cells. Nanoparticles were characterized and some preliminary *in vitro* studies were done, showing that nucleic acids are efficiently condensed with this system, which appears to have a potential use in cancer gene therapy.

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A72
Reduced transgene persistence and trafficking to nuclear periphery are barriers to transfection in lipid substituted nonviral cationic polymer

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Background: Polyethylenimine (PEI) is one of the most sought after cationic polymer for nonviral gene delivery owing to its ability to transfect a variety of cell types efficiently. The amine groups found on the polymer renders high density of cationic charges, which facilitates efficient binding to DNA, while allowing polymer to be derivatized conveniently. Recently, our lab has derived a novel amphiphilic polymer by grafting linoleic acid (LA) to a low molecular weight PEI (2 kDa). The resulting polymer, PEI2k-LA, displayed significant improvement in transfection efficiency in HEK 293T cells over the ineffective, unmodified 2 kDa PEI. However, when PEI2k-LA was used to transfect rat bone marrow stromal cell (rBMCS), low transfection was observed despite 80% of the cells showing polyplex uptake. We aim to further improve PEI2k-LA transfection efficiency in primary cell line by gaining better understanding of its intracellular kinetics in transfection. In this study, we compared the efficiency of polyplexes trafficking to the nuclear periphery with respect to cellular uptake and transgene expression. Polyplexes routing to the nuclear periphery may facilitate passive nuclear uptake of transgene DNA following mitosis, which may increase the probability of transgene expression. *Methods*: A mammalian expression vector encoding the green fluorescent protein is covalently labeled with Cy5 (Mirus Bio Label IT® Tracker). Plasmid DNA labelled using this method maintains transcriptional activity, permitting simultaneous tracking of DNA and transgene expression. Labelled DNAs are complexed with PEI2k-LA or 25 kDa branched PEI (bPEI25k) to transfect tissue cultured rBMSC; cells and nuclei are processed for analysis by flow cytometry at 0.16, 1, 4, and 7 days to assess for DNA uptake and transgene expression. *Results and discussion*: GFP-expression was detected in bPEI25k transfected cells, but not with PEI2k-LA treated cells. PEI2k-LA was able to deliver DNA with similar efficiency as bPEI25k; both carriers delivered DNA to >90% of cells by Day 1. However, the percent of cells with DNA uptake reduced to <50% at an earlier time point with PEI2k-LA than with bPEI25k (~1.7-fold difference between carriers by Day 4). There were significantly fewer nuclei with plasmid DNA associated from PEI2k-LA treated cells than bPEI25k (6% versus 43%, Day 7). Further, the nuclei from PEI2k-LA treated cells had, on average, fewer amounts of DNA associated (~11-fold lower). Taken together, these data suggest that the lack of transfection in rBMSC by PEI2k-LA may be attributed to reduced transgene trafficking to the nuclear periphery and reduced intracellular retention of transgene DNA. Carrier efficiency in transfection may be improved by concurrently enhancing its DNA protective ability and nuclear routing capability.

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A74
Utilising the fluorescent properties of Laurdan to study plasma membrane fluidity in cells treated with the cell penetrating peptide R8

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Despite a large body of research, the mechanism of CPP translocation across biological barriers remains unclear. CPP interactions with membrane lipids have been studied by numerous groups and are hypothesised to be critical determinants for internalisation into cells. The possibility exists that cationic CPPs such as octaarginine (R8) and HIV-TAT, at certain concentrations, affect the phase behaviour of the membrane bilayer [1,2]. This phenomenon may explain our earlier studies with leukaemia cells; R8 freely crosses the plasma membrane at concentrations >5 μM, in cells depleted of cholesterol and also at low temperatures [3]. We therefore determined what effects different temperatures, and cholesterol manipulations had on the fluidity and phase behaviour of the plasma membrane of leukemic KG1a and K562 cells and then compared the data with that obtained from experiments in cells incubated with R8. Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) is a fluorescent membrane probe that possesses different spectral properties depending on the phospholipid phase state of the membrane. Upon passing from the gel phase to the liquid crystalline phase a shift of the emission maxima is observed, from 440 nm to 490 nm and the emission/excitation values obtained can be used to determine membrane fluidity. The results confirm that for both cell lines, over the temperature range of 4–37 °C, the plasma membrane fluidity increased with increasing temperature. Extraction of plasma membrane cholesterol results in an influx of R8-Alexa488 into the cytosol of cells incubated at 37 °C with 2 μM peptide but this effect can be reversed by adding back cholesterol to cholesterol depleted cells. MβCD treatment caused an increase in plasma membrane fluidity but this was unchanged in cells in which had been incubated with MβCD:Chol. Direct plasma membrane translocation of R8-Alexa488 was previously seen in the majority of both KG1a and K562 cells within 10 min of peptide addition (10 μM) while the peptide was restricted to intracellular vesicles at 2 μM thus raising the possibility that translocation at high concentration was the result of peptide induced effects on membrane fluidity. This was however not the case as no effects on membrane fluidity were observed when similar Laurdan measurements were performed in R8 treated cells. Overall the data show that under conditions where direct translocation of R8 is observed, the fluidity of the plasma membrane is unperturbed.

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