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The development of simple flow injection analysis tandem mass spectrometric methods for the cutaneous determination of peptide-modified cationic gemini surfactants used as gene delivery vectors

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ABSTRACT

Diquaternary ammonium gemini surfactants are a class of non-viral gene delivery vectors, primarily studied for their dermal applications. However, their biological fate has rarely been investigated. In this work, we developed simple flow injection analysis tandem mass spectrometric methods, (FIA)-MS/MS, to understand the fate and biodistribution of topically applied gemini surfactant-based therapeutics in an ex-vivo skin model.

Three peptide-modified gemini surfactants with varied structures and transfection efficiencies were evaluated. For each compound, two methods were developed to quantify their presence in skin tissue and in phosphate buffered saline (PBS). The methods were developed using single-point calibration mode. Skin penetration was assessed on CD1 mice dorsal skin tissue mounted in a Franz diffusion cell after extraction. Amongst the five evaluated liquid-liquid extraction protocols, the Folch method provides the highest extraction efficiency for all compounds. Weak cationic exchange solid phase extraction was also used to further isolate gemini surfactants from endogenous skin lipids. FIA–MS/MS analysis of the skin revealed that all compounds were detected in the skin with minimal partition into the PBS compartment, which represents circulation. Interestingly, the detected amounts of gemini lipids in the skin were correlated with their transfection efficiencies.

1. Introduction

Recent advances in discovering the genetic basis of many dermatological disorders have found cutaneous gene therapy to be a promising therapeutic option [1]. Cutaneous delivery of genetic material offers numerous advantages over other routes of administration, such as minimizing systematic toxicity, bypassing first-pass metabolism, and avoiding rapid clearance from the systemic circulation [2]. Despite these advantages, skin is a formidable barrier to foreign materials, such as nanotechnology products [3]. Therefore, effective delivery systems capable of penetrating the cutaneous barrier and facilitating recombinant DNA uptake into the skin are needed to achieve gene expression – the ultimate goal of gene therapy.

Among topical delivery modalities currently being explored, lipid-based delivery vectors are at the forefront [4]. They have the ability to encapsulate, protect, and compact negatively charged nucleic acids, whereby forming nano-sized lipoplexes. Furthermore, the chemical composition of lipid-based nanocarriers bears some similarity to skin lipids, which enables them to fuse with the lipids in the stratum corneum, the outer layer of the skin; destabilizing the lipid matrix and enhancing drug penetration [5]. Diquaternary ammonium gemini surfactants are a class of lipid-based delivery systems that are composed of dimeric surfactants with positively charged head groups and hydrocarbon tails linked by a spacer chain (Fig. 1) [6]. The structure of gemini surfactants can be tailored to overcome skin barrier functions [7]. The topical application of gemini surfactant-based nanoparticles demonstrated a promising potential in the treatment of localized cutaneous scleroderma [7,8]. Nanoparticles of *N,N'*-bis(dimethylhexadecyl)-1,3-propanediammonium dibromide gemini surfactant complexed with pDNA encoding for interferon gamma (INF-γ) showed a significant increase in the level of INF-γ in mice [7,8].

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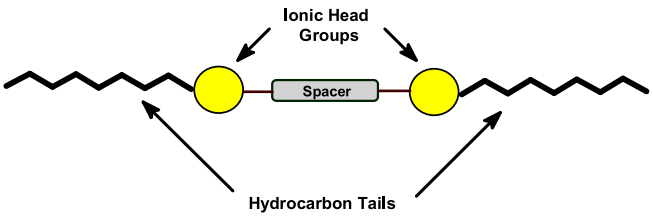

Fig. 1. Schematic representation of gemini surfactants showing the two ionic head groups, hydrocarbon tails and the spacer.

Despite advancements in the design of lipid-based nanocarriers, their biodistribution and biological fate have been less explored. Upon topical application, lipid-based nanoparticles distribute within various layers and cellular components. At present, the biodistribution, intracellular trafficking, and the ultimate fate of the lipid vector, after releasing its therapeutic cargo, are not fully understood. A fundamental understanding of the behavior of the lipid-based vectors in complex biological environments is essential in guiding the design of safer and more effective nanoparticles.

To track the fate and distribution of lipid-based nanoparticles, fluorescently labeled and radiolabeled carriers are the most commonly used strategies. However, labeling techniques have drawbacks, particularly their tendency to alter the physicochemical properties of the delivery system. These modifications, in turn, change the pharmacokinetic profile of the nanocarriers [9]. Furthermore, they are unable to distinguish between the localization of a labeled molecule and the metabolites that retain the fluorescent or radioactive probes [10]. Therefore, a more robust and sensitive analytical technique should be employed to identify and quantify gene-based carriers in complex biological samples.

Mass spectrometry (MS) is an ideal technique to monitor the fate of gemini surfactants in the skin [11,12]. It is a label-free technique with a powerful chemical identification capability and is gaining popularity in pharmaceutical sciences due to its high selectivity and sensitivity [13]. Coupling liquid chromatography to tandem mass spectrometry (LC—MS/MS) allows for reliable high throughput qualitative and quantitative analysis [14]. In fact, it is the gold standard technology for the quantification of pharmaceuticals in complex biological matrices [15].

In our laboratory, we developed two LC—MS/MS methods for the quantification of unsubstituted diquaternary ammoniums gemini surfactants (*N,N*-bis(dimethylalkyl)-α,ω-alkane-diammonium), amine substituted diquaternary ammoniums compounds, and heterocyclic headgroup gemini surfactants (bis(alkyl-pyridinum) in epidermal keratinocytes [16,17]. These methods provided essential information about the rate of cellular uptake and intracellular depletion of gemini surfactants [16,17]. Currently, these methods are being employed to determine the subcellular localization of gemini surfactants and identify any potential metabolites. Recently, a new series of peptide modified diquaternary ammoniums gemini surfactants was found to exhibit superior transfection efficiency compared to previous generations of gemini surfactants [18]. Their collision-induced dissociation (CID)-MS/MS behaviour was evaluated, establishing a universal mass spectrometric fingerprint, essential for the development of targeted LC—MS qualitative and quantitative methods [11].

Herein, we resolved a significant analytical challenge, the efficient extraction of gemini surfactants from lipid rich skin tissues. Efficient analytical platforms are needed to guide the development of effective pharmaceutical formulations. Three representative compounds were selected with high, low, and moderate transfection efficiencies. Subsequently, rapid and simple flow injection analysis (FIA)-MS/MS methods were developed to detect and quantify peptide-modified gemini surfactants in skin tissues as well as in phosphate buffered saline (PBS).

2. Materials and methods

2.1. Materials

The evaluated peptide-modified gemini surfactants, designated as 16-7N(R)-16 where 16 is the alkyl chain length and R is the peptide-containing moiety: R = glycyl-lysine, glycyl-hexyl-trilysine and glycyl-undecyl-trilysine (Fig. 2), were synthesized using previously reported synthetic methods [18]. The corresponding internal standards were synthesized using the same synthetic procedure with the incorporation of deuterated lysine moiety bearing four deuterium atoms (Fig. S1, Supplementary material). The plasmid pGThCMV.IFNGFP (pDNA), encoding for murine interferon gamma (IFN-γ) and green fluorescent protein (GFP) was utilized in this work [7].

The helper lipid 1,2-dioleoyl-snglycerol-3-phosphoethanolamine (DOPE) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Sucrose, used as a stabilizing agent, and phosphate buffered saline (PBS) were purchased from Sigma Aldrich (Oakville, ON, Canada). Mass spectrometry-grade methanol, water, and acetonitrile were purchased from Fisher Scientific (Nepean, ON, Canada). Formic acid (purity 90%) was obtained from EMD Chemicals Inc. (Merck KGaA, Darmstadt, Germany). Anhydrous chloroform and methyl tert-butyl ether (MTBE) used as extraction solvents were purchased from Sigma Aldrich (Oakville, ON, Canada). Solid phase extraction cartridges, Bond Elute® CBA, were obtained from Agilent Technologies (Mississauga, ON, Canada).

2.2. Preparation of topical formulations

Cationic gemini lipids were combined with 16 μg pDNA at a negative to positive charge ratio (N/P) of 1:5 in the presence of a helper lipid DOPE to create pDNA/gemini lipid/helper lipid (P/G/L) nanoparticles. The concentration of the gemini surfactants was calculated so that the amount of gemini surfactants is enough to achieve the required charge ratio. As such, an appropriate amount of 30 mM aqueous solutions of gemini surfactant was added to 2 mg/mL pDNA solution and incubated for 20 min at room temperature (P/G complex). 2 mM DOPE was prepared as described previously [19] then concentrated to 10 mM using Eppendorf concentrator 5301(Eppendorf, Hamburg, Germany). The concentrated DOPE was added to P/G complexes at a gemini surfactant to DOPE molar ratio of 1:16 to form the final nanoparticles (P/G/L) and incubated at room temperature for 20 min.

2.3. Ex vivo *skin penetration study*

Dorsal skin tissues were collected from female CD1 mice (Charles River Laboratories, Saint-Constant, QC, Canada) weighing around 22–24 g. Approval for this study was granted by the University of Saskatchewan's Animal Research Ethics Board in adherence to the Canadian Council on Animal Care guidelines for humane animal use (protocol # 20090081). The animals were shaved and the skin was collected and stored at −80 °C until use.

Skin penetration was evaluated using multi-station Franz diffusion cell system with 64 mm^2 surface area (PermeGear Inc., Hellertown, PA, USA). The skin tissue was mounted between the donor and receptor compartments of the Franz cell with the stratum corneum facing the donor compartment. The receiving chamber was filled with 5 mL PBS, avoiding any air bubbles between the skin and the solution. The skin tissues were allowed to equilibrate for 10 min before applying any formulation. A total