New gemini surfactant - SS14 - containing liposomes for gene delivery

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Statement of Purpose: The main hurdles towards implementing an effective gene therapy using non-viral vectors are their low efficiency and the inhibitory effect of serum. Herein we describe the *in vitro* development of two different liposome formulations of SS14 gemini surfactant.

Methods: SS14 gemini surfactant was previously synthesized by our group.[1] 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. Two different lipid formulations were chosen: DMPC/SS14:0.75/0.25 and DMPC/DPPE/SS14: 0.5/0.25/0.25 molar ratios. The size of SS14 containing liposomes was determined by photon correlation spectroscopy (PCS) with a Malvern ZS ZEN3500 particle sizer. The surface charge of liposomes (Zeta Potential) was obtained by Laser Doppler Velocimetry (Malvern ZS ZEN3500 apparatus). Each lipoplex sample was prepared at r.t. by adding a stock solution of nucleic acid (pCMV-GLuc, encoding for the secreted Gaussia Luciferase) to a suspension of SS14 containing liposomes, at the desired lipid concentration. This yielded different charge ratios (CR, +/-). DNA binding ability of liposomes was assessed by gel retardation assay.[2] For transfection procedure, U87-MG (glioblastoma) cells were seeded at a density of 10^4 cells/cm^2 the day before transfection. The day after, medium was replaced either with serum-free medium (Opti-MEM®) or with D-MEM 10% FBS (complete medium), both containing lipoplexes (lipid/DNA complexes); the DNA dose was 80 ng/cm^2. Following a 4-hour incubation in Opti-MEM®, cells were washed with PBS and incubated 44 hours post-transfection in complete medium; otherwise cells were maintained in complete medium containing lipoplexes. 48 h post-transfection, the medium was harvested and the cytotoxicity was tested according to MTT viability assay.[2] Transfection efficiency was evaluated by measuring the luminescence of the samples according to Gaussia Luciferase Assay kit procedures.

Results: The size distribution intensity of DMPC/SS14 liposomes showed a main population centered at d=142 nm (95% by integrated intensity), together with a much smaller population of large objects 5-6 μm (diameter, 5% of total liposomes) (polydispersity index (P.I.) = 0.45-0.55). In agreement with light scattering data, the Zeta Potential of DMPC/SS14 liposomes was spread along a large distribution of values with a high surface charge of +57 ± 10 mV. The size distribution obtained for three-component DMPC/DPPE/SS14 liposomes was markedly narrower than that of DMPC/SS14 liposomes. Indeed, in this case, a single population was found with a mean size of 144 nm and P.I. = 0.29. The measured Zeta Potential of the three component liposomes were within experimental values the same as for DMPC/SS14 liposomes, both in value and dispersion. By electrophoretic gel retardation assay, it was apparent that DMPC/DPPE/SS14 liposomes began to efficiently retain the DNA at CR2 but complexes were effectively and completely formed at CR4. Instead, for DMPC/SS14 liposomes, complete retention was effective at CR2. Transfection experiments demonstrated that CR2 was the most effective in transfecting cells for all the formulations, combining the highest viability with the highest transgene expression. Transfection efficiency and viability in complete medium were both increased compared to lipofections carried out in Opti-MEM®, demonstrating that the produced liposomes maintained the serum resistance, characteristic of non formulated SS14, as shown in Figure 1.

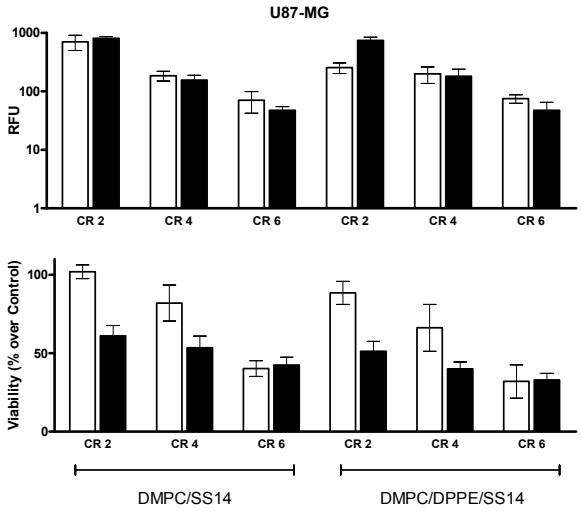

Figure 1. Transfection efficiency and cytotoxicity of DMPC/SS14 and DMPC/DPPE/SS14 liposomes in complete medium (□) versus Opti-MEM® (■).

Conclusions: Two different liposome formulations were prepared with the SS14 gemini surfactant, previously used non formulated to transfect cells.[1] Transfection experiments demonstrated that CR2 was the most effective in transfecting cells for both formulations. On the other hand, comparing liposomes with non formulated SS14 in terms of transfection efficiency, a sharp difference could still be found. Moreover, non formulated SS14 was more cytotoxic than DMPC/SS14 and DMPC/DPPE/SS14 liposomes (not shown). These promising results obtained by transfecting the U87-MG cell line prompt us to extend our investigations to other cell lines and primary-derived cells. Moreover, we will investigate the transfection effectiveness of DMPC/SS14 and DMPC/DPPE/SS14 liposomes of different size.

References:

[1] Candiani G. ChemMedChem. 2007; 2(3): 292-6.
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