Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supporting Information

Effects of Spatial Distribution of Nuclear Localization Sequence on Gene Transfection in Catiomer/Gene Polyplexes

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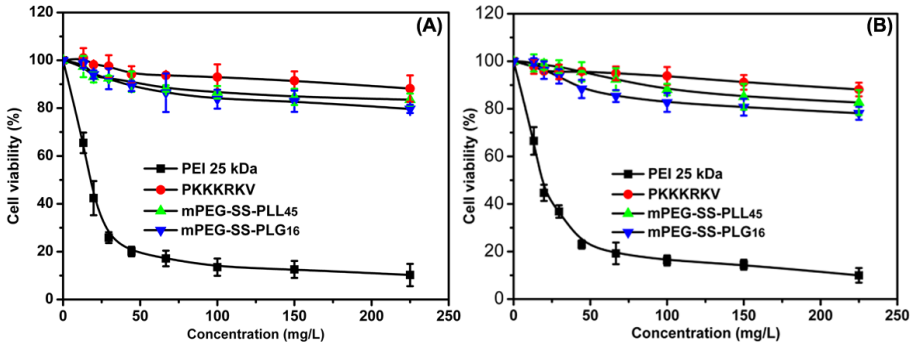

Fig. S1 Dose-dependent cytotoxicity of mPEG-SS-PLL$_{45}$-based catiomers and PKKKRKV. Viability of 293T (A) and HeLa (B) cells following a 24 h incubation with various concentrations of PEI (positive control), PKKKRKV (NLS) or mPEG-SS-PLL$_{45}$-based polymers was quantified using the WST-1 assay. Data are shown as mean ± SD (n = 6).

As shown in this figure, NLS does not exhibit apparent cytotoxicity, throughout the entire concentration range. The cell viability is maintained at a level greater than 90% following 24 h incubation. Cytotoxicity of bPEI-25k is found to be high, and abruptly decreasing to a limiting value around 20% at concentrations > 25 mg/L, attributable to its high cationic charge density.[1] In contrast to bPEI-25k, mPEG-SS-PLG$_{16}$ catiomers exhibits a negligible cytotoxicity like scaffold polymer, mPEG-SS-PLL$_{45}$, and the cellular viability is maintained at 80% even at concentrations of 225 mg/L. These results are in good agreement with our previous reports that owing to the PEG-shielding effect, limited interactions can reduce cytotoxicity,[2] which is a favorable clinical condition for gene delivery applications.

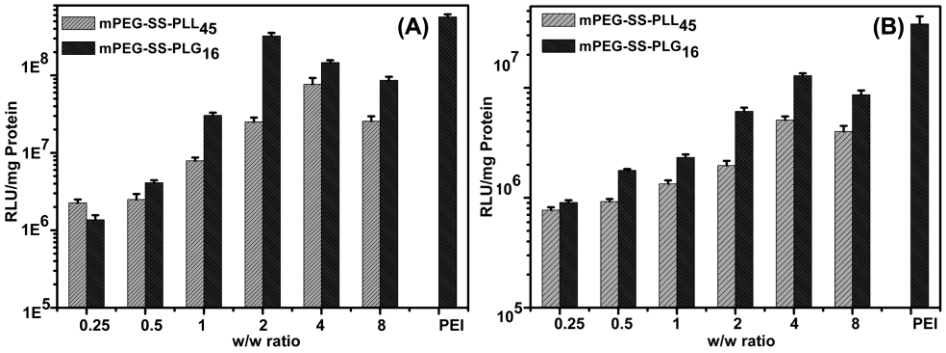

Fig. S2 Transfection efficiency of mPEG-SS-PLG_{16}/pDNA polyplexes. 293T (A) and HeLa (B) cells are incubated for 4 h with mPEG-SS-PLL_{45}/pGL-3 or mPEG-SS-PLG_{16}/pGL-3 polyplexes fabricated at different weight ratios ranging from 0.25:1 to 8:1. PEI/pGL-3 (1.3:1, w/w) is used as control. The luciferase activities of these polyplexes are identified 44 h after transfection by luciferase assay and normalized to viable control cells. Data are shown as mean ± SD (n=5).

Weight ratio and cell type dependence of gene transfection efficiency are shown in this figure. It can be seen that a higher transfection activity is observed in 293T cell lines. The gene expression increases with increasing catiomer concentration and gradually approaches saturation. All polyplexes exhibit the highest gene transfection efficiencies in the range between 2 and 4. It is to be noted that the highest efficiency for mPEG-SS-PLL_{45} is 7.7 x 10^7 RLU/mg protein, while the transfection efficiency of mPEG-SS-PLG_{16} is up to 3.3 x 10^8 RLU/mg protein, approaching the same order of transfection efficacy from bPEI-25k (5.6 x 10^8 RLU/mg protein at weight ratio of 1.3:1). These results indicate highly enhanced biological efficacy of mPEG-SS-PLL_{45} by guanidinylation.

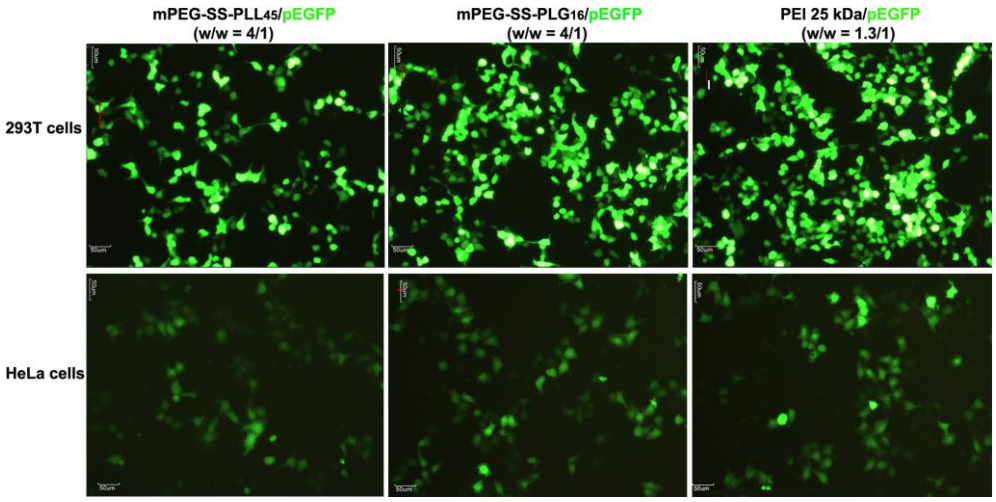

Fig. S3 Transfection efficiency of mPEG-SS-PLG$_{16}$/pDNA polyplexes. 293T and HeLa cells are incubated for 4 h with mPEG-SS-PLL$_{45}$/pEGFP or mPEG-SS-PLG$_{16}$/pEGFP polyplexes fabricated at a weight ratio of 4:1. PEI/pEGFP (1.3:1, w/w) is used as control. pEGFP-positive cells are indentified 44 h after transfection by fluorescence microscopy.

This picture shows the typical fluorescence images of the transfected 293T and HeLa cells. The high density of fluorescent cells visible under the microscope suggests successful delivery of pEGFP into the nucleus of 293T cells after transfection with both mPEG-SS-PLG$_{16}$ and PEI-based polyplexes.

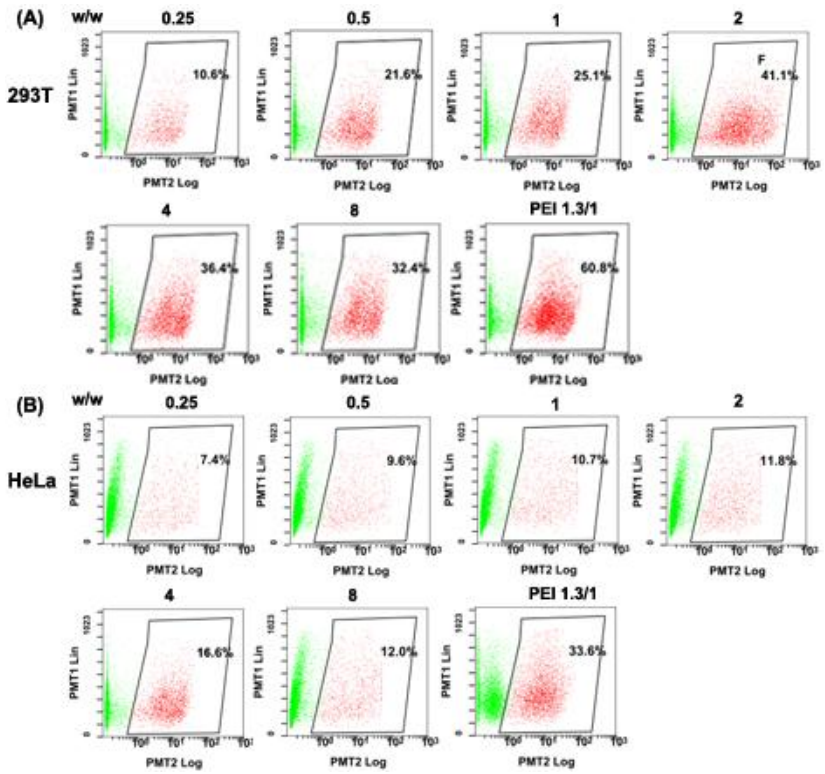

Fig. S4 Transfection efficiency of mPEG-SS-PLG$_{16}$/pDNA polyplexes. 293T (A) and HeLa (B) cells are incubated for 4 h with mPEG-SS-PLL$_{45}$/pEGFP or mPEG-SS-PLG$_{16}$/pEGFP polyplexes fabricated at different catiomer/pDNA weight ratios ranging from 0.25:1 to 8:1. PEI/pEGFP (1.3:1, w/w) was used as control. Viable pEGFP-positive cells were quantified using flow cytometry and normalized to viable control cells. Data are shown as mean ± SD (n = 3).

EGFP expression exhibits a weight ratio and cell type dependence manner. A high transfection activity is observed in 293T cell line, which is in accordance with the luciferase assay results. The calculated transfection efficiency of viable cells in HeLa cells is between 8% and 17% for the novel catiomer, and ~33% for PEI-based polyplexes. In contrast, in 293T cells, the calculated transfection efficiency for the novel catiomer is mainly localized between 20% and 40%. It should be noted that a slight decrease in transfection efficiency with increasing catiomer concentration implies that excess cationic polymer limits pDNA release from the polyplexes, possibly as a consequence of the increased stability of these association polyplexes.[3] Furthermore, the gene transfer ability can also decrease at high zeta potentials due to increased cytotoxicity or activation of some unknown deleterious cellular response.

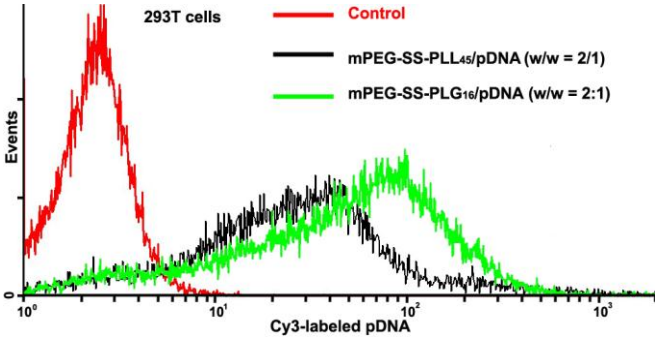

Fig. S5 Cellular uptake result by flow cytometry in 293T cells. Cy3-labeled pEGFP was used. Red peaks: cell only; black peaks: mPEG-SS-PLL$_{45}$/pDNA polyplexes; green peaks: mPEG-SS-PLG$_{16}$/pDNA polyplexes.

mPEG-SS-PLG$_{16}$/pDNA polyplexes exhibit higher cellular uptake compared to that of the mPEG-SS-PLL$_{45}$/pDNA polyplexes. This difference is a direct evidence of guanidinylation being beneficial to enhanced transfection efficiency of mPEG-SS-PLL$_{45}$-based polyplexes.

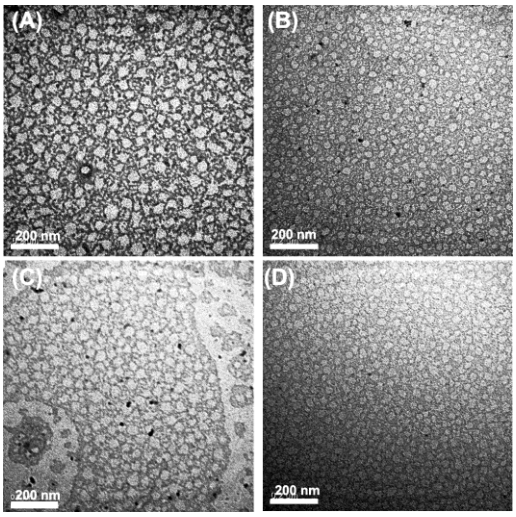

Fig. S6 Representative TEM images of mPEG-SS-PLG_{16}/pDNA polyplexes (w/w, 2:1) (A), NLS-out polyplexes (B), NLS-random polyplexes (C), and NLS-inside polyplexes (D). Ternary polyplexes were prepared at a mPEG-SS-PLG_{16}/pDNA weight ratio of 2:1, NLS/pDNA weight ratio of 16:1.

In this figure, all polyplexes are visible as spherical aggregates with diameters between 40 ± 10 nm. The addition of NLS is found to have no effect on the morphologies of mPEG-SS-PLG_{16}/pDNA polyplexes.

References

1. D. Fischer, T. Bieber, Y. Li, H. Elsasser, T. Kissel, *Pharm. Res.* **1999**, 16, 1273.
2. X. J. Cai, H. Q. Dong, W. J. Xia, H. Y. Wen, X. Q. Li, J. H. Yu, Y. Y. Li, D. L. Shi, *J. Mater. Chem.* **2011**, 21, 14639.
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