Enhanced Gene Expression, Transfection and Reduced Cytotoxicity of Novel Hyaluronic Acid-PEI-Cyclodextrin Polyplexes

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ABSTRACT SUMMARY
The present study explores the potential of novel Hyaluronic acid-PEI-Cyclodextrin (HA-PEI-CyD) polyplexes, as a non-viral vector for site specific gene delivery. The developed polyplexes exhibited excellent stability against DNase I and serum. Subsequently, HA-PEI-CyD exhibited significantly higher transfection efficiency and cell viability (>90%) in comparison with plain PEI and PEI-CyD polyplexes in HeLa, HEK-293 and MCF-7 cell lines. Furthermore, excellent in-vivo gene expression in tumor bearing animals revealed its suitability as gene delivery reagent.

INTRODUCTION
Gene therapy has been evolved as a potential therapeutic strategy for various stubborn human diseases since last few decades. Safety risk and immunogenicity associated with conventional viral vectors resulted in emergence of non-viral vectors as a potential alternatives. Polyethylenimine (PEI) has been recognized as an efficient non-viral vector due to its excellent transfection efficiency. However clinical applicability of this polymer is fraught with its inherent cytotoxicity and proclivity towards aggregation in presence of negatively charged blood components. Herein we explored the potential of novel HA-PEI-CyD polyplexes as non-viral vectors for targeted gene delivery.

EXPERIMENTAL METHODS
Sequential conjugation of CyD and HA with PEI resulted in formation of HA-PEI-CyD conjugate. Polyplexes were formed at different N/P ratios (1, 5, 10, 15 and 20) by simply mixing the varying concentration of HA-PEI-CyD with pDNA (encoded for enhanced green fluorescence protein) and characterized for size, PDI and surface morphology using zeta sizer and SEM respectively. DNase I protection assay was performed by incubating complex at desired weight ratio (Polyplexes/DNA = 1.5) in presence of DNase I (1000 units/mL). Furthermore, serum stability was determined using EtBr intercalation assay. Transfection efficiency and cytotoxicity of developed polyplexes were evaluated on HeLa, HEK-293 and MCF-7 cell lines using CLSM, spectrofluorimeter and MTT assay, respectively. In-vivo gene expression was measured by whole body photon imager in tumor bearing animals following intratumoral injection of polyplexes.

RESULTS AND DISCUSSION
The structure of PEI-CyD and HA-PEI-CyD was confirmed by NMR spectroscopy. An N/P ratio of 10 was found to be optimum for polyplex formation. SEM analysis confirmed the formation of almost spherical polyplexes. Electrophoretic mobility of DNA was maintained in all the polyplexes which, however, was lost in case of naked DNA (Fig. 1) indicating excellent protective potential of polyplexes against DNase I.

![Figure 1: DNase protection assay. kbp represents kilo base pair. Lane 1: pDNA without DNase treatment; lane 2: pDNA incubated with DNase; lane 3-4: PEI and PEI-CyD; lane 5-7: HA-PEI-CyD (1-3)](image)

Developed polyplexes exhibited excellent serum stability as evidenced by significantly lower change in EtBr fluorescence (Fig. 2).

![Figure 2: Stability of different polyplexes in presence of serum](image)
HA modified complexes exhibited significantly higher transfection in comparison with PEI and PEI-CyD in all the cell lines. However, transfection observed in HeLa and HEK-293 cell lines was much higher in comparison with MCF-7 cell lines which might be the consequences of hyaluronate receptors (CD44/RHAMM) expression in HeLa and HEK-293 cell lines in contrast to MCF-7 cells. Amongst all polyplexes HA-PEI-CyD1 exhibited the highest transfection, which was 39.5, 41.5 and 8.8 folds higher as compared to PEI while 9.6, 6.3 and 1.4 folds higher as compared to PEI-CyD in HeLa, HEK-293 and MCF-7 cell lines, respectively. Higher transfection observed in case of HA modified PEI-CyD could be attributed to the enhanced uptake of the polyplexes through receptor mediated endocytosis. (Fig. 3 and Fig. 4).

Figure 3: Confocal micrographs of HeLa, HEK-293 and MCF-7 cells. For each incubation type, the left and right panels represent GFP fluorescence (green) and overly of GFP and DIC (Differential interference contrast image)

Figure 4: Transfection efficiency of PEI, PEI-CyD and HA-PEI-CyD (1-3) polyplexes in different cell lines

In case of PEI, the observed cellular viability (%) was only 25. The viability, however, was markedly increased in case of PEI-CyD (~ 70%) and HA-PEI-CyD (> 90%) polyplexes (Fig. 5) demonstrating improved cytocompatibility of the complexes as compared to PEI.

Amongst all the polyplexes, HA-PEI-CyD exhibited the highest GFP expression level in-vivo demonstrating excellent tumor targeting potential of the synthesized polyplex (Fig. 6).

Figure 5: In-vitro cell viability of HeLa, HEK-293 and MCF-7 cells incubated with various polyplexes (a; in comparison with PEI, b; in comparison with PEI-CyD) (*** p<0.001, * p<0.05)

Figure 6: In-vivo gene expression observed in excised tumor.

**CONCLUSION**

In conclusion we have successfully combined the tumor avidity of HA with high transfection efficiency of PEI to produce the novel tumor targeted non-viral gene delivery vector with enhanced transfection efficiency and reduced toxicity.

**REFERENCES**


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