

PEGylated cationic liposome – DNA nanoparticle assembly in cell culture media: pathway effects and clues to enhanced control and transfection efficiency optimization

Cláudia Botelho^{1;2;3*} and Bruno F.B. Silva¹

¹INL – International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n., 4715-330 Braga, Portugal,

²Center of Biological Engineering (CEB), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

³Center of Molecular and Environmental Biology (CBMA), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

*e-mail: claudia.botelho@inl.int

Introduction: Cationic Liposome – DNA nanoparticles constitute a promising approach for safe and efficient delivery of genes for therapeutic applications. In order to be used *in vivo*, these particles can be coated with an inert and hydrophilic polymer, such as polyethylene-glycol (PEG), which improves blood circulation time by providing steric stabilization against removal by the immune system. In this work we study the influence of the initial salt concentration, which controls the electrostatic attraction between cationic liposomes and anionic DNA, on the structure of PEGylated CL–DNA nanoparticles.

Materials and Methods: The lipids DOPC, DOTAP, and 18:1 PEG2000 PE were purchased from Avanti Polar Lipids and used as received. Two parameters are systematically changed in the lipid composition: (i) the lipid membrane charge density (σ_M), which is proportional to the amount of DOTAP in the membrane; and (ii) the amount of PEG2K lipid (0, 5 and 10% molar fraction). Two CL–DNA preparation methods are studied in this work. In the first, the complexes are prepared in water, and transferred later to saline media. In the second, when cationic lipid and DNA are mixed, they are already in saline media. Small-angle X-ray Scattering (SAXS) and cryo-TEM are the main characterization techniques.

Results and Discussion: Previous results have shown that if non-PEGylated or PEGylated CL–DNA lamellar complexes are prepared in water, their structure is well defined with a high number of lipid membrane–DNA layers [1,2]. Here we show that if these complexes are transferred to saline media (150 mM NaCl or DMEM, both near physiological conditions), this structure remains nearly unchanged. Contrariwise, if PEGylated complexes are prepared in saline media from the beginning, their lamellar structure is much looser, with a small number of layers [3]. This pathway-dependent behavior of PEGylated complex formation in salty media is controlled by the liposome membrane charge density and the mole fraction of PEG in the membranes, with the average number of layers decreasing with increasing salt concentration and increasing amount of PEG-lipid. Each of these structures (high and low number of layers) is stable with time, suggesting that in spite of particle formation being thermodynamically favored, the assembly process between DNA and cationic PEGylated membranes is largely controlled by kinetics.

Conclusion: One important result of these findings is the realization that subtle differences in the preparation protocol (differences which are common between different laboratories and researchers), can lead to important differences over the structure of PEGylated CL–DNA–NPs, and possibly to the way these particles interact with cells, influencing gene delivery. Ongoing work is aimed at elucidating the influence of these differences in the transfection efficiency, and how they can be exploited towards achieving more efficient therapeutic formulations.

[1] Rädler JO, Koltover I, Salditt T, Safinya CR. *Science* 1997, **275**, 810.

[2] Martin-Herranz A, Ahmad A, Evans HM, Ewert K, Schulze U, Safinya CR. *Biophys J.* 2004, **86**, 1160.

[3] Silva BFB, Majzoub RM, Chan C-L, Li Y, Olsson U, Safinya CR. *Biochim. Biophys. Acta-Biomembr.* 2014, **1838**, 398.