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Nanodevices for Cytosolic Delivery of siRNA

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Extended Abstract

Release from the endo-/lysosomal compartment after endocytosis is an important barrier to efficient cytosolic siRNA delivery with polymeric nanocarriers. Since polymers can be readily modified to address this problem, several approaches which mediate increased endosomal release have been described in the literature and were recently reviewed by us (Merkel and Kissel 2014).

Amphiphilicity of nucleic acid carriers has attracted strong interest. Therefore, three groups of nylon-3 copolymers (poly-β-peptides) possessing different cationic/hydrophobic content were evaluated as siRNA delivery agents in this study. Their ability to condense siRNA was determined in SYBR Gold assays. Cytotoxicity of the polymer/siRNA polyplexes was tested by MTT assays in a cell line stably expressing luciferase (H1299/Luc). AlexaFluor-488 labeled siRNA was used to quantify cellular uptake in presence and absence of uptake inhibitors, and transfection efficacies of siRNA formulations were studied by luciferase knockdown. Endosomal release was determined by confocal laser scanning microscopy after staining the treated cells with a lysotracker.

All polymers efficiently condensed siRNA at nitrogen to phosphate (N/P) ratios of 2 or 5 and formed polyplexes with smaller hydrodynamic diameters compared to N/P 1. Although several formulations had negative zeta potentials at N/P 1, G2C and G2D polyplexes yielded >80% uptake in H1299/Luc cells according to flow cytometry. Luciferase knockdown (20-65%) was observed after transfection with polyplexes made of high molecular weight polymers with the highest proportion of hydrophobic subunits. The ability of nylon-3 polymers to deliver siRNA intracellularly even at negative zeta potential implies that they mediate transport across cell membranes based on their amphiphilicity. The cellular uptake route was determined to strongly depend on the presence of cholesterol in the cell membrane. These polymers are therefore very promising for siRNA delivery at reduced surface charge and toxicity.

Our study identified nylon-3 formulations at low N/P ratios for effective gene knockdown and implied nylon-3 polymers as a new type of promising gene delivery agents.

Reference

Merkel, O. M. and T. Kissel (2014). "Quo vadis polyplex?" J Control Release, in press.