

TRACKING CELLULAR UPTAKE OF PLASMID/POLYETHYLENIMINE-CY5 COMPLEXES IN HEK CELLS: INFLUENCE OF PEI MOLECULAR WEIGHT

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Gene therapy is a promising strategy for delivering genetic material into cells to treat various diseases or regulate cellular functions. Among gene delivery approaches, non-viral vectors offer several advantages over viral systems, including lower pathogenicity, reduced cost, and simpler production. Polyethylenimine (PEI) is a synthetic polymer widely used as a model polycation in non-viral gene delivery studies due to its high transfection efficiency, compatibility with serum-free media, and cost-effectiveness. Owing to its high density of protonatable amino groups, PEI carries a strong cationic charge that allows efficient condensation and stabilization of nucleic acids into nanoparticles, commonly known as polyelectrolyte complexes. Despite the extensive use of PEI, a critical gap remains in understanding how the internalization of PEI/nucleic acid complexes relates to endosomal escape and subsequent transfection efficiency. While internalization can be quantified, the relationships between plasmid internalization, intracellular trafficking, and the resulting gene expression are still poorly understood. To address this gap, we examined the relationship between internalization and transfection efficiency of peGFP-C3-based complexes using PEI samples of 0.8, 20, 25, and 60 kDa (both in their native form and conjugated to the fluorophore Cy5-NHS). Our findings demonstrate that the physicochemical properties of PEI/peGFP-C3 complexes, as well as their cellular internalization and transfection efficiency, depend on the molecular weight and chemical structure of PEI. Complexes formed with the PEI 25 kDa sample were smaller than those formed with other molecular weights, whereas complexes made with PEI 0.8 kDa and PEI 20 kDa exhibited lower surface charges. Notably, PEI 25 kDa achieved the highest transfection efficiency in HEK293T cells, despite all PEI samples showed a similarly high percentage of Cy5-positive cells. These results suggest that, although overall cellular uptake was comparable with all PEI molecular weights, the highest transfection efficiency of PEI 25 kDa may be linked to more favorable intracellular trafficking, including most probably an improved endosomal escape. A systematic study exploring different charge ratios ($R = [N]/[P]$) and the kinetics of internalization and trafficking will be performed in future work.

Keywords: Polyplexes, Gene Delivery, Intracellular Trafficking

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