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Formulation of new reducible liposomes for gene delivery

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Gene therapy aims to eradicate causes rather than symptoms of diseases and is believed by many to be the therapy of the future. Improved liposome formulations are a valuable alternative to viral gene delivery vectors and the rapid disulfide linkages cleavage by the intracellular reductive environment can induces fast reducible lipoplex dissociation and efficient DNA release, yielding increased gene expression. On the light of these findings, we developed four different liposome formulations based on SS14, a reducible cationic gemini-like surfactant. SS14 was previously synthesized by our group [1,2]. Helper lipids bearing different alkyl chain and/or polar head types were chosen and four formulations were investigated: 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. SS14-containing liposomes were prepared by repeated extrusions through polycarbonate filters of 100 nm pore diameter. Three out of four liposome formulations showed a size distribution with a monodisperse population (polydispersity index, P.I. ≤ 0.3) while in DMPC/DMPE/SS14 liposomes, large aggregates ($\varnothing > 1$ μm) were found together with the main liposome population, possibly due to fluctuating lamellar sheets. All liposome dimensions were between 95 and 120 nm. Zeta potential, within experimental error, was the same for all the formulations, ranging from $+39 \pm 7$ mV and $+55 \pm 8$ mV. By monitoring the displacement of SYBR-Green I from DNA, a negative trend of fluorescence in function of CR was noticed for each formulation with a plateau reached beyond CR5. Since between the reducing intracellular space and the oxidizing extracellular environment a high redox potential difference exists (~100–1000-fold), by agarose gel electrophoresis we demonstrated the ability of GSH to enable DNA release. Transfection activity and cytotoxicity of the four formulations were compared at CR5 and CR15 on U87-MG, Cos-7, HeLa and MG63 cell line using pEGFP-N1 as plasmid DNA. Firstly, liposome effectiveness was not inhibited by the presence of serum in transfection experiments. Secondly, the introduction of helper lipids bearing PE polar heads in two-component liposome formulations increased significantly transfection efficiency up to 7-fold ($p < 0.05$). This may be due to the high fusogenic properties of their phosphoethanolamine (PE) polar head. Finally, three-component formulations were more cytotoxic. In particular, DOPC/DOPE/SS14:0.5/0.25/0.25 CR5 liposomes demonstrated superior transfection efficiency (24.4 ± 2.7% by FACS analysis on U87-MG cells) and modest cytotoxicity. The mechanisms beneath intracellular reduction, transfection enhancement and increased cytotoxicity will be the subject of further investigation.

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Strategies for microsphere-mediated delivery of oligonucleotides

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An efficient intracelllar delivery of oligonucleotides is a vital step for gene therapy. Many technologies have been developed to design efficient transfection agents. Many of these agents are promising tools *in vitro* but they fail when *in vivo* assays are carried out. Recently we have developed a polystyrene microsphere-based system designed to efficiently deliver biological materials into a broad range of cell lines. Additionally, these particles have been successfully test *in vivo*. The fact that these polymer particles are easy to functionalise with high controllability over the cargo loading, showing any undesired cytotoxic effect, make them enormously attractive as delivery system. Our recent advances in the design of strategies for the delivery of oligonucleotides using microspheres as transfection system will be presented.

See reference below for additional reading
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Microsphere-mediated delivery of therapeutic peptides on neuronal cells

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Many proteins exert their biological roles as components of complexes, and the functions of proteins are often determined by their specific interactions with other proteins. The identification of inhibitory peptides and derived peptidomimetics has been developed as potent inhibitors of protein–protein interaction. More specifically protein–protein interaction domains that couples the NMDA receptor to intracellular proteins are potential targets for the development of new therapies to combat neurodegenerative diseases [1]. Different studies of the PDZ domain in nNOS inhibitors have been carried out. The peptidic nature of these compounds has obstructed their uptake into the cell. Amino cross-linked microspheres have been used previously for the delivery of therapeutic molecules [2–5]. The design, synthesis and biological evaluation of microspheres as carrier systems to facilitate the cellular uptake of these peptidic sequences on SH-SY5Y neuroblastoma cells will be presented.

See reference below for additional reading
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