Development of new Reducible Cationic *Gemini* Surfactant – SS14 – Containing Liposomes for Gene Delivery

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Introduction.

Gene therapy has become one of the most intensively developing strategies in current clinical research because it offers new treatment possibilities for many common diseases. Improved liposome formulations for gene delivery are a valuable alternative to viral vectors and avoid several problems associated with viral delivery. Herein we describe the development of different liposome formulations based on SS14, a reducible cationic *gemini* surfactant.

Materials and Methods.

SS14 was previously synthesized by our group.[1, 2] Its structure is reported in Fig. 1.

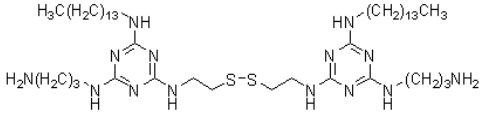

Figure 1. SS14 structure

SS14-containing liposomes were prepared by the well established extrusion method through polycarbonate filters of 100 nm pore diameter, which gives monolamellar aggregates of low polydispersity in size. Four formulations with different alkyl chains and/or polar head types were chosen: DMPC/SS14:0.75/0.25; DOPC/SS14:0.75/0.25;
DMPC/DMPE/SS14:0.5/0.25/0.25;
DOPC/DOPE/SS14:0.5/0.25/0.25 molar ratios.

Size and surface charge of liposomes were determined by Photon Correlation Spectroscopy (PCS) and Laser Doppler Velocimetry (LDV). Each lipoplex sample was prepared by adding a stock solution of plasmid (pEGFP-N1 or pCMV-GLuc) to the liposome suspension, at the desired lipid concentration, yielding different Charge Ratios (CR, +/-). The DNA binding ability of liposomes was assessed fluorimetrically by monitoring the displacement of SYBR-Green I from DNA. In parallel, lipoplex disassembly was assessed by adding a reducing agent (10 mM DTT *vs* threitol) to lipoplex suspension.

For transfections, U87-MG cells were seeded at a density of 10^4 cells/cm^2 the day before transfection. The day after, the medium was replaced by complete medium (DMEM with 10% FBS) or Opti-MEM® containing lipoplexes. The DNA dose was 80 ng/cm^2. Cytotoxicity was evaluated by AlamarBlue® assay and EGFP expression was estimated by fluorescence-activated cell sorter (FACS, Becton Dickinson), 48 h post-transfection and analyzed using WinMDI 2.9 software. Statistical analysis was performed by ANOVA test. Significance was retained when $p<0.05$.

Results and Discussion.

The size distribution for three out of four liposome formulations showed a monodisperse population (Polydispersity Index, P.I. ≤ 0.3). In DMPC/DMPE/SS14 liposomes, together with the main liposome population, large aggregates (Ø > 1μm) were found, possibly due to fluctuating lamellar sheets. All liposome dimensions were between 95 and 120 nm for the 100 nm extruded ones. Zeta Potential, within experimental error, was the same for all the formulations, ranging from +39±7 mV and +55±8 mV.

By monitoring the displacement of SYBR-Green I from DNA in each formulation, we noticed a negative trend of fluorescence in function of CR that plateaud at its lower limit beyond CR5.

Because of the high redox potential difference (~100-1000 fold) existing between the reducing intracellular space and oxidizing extracellular milieu, we evaluated that DTT enabled DNA release from lipoplexes (Fig. 2).

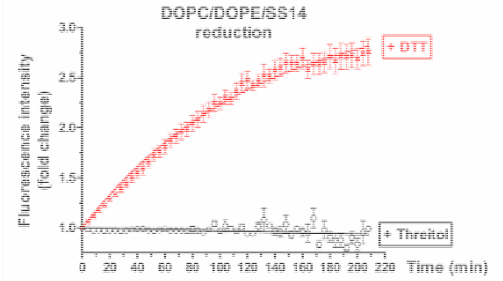

Figure 2. DNA release after lipoplex reduction.

Transfection activities of the four formulations complexed at CR5 and CR15 were compared. Firstly, the presence of serum in transfection experiments did not inhibit liposome effectiveness. Finally, the introduction of DOPE and DMPE in two-component liposome formulations improved significantly transfection efficiency (19.5±1.8 and 24.4±0.7, respectively; $p<0.05$) (Fig. 3). This may be due to the high fusogenic properties of their phosphoethanolamine (PE) polar head. On the other hand, three-component formulations were more cytotoxic than both DMPC/SS14 and DOPC/SS14 liposomes.

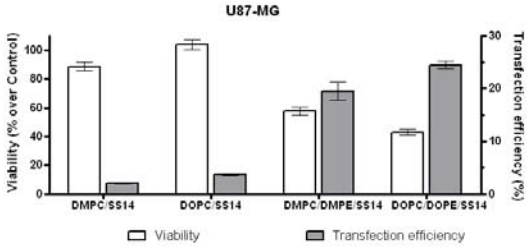

Figure 3. Transfection efficiency and viability of liposomes extruded at 100 nm and complexed at CR5.

Conclusions.

Four different liposome formulations containing the cationic *gemini*-like surfactant, SS14, were prepared and characterized for cell transfection. Among the chosen four formulations, DOPC/DOPE/SS14 liposomes demonstrated superior transfection efficiency and modest cytotoxicity on U87-MG cell line. The mechanisms beneath the enhancement of efficiency will be the subject of further investigation.

[1] Candiani G et al., *ChemMedChem*. 2007; 2(3): 292-6.
[2] Candiani G., *J Gene Med*. 2008; 10(6): 637-45.