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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.

Reference
1. Candiani G. *ChemMedChem* 2007;**2**:292–6.
2. Candiani G. *J Gene Med* 2008;**10**:637–45.

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A61
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
1. Sánchez-Martín RM, et al. *ChemBioChem* 2005;**6**:1341–5.
2. Sánchez-Martín RM, et al. In: Pignataro B, editor. *Ideas in Chemistry and Molecular Sciences: Where Chemistry Meets Life*. Wiley VCH Ed.; 2010. p. 117–40. Chapter 5.
3. Alexander LM, et al. *Bioconjug Chem* 2009;**20**:422–6.
4. Tsakiridis A, et al. *Biomaterials* 2009;**30**:5853–61.

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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
1. Pawson T, Scott JD. *Science* 1997;**278**:2075–80.
2. Sánchez-Martín RM, et al. In: Pignataro B, editor. *Ideas in Chemistry and Molecular Sciences: Where Chemistry Meets Life*. Wiley VCH Ed.; 2010. p. 117–40. Chapter 5.

3. Sánchez-Martín RM, et al. *Angew Chem Int Ed* 2006;**45**:5472–4.
4. Alexander LM, et al. *Bioconjug Chem* 2009;**20**:422–6.
5. Sánchez-Martín RM, et al. *ChemBioChem* 2009;**10**:1453–6.

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A63
siRNA versus pharmacological inhibition of endocytic pathways for studying cellular uptake of cell penetrating peptides and other drug delivery vectors

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Cell-penetrating peptides (CPPs) have the potential to deliver numerous therapeutic macromolecules into cells including peptides, proteins, and nucleic acids. Under defined conditions endocytosis is thought to be of significant importance for CPP entry but identifying the exact uptake mechanism and pathway(s) involved has been difficult. Multiple pathways have been reported to contribute to uptake, including macropinocytosis and those regulated by clathrin and cavaeolin-1. This project aims enhance the use of cell penetrating peptides as drug delivery vectors by developing new technologies to study their mechanisms of uptake. Traditionally studies investigating the uptake of these molecules, and other drug delivery vectors, have been performed using chemical inhibitors but these are often toxic and lack specificity [1]. We have developed siRNA-based assays to silence endocytic proteins that have previously been shown to regulate distinct endocytic pathways. The effect of depleting these proteins was then assessed to investigate their roles in mediating the uptake of well characterised endocytic probes and CPPs. Two cell lines were predominantly used, HeLa (cervical cancer epithelial) and A431 (human epithelial carcinoma). Endocytic proteins clathrin heavy chain, flotillin-1, dynamin II, caveolin-1 and P21-activated kinase (PAK-1) were depleted using single siRNA sequences; siRNA against GFP was used as a control. In siRNA treated cells the uptake of fluorescent endocytic markers including; Alexa488-transferrin (clathrin mediated endocytosis), 40 kDa FITC Dextran (fluid phase uptake and macropinocytosis), FITC conjugated anti-CD59 antibody (flotillin-1 dependent uptake) and the uptake of Alexa488 CPPs (RRRRRRRRGC-Alexa488-R8, and GRKKRRQRRRPPQ-Alexa488-HIV-TAT) were measured by flow cytometry. Protein depletion was assessed from protein lysates using SDS PAGE and Western blotting. Overall, the data shows that siRNA transfection method could effectively reduce expression of clathrin heavy chain, caveolin-1 and flotillin-1 from HeLa cells and this then allowed for us to study effects on endocytosis of various probes. PAK-1 has been shown to regulate macropinocytosis and we show that the induction of macropinocytosis and PAK-1 expression are highly cell line dependent. Paralleled with this was our findings that cationic CPPs induce an increase in fluid phase uptake of dextran and the extent of this was cell line dependent. Comparative analysis of these experiments with those performed using pharmacological inhibitors, allowed us to determine the usefulness of this approach for drug delivery research.

Reference

1. Vercauteren D, et al. The use of inhibitors to study endocytic pathways of gene carriers: optimisation and pitfalls. *Mol Ther* 2010;**18**:561–9.

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A64
SAINTargs, a novel lipid-based targeting device for siRNA delivery

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The endothelium represents an important therapeutic target because its pivotal role in many diseases such as chronic inflammation and cancer and its accessibility for systemic administration. RNA interference by small interfering RNA (siRNA) has become in the last decade a very powerful tool in basic research, and has huge potential to become an important new class of therapeutics for humans. However, due to their size and charge, siRNAs have no bioavailability to enter unperturbed cells. To overcome this problem, our laboratory developed a non-viral lipid-based targeting device which efficiently and specifically delivers siRNA into endothelial cells. Molecular determinants expressed on the surface of inflammation-activated endothelial cells, like certain adhesion molecules and receptors involved in endocytosis, are excellent candidates to increase carrier-mediated siRNA uptake. Therefore we conjugated monoclonal anti-E-selectin antibodies to the cationic amphiphilic lipid, 1-methyl-4-(cis-9-dioleyl)methyl-pyridinium-chloride (SAINT-18) which was complexed in a 1:2000 molar ratio with the transfection agent SAINT-MIX (SAINT:DOPE, 1:1), and siRNA, resulting in a siRNA containing lipoplex called anti-E-selectin-SAINTarg [1]. Our findings demonstrate that anti-E-selectin-SAINTargs maintained the antigen recognition capacity of the parental antibody and showed increased siRNA uptake in otherwise difficult-to-transfect primary human umbilical vein endothelial cells (HUVEC) as compared to non-targeted SAINT-MIX. Moreover, anti-E-selectin-SAINTargs superior binding and uptake efficiency was corroborated by improved silencing of both gene- and protein expression of VE-cadherin in activated HUVEC. The VE-cadherin gene expression could be silenced up to 95% by VE-cadherin specific siRNA, at low siRNA concentrations (30 pmol/ml). Furthermore, no non-specific silencing by scrambled or VE-cadherin specific siRNA was observed. To optimize siRNA delivery into activated endothelial cells we also synthesized anti-VCAM-1-SAINTargs which were as efficient in VE-cadherin silencing as anti-E-selectin-SAINTargs. Because of the heterogeneous expression of adhesion molecules on inflammation-activated endothelial cells *in vivo*, a combination of these two SAINTargs may result in enhanced siRNA effects. Taken together, SAINTargs demonstrate specific and efficient targeting to inflammation-activated difficult-to-transfect primary endothelial cells and results in strong siRNA specific gene silencing at low siRNA concentrations.

Reference

1. Asgeirsdottir SA, Talman EG, de GI, Kamps JA, Satchell SC, Mathieson PW, Ruiters MH, Molema G. Targeted transfection increases siRNA uptake and gene silencing of primary endothelial cells in vitro - A quantitative study. *J Control Release* 2010 Jan 25;**141**(2):241–51.

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