and active compounds has received significant interest in the medical and pharmaceutical fields. However, the incorporation of molecules in the skin is difficult due to the barrier function of the superficial layer of this tissue, the stratum corneum (SC) [1]. The incorporation of lipophilic molecules is facilitated by their dissolution into intercellular lipids around the cells of the SC [2]. Additionally, lipid vehicles are frequently used to facilitate the incorporation of different active compounds in the skin [2,3].

Bicosomes are phospholipid assemblies based on mixtures of spherical vesicles with diameters of approximately 100–200 nm and discoidal structures with sizes of approximately 15–25 nm in diameter and 5.4 nm in thickness (Fig. 1). In this sense, bicosomes combine the advantages of disks and spherical vesicles [4]. Lipid vesicles have been used in dermatological applications for decades as delivery systems with different active compounds in the skin [5], and discoidal lipid structures have recently demonstrated a significant potential as carriers and modifiers of skin permeability [6,7]. The combination of discoidal and vesicular assemblies forming bicosomes could potentiate the effects of both nanostructures on the cutaneous tissue.

Fourier-transform infrared spectroscopy (FTIR) has been used to evaluate skin composition as well as penetration of different substances in this tissue [8,9]. This technique is employed to investigate the weak energies involved in vibrational levels. The IR spectrum of the skin shows at least three bands associated with different molecules from polypeptides and proteins, as follows: amide A (NH vibration, approximately 3300 cm^{-1}), amide I (CO vibration, approximately 1650 cm^{-1}), and amide II (CN vibration, approximately 1550 cm^{-1}). The bands associated with the alkyl chains

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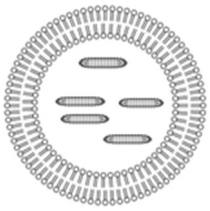

Fig. 1. Bicosome structure.

of skin lipids through the CH_3 and CH_2 stretching vibration are also present (approximately 2920 and 2850 cm^{-1} for asymmetric and symmetric, respectively) [8,9]. Due to the chemical similarity between lipids from different vehicles and skin lipids (both have CH_3 and CH_2 stretching), their characteristic vibrational features cannot be separated. Consequently, in a previous work, the deuteration of lipids from different vehicles was performed to differentiate the vibrations of endogenous (skin lipids) and exogenous lipids [8,9]. This method allowed for the detection of lipids from vehicles in the skin but not the study of the penetration of other active compounds in these lipid systems. An IR active probe with vibrational levels that do not interfere with those from the skin and carrier is needed for this evaluation.

In this work, the use of another type of lipid tagging metal–CO probes that are appropriate for IR imaging is proposed [10–12]. Our research group recently showed that $Re(CO)_3$ is a useful probe for IR and luminescent imaging. These compounds can be used for tagging different molecules, including alkyl chains of various lengths, tamoxifen-like derivatives, oestrogen, or peptides, to image them in cells or tissue using both IR microscopy and fluorescence imaging [13–19]. Interestingly, these $Re(CO)_3$ probes, which are very stable in biological environments, show a very intense signal in the region of 1900–2000 cm^{-1}, that does not interfere with the IR signals from the skin and can be easily quantified [13]. For these reasons, they are the probes of choice for detection and quantification in the IR and have been considered for tagging bicosomes and monitoring their penetrations.

A $Re(CO)_3$ derivative can be attached to several different molecules and provides a way to mark and follow these molecules for skin penetration studies. Recently, a nona-arginine was conjugated with a $Re(CO)_3$ derivative and imaged in skin after permeation [16]. Moreover, the synthesis, sub-cellular imaging and quantification in cells of a $Re(CO)_3$ derivative appended with a $C_{12}N_3$ chain were previously described. In this study, this lipophilic molecule was simultaneously detected using FTIR and fluorescence spectroscopy [13–16,18].

From a spectroscopic point of view, $C_{12}Re(CO)_3$ (Fig. 2) shows a specific vibrational signature with two bands at 1920 cm^{-1} and 2020 cm^{-1}, where no absorption from skin constituents occurs. The first band (E-band at 1920 cm^{-1}) corresponds to asymmetric stretching vibrations, and the second (A_1-band at 2020 cm^{-1}) comes from symmetric stretching vibrations [16,18]. Therefore, this lipophilic molecule can be detected without interference in the skin using FTIR.

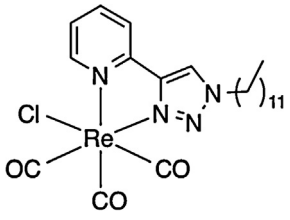

Fig. 2. $C_{12}Re(CO)_3$: (fac-[$Re(CO)_3Cl$(2-(1-dodecyl-1H-1,2,3,triazol-4-yl)-pyridine)]). See synthesis in [10].

In the present study, the lipophilic $C_{12}Re(CO)_3$, which is composed of a $Re(CO)_3$ derivative attached to a dodecyl chain, was used. This lipophilic derivative can be inserted into the bicosome membrane. This strategy enables the study of the penetration and location of this molecule inside the skin and, at the same time, the evaluation of bicosome systems as carriers for the incorporation of lipophilic molecules in the skin.

2. Experimental

2.1. Chemicals

Bicosome systems were formed using 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Cholesterol (CHO) was obtained from Sigma–Aldrich (St. Louis, MO, USA), and Lipoid S-100, whose main component (>94%) is phosphatidylcholine (PC), was obtained from Lipoid GmbH (Ludwigshafen, Germany). The $C_{12}Re(CO)_3$ was synthesized and obtained from the Ecole Normale Supérieure (Paris, France) [13,18]. Chloroform and dimethyl sulfoxide 99% (v/v) (DMSO) were purchased from Merck, and purified water was obtained using an ultra-pure system, Milli-Q plus 185 (Millipore, Bedford, MA, USA).

2.2. Preparation of bicosomes incorporating $C_{12}Re(CO)_3$

Bicosomes incorporating $C_{12}Re(CO)_3$ were prepared by mixing an appropriate amount of DPPC, DHPC and the $Re(CO)_3$ derivative in a chloroform solution to reach a molar ratio of DPPC/DHPC of 3.5. After the components were mixed, the chloroform was evaporated with a rotary evaporator, and the resulting lipid film was hydrated. The resulting solution was subjected to several cycles of sonication and freezing until the sample became transparent.

After that, 80% (w/v) Lipoid S-100 and 20% (w/v) cholesterol in chloroform were mixed and the solvent was removed with a rotary evaporator. The resulting film was hydrated with the previously formed solution [4].

The total lipid concentration in the bicosomes was 15% (w/v) and the concentration of $C_{12}Re(CO)_3$ was 1% (w/v).

The solution in DMSO was prepared by dissolving the appropriate amount of $C_{12}Re(CO)_3$ in DMSO to reach the same concentration as the bicosomes (1%, w/v).

2.3. Dynamic light scattering (DLS)

The sizes of the bicosomes incorporating $C_{12}Re(CO)_3$ were measured by detecting the hydrodynamic diameter (HD) using a Zetasizer nano ZS90 (Malvern Instruments, Malvern, Worcestershire, UK). For comparative purposes, the size of the bicosomes without any incorporated molecule was also measured.

DLS measures the Brownian motion of the particles and correlates this to the particle size. The relationship between the size of a particle and its speed due to Brownian motion is defined by the Stokes–Einstein equation:

$$\mathrm{HD} = \frac{k_B T}{3\pi\eta D}$$

where HD is the hydrodynamic diameter of a hypothetical hard sphere that diffuses with the same speed as the particle in the experiment, D is the translational diffusion coefficient (m^2/s), k_B is the Boltzmann's constant ($1.3806503 \times 10^{-23}$ $J\,K^{-1}$), T is the absolute temperature (K) and η is the viscosity (mPa s) [20].

DLS measurements were performed in triplicate and the mean size and standard deviation (SD) of the different populations in the distribution curves were obtained.