Optimizing trnsfection of primary human umbilical vein endothelial cells using facial amphipathic deoxycholic acid-conjugated polyethyleneimine

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Introduction: Currently, RNA interference (RNAi)-based gene therapy has been investigated for treating various disease conditions. However, successful application of RNAi including siRNA or miRNA has been limited by several factors *in vitro* and *in vivo*. To overcome these challenges, various non-viral carriers have been developing for efficient RNAi-mediated gene silencing. However, it is necessary to improve the efficacy of these non-viral strategies to achieve desired therapeutic effect. In this study, we carried out synthesis, characterization, and optimization of a polymeric conjugate based on low molecular weight polyethylenimine which was modified by high membrane permeable deoxycholic acid (PEI-DA) for efficient delivery of RNAi-based therapeutics into primary human umbilical vein endothelial cells (HUVECs). **Methods:** Herein, DA-PEI conjugate was synthesized based on DCC/NHS chemistry at various DA to PEI molar ratios of 2 to 4 and used for delivery of a fluorescent labeled siRNA into HUVECs. Conjugates were characterized for chemical structure, size, and cell cytotoxicity. The effect of various parameters including DA/PEI molar ratio, polymer/siRNA weight ratio, and different buffer solutions was investigated on transfection efficacy of conjugates. **Results:** DA was conjugated to the terminal amine groups of the $PEI_{1.8}$ via amide bonds. The polyplexes had smaller sizes (about 130~150 nm) than the parent $PEI_{1.8}$ at different weight ratios. MTS assay revealed that the conjugates were non-toxic at polymer concentrations used in transfection experiments. The higher intracellular uptake and transfection efficiency were achieved by the conjugates synthesized in DA/PEI molar ratio of 3 or 4, polymer/siRNA weight ratio of 5 when they were prepared in salty buffers. **Conclusion:** These results suggest that the DA-PEI1.8 conjugate can be applied as a promising candidate to enhance delivery of RNAi therapeutics into primary endothelial cells under the optimized transfection conditions.

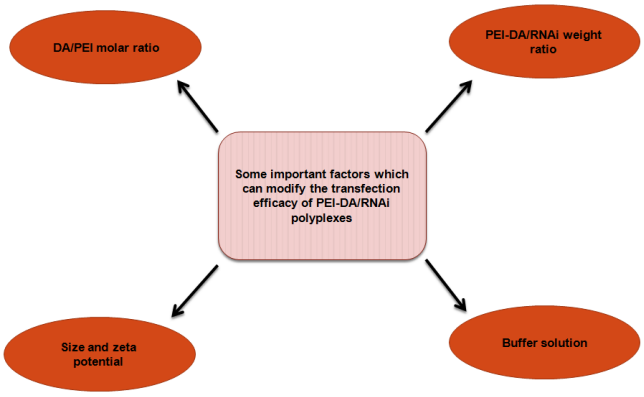


Figure 1: There are many different parameters that can affect intracellular uptake and transfection efficacy of polymeric based carriers such as high membrane permeable DA-conjugated low molecular weight PEI.

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Biography:

Fatemeh Radmanesh is a member of Royan Cardiovascular and Cell Engineering Groups. She studied Biotechnology in her BSc and graduated in MSc of Microbial Biotechnology from Isfahan University. Now, she is PhD student of Medical Biotechnology at Shahid Beheshti University of Medical Sciences. She is interested in nanoparticulate delivery strategies, specially for RNAi-based therapeutics delivery. She joined Royan Institute at 2015 to do her PhD thesis on using polymeric nanoparticles to deliver RNAi therapeutics.

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