Fluorinated Cholesterol based system with DOTAP for DNA Transfection

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Cationic liposomes are extremely advantageous for DNA transfection due to stability and health safety when compared with viral vectors. Liposomal delivery systems are formed by a cationic lipid bilayer usually combined with helper lipids, such as cholesterol, in order to improve their stability, reduce the toxicity of cationic lipids and increase their plasma half-life time. One of the best properties of cationic liposomes is that they interact efficiently with negatively charged molecules, such as DNA, mainly by electrostatic interactions. This becomes a fast and easy method to prepare delivery systems which do not require an encapsulation step.

A new system has been developed and characterized using the cationic lipid DOTAP and fluorinated cholesterol (F7-cholesterol), as the helper lipid. Liposomes were prepared by the lipid film hydration method and extruded through polycarbonate membranes of pore size from 800 to 100 nm. The system was characterized by dynamic light scattering and zeta-potential measurements. The study confirms the formation of a liposome-monodisperse sized population and a positive zeta-potential (55 ± 5 mV) of the system. Their positively charged surfaces efficiently compact DNA by means of a strong entropically driven surface interaction that yields the formation of lipoplexes as confirmed by zeta-potential values.

Langmuir monolayers of the lipid system were obtained and have shown that the fluorinated cholesterol does not induce a first order phase transition, although its addition has an ordering effect in DOTAP layers. The results obtained by Brewster angle microscopy evidence a complete miscibility between the compounds, which confirms the previous results. It was noticed that for the same pressure, the area per molecule is smaller than the expected at all ratios tested (DOTAP:F7-cholesterol 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4 and 1:9), which indicates a strong interaction between DOTAP and fluorinated cholesterol reducing the area per molecule occupied by the compounds.

The infrared reflection absorption spectroscopy (IRRAS) studies show the adsorption of DNA to lipid monolayers of pure DOTAP and mixtures DOTAP:F7-cholesterol 2:1, 1:1 and 1:2. Pure F7-cholesterol monolayer does not show any DNA at the interface. The DNA migrates to the interface due electrostatic interactions. For the tested mixtures, the amount of DNA at the interface decreases with the decreasing amount of DOTAP. However, the amount of absorbed DNA is higher than the values expected by the linear relation, meaning that even when DOTAP was combined with F7-cholesterol, it has enough free charges to establish electrostatic interactions with DNA.