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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),
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,6-diamidino-2-phenylindole (DAPI) was added to highlight the DNA blue. Both endosomal models formed spherical GUVs approximately 10–90 m 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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