used to transfect an airway epithelial cell line, Calu-3 cells. A range of targeted, mannosylated liposomes were also synthesized, fl-siRNA was encapsulated therein and these complexes were used to transfect an alveolar macrophage-like cell line, THP-1. Each of the systems was characterised for size, zeta-potential and encapsulation efficiency prior to transfection. To determine the efficiency of fl-siRNA transfection facilitated by these nanoparticles a protocol was specifically designed to qualitatively and quantitatively monitor siRNA uptake using InCell 1000 high content screening. *Results*: A number of the PEI-PEG nanoparticles significantly increased siRNA uptake into Calu-3 cells and a number of the mannosylated liposomes were capable of efficiently transfecting alveolar macrophages, a particularly difficult to transfect cell type. *Conclusion*: HCS facilitated the screening of a large number of novel nanoparticles rapidly and comprehensively for siRNA delivery efficiency, providing both high quality cell images and quantitative data on siRNA uptake, thereby avoiding the need for separate microscopy and quantification studies.

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A78
Endosomal DNA release studies using giant unilamellar vesicles as model endosomal membranes

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Endosomal DNA release is one of the main barriers to successful non-viral gene delivery, since the inability of DNA to escape from the endosome at an early stage leads to its degradation through trafficking to the lysosomal compartment. It is therefore essential to understand the interactions between commonly used gene delivery vectors and endosomal membranes. While membrane interactions are often studied using small unilamellar vesicles (SUVs) as model bilayers, it is proposed that giant unilamellar vesicles (GUVs) present more realistic models due to their larger size, their superior lipid packing due to reduced surface curvature and the ability to visualise them using light or confocal microscopy. GUVs composed of a mixture of neutral or neutral and negatively charged lipids, representing early or late stage endosomal membranes respectively were prepared by electroformation in calcein, followed by the addition of cobalt chloride to quench background fluorescence. GUVs were then observed by confocal fluorescence microscopy before and after the addition of lipid:DNA complexes composed of equimolar mixture of dimethyldioctadecylammonium bromide (DDAB) with the helper lipid dioleoylphosphatidyl-ethanolamine (DOPE) incorporating a 10 mol% rhodamine-labelled DOPE at a 4:1 lipid:DNA charge ratio. Furthermore, in order to visualise the DNA in relation to the encapsulated calcein (green) and the lipid (red), 4′,6-diamidino-2-phenylindole (DAPI) was added to highlight the DNA blue. Both endosomal models formed spherical GUVs approximately 10–90 μm in diameter and were visible as green calcein-encapsulating vesicles. Upon the addition of lipid:DNA complexes to the early endosomal model, a large number of GUVs were shown to lose fluorescence due to calcein leakage, which was concentration dependent first order kinetics. This was also associated with visible alignment of the lipid (red) and the DNA (blue) around the GUV with possible pore formation and DNA translocation across the endosomal membrane. When lipid:DNA complexes were added to the late endosomal membrane model (which incorporated a small percentage of anionic lipid), calcein leakage and pore formation on the surface of the GUV membranes were clearly visible. Additionally, and exclusively to this model, however, a high number of GUVs were shown to deform after the addition of the complexes with or without calcein leakage. This was thought to be due to electrostatic interactions between the cationic DDAB and the anionic lipid domains of the endosomal membrane. In conclusion, it is thought that DDAB-DOPE:DNA complexes interact with both early and late endosomal membranes, causing pore formation and DNA translocation across the membrane, however the nature of the interaction changes as the endosomes traffic from early to late stages.

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A79
Characterisation of a cytosolic shuttle based upon ricin toxin

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We have cloned and codon optimised both modified ricin B chain (containing N-terminal 6 His and V5 motifs) and disarmed ricin A chain (containing either a deletion (deleted amino acids 177–183) or mutation (amino acids 177–183 mutated to Gly) within the active site). These molecules were expressed in *Escherichia coli* BL21*DE3 and affinity purified from *E. coli* lysate using Talon affinity resin. Following an initial round of characterisation by SDS PAGE and Coomassie brilliant blue staining, Western blotting (using commercially available anti-ricin A or B chain antibodies as well as antibodies specific for N- and C-terminal epitopes) was successfully used to confirm the production of both species of molecule. Both ricin A and B chains were tested for toxicity against a panel of cell lines either individually, after mixing the A and the B chains, or after re-folding using published protocols. Having ascertained that, relative to wild-type ricin A chain, minimal toxicity was displayed by the disarmed A chain analogues, further controls were undertaken to investigate the character of the recombinant B chain. These studies are reported here and show that the recombinant B chain demonstrates both lectinic activity and the ability to translocate to the Golgi, being localised to GM130 positive structures as depicted by epifluorescence microscopy. Further, crude subcellular fractionation and Western blotting of Vero cells exposed to refolded ricin toxin containing disarmed A chain show the disarmed A chain in the cytosol and the differential sedimentation of the B chain within membrane delimited structures. This data suggests the potential of these materials as cytosolic delivery vehicles suitable for use with gene medicines such as antisense oligonucleotides or RNAi agents.

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