Supplementary Information

PR_b functionalized stealth liposomes for targeted gene delivery to metastatic colon cancer

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Sizing and zeta potential of condensed DNA particles

Particles were sized using dynamic light scattering (DLS) (Brookhaven Instruments Corporation), and the data analyzed using the ZETAPALS particle sizing software (Brookhaven Instruments Corporation). Zeta potential was measured using the PALS zeta potential analyzer (Brookhaven Instruments Corporation). Dynamic light scattering data of condensed DNA particle sizes formed at different amine to phosphate (N/P) ratios is presented in Fig. S1 (A, B). The goal of these experiments was to identify the optimal N to P ratio to form small condensed DNA particles that can be efficiently encapsulated into a 200 nm liposome, and have a net

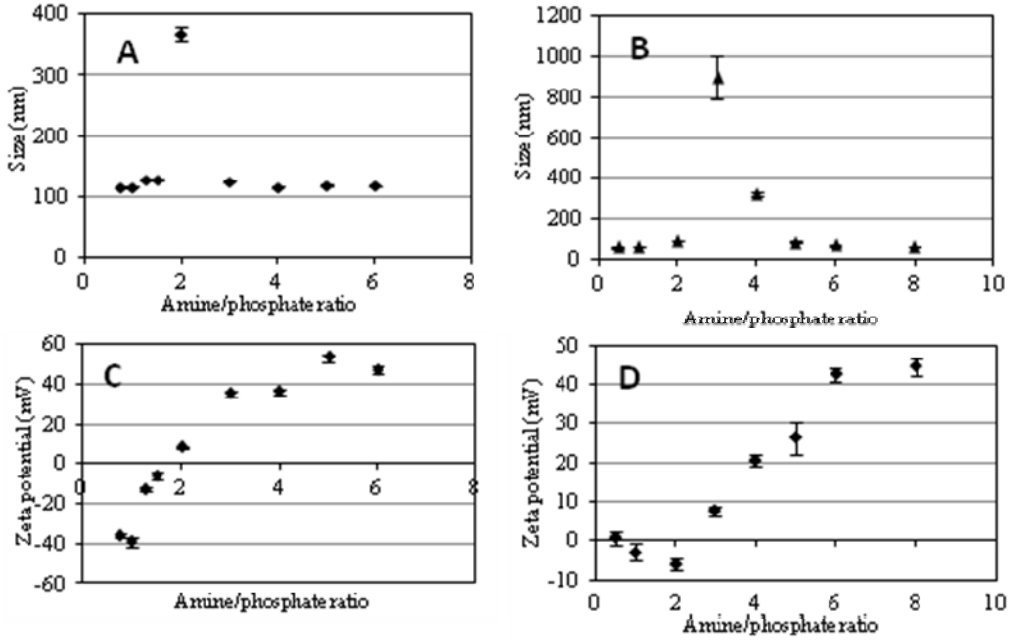

Fig. S1 Characterization of condensed DNA. DLS size measurement of pLL (A) and bPEI (B) condensed DNA particles at different amine to phosphate ratios. Corresponding zeta potential charge measurements for pLL (C) and bPEI (D) condensed DNA particles. Values represent the mean ± SD (n =3).

positive charge for better transfection. pLL condensed DNA particles were approximately 100 nm for all the N/P ratios tested, except around 2, where large visible aggregates were formed resulting in the increased particle size (Fig. S1A). As others have suggested before [1], this aggregation happens at N/P ratios that result in nearly charge neutral particles which experience no repulsion as they come together. This is verified by the zeta potential data presented in Fig. S1C where the net charge of the particles increases from negative to positive as the ratio of the positive polymer is increased and is seen to go through zero around an N/P ratio of 2. Similarly, for the bPEI condensed DNA particles, this aggregation effect was seen around a N/P ratio of 3, with particles sizes less than 100 nm for other ratios away from this aggregation zone (Fig. S1B). The zeta potential of the particles at these same N/P ratios show similar trends to the pLL condensed DNA system, with a net neutral zone occurring around a ratio of 3 (Fig. S1D).Overall the bPEI particles were seen to be smaller than the pLL condensed particles at N/P ratios away from the aggregation ratio. In order to get small positively charged particles to be encapsulated in liposomes, we chose a N/P ratio of 4 for the pLL condensed DNA and a ratio of 8 for the bPEI condensed DNA system.

Toxicity of encapsulated or free bPEI-DNA

DLD-1 cells were seeded overnight in a clear 96 well plate at 5000 cells/well. After a medium change the next morning, cells were treated with 100 ng/well bPEI- DNA encapsulated in PR_b functionalized stealth liposomes. For comparison, unencapsulated bPEI-DNA was delivered at 100 ng/well. Untreated cells were used as the control and media alone was used as the background. After 40 hours of incubation at 37 ºC, 10 µl WST-1 reagent (Clontech, Mountain View, CA) was added to each well and incubated after a gentle shake at 37 ºC for 2 more hours. Following incubation, the absorbance of the plate was read at 450 nm using a Spectramax Plus spectrophotometer (Molecular Devices). Cell viability is reported as the sample absorbance signals and shown as a percentage of the signals from the untreated cells.

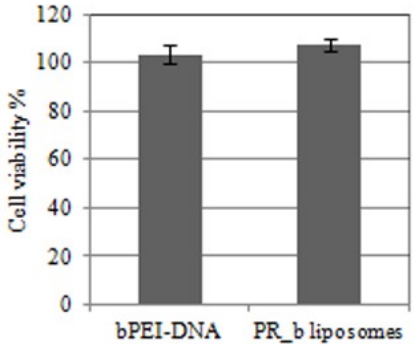

Fig. S2 Effect of delivery of the bPEI-DNA on cell viability. 100% cell viability is representative of untreated cells. DLD-1 cells were treated with PR_b (3.9, 4.1 and 6.1 mol%) functionalized stealth liposomes (5 mol% PEG2000) encapsulating bPEI-DNA or free bPEI-DNA followed by WST-1 treatment and absorbance measurement. The values represent mean ± standard error of three separate liposomal experiments (n =3), each done in replicates of six.

Transfection subjects cells to high stress levels that scale with the amount of genetic material being introduced into the cells [2]. However, at the concentrations of DNA used in our experiments the PR_b functionalized stealth liposomes are able to increase transfection efficiency without adversely affecting cell viability (Fig. S2). Previous studies have shown that the electrostatic method of intracellular DNA uptake may be detrimental to cell viability [2]. For PEI, the stronger the electrostatic attraction between the delivery vehicle and the cells, the higher the transfection level at the expense of cell viability. The non-electrostatic uptake of PR_b functionalized liposomes could be a reason for high cell viability concurrent with high transfection.

References

1. M. Ikonen, L. Murtomaki and K. Kontturi, *Colloids and Surfaces B-Biointerfaces*, 2008, **66**, 77-83.
2. W. T. Godbey, K. K. Wu and A. G. Mikos, *Biomaterials*, 2001, **22**, 471-480.