ABSTRACT

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Gene therapy is a promising field offering potential in several currently incurable diseases. Gene therapy is mediated by modulation of gene expression in specific cells by delivering exogenous nucleic acids. One of current tasks of nucleic acid delivery is exploring several synthetic vectors which would have a potential to overcome the disadvantages of commonly used viral vectors. The present study focused on different types of polyethyleneimine-based nanoparticles for plasmid DNA (pDNA) and small interfering RNA (siRNA) delivery.

Integration of imaging contrast agents with gene delivery vehicles is advantageous for tracking the gene delivery process both *in vivo* and *in vitro*. Gadolinium based contrast agents (e.g. Gadoteric acid) have shown potential for magnetic resonance imaging (MRI) applications. However, conjugation of gadoteric acid to polyethyleneimine may affect its ability for transfection. Towards this goal, polyplexes based on linear polyethylenimine (LPEI) labelled with gadoteric acid (LPEI-DOTA-Gd) were tested for evaluation of pDNA transfection efficiency. The transfection efficiency, studied in A549 and CT26 cells and determined by Firefly luciferase reporter gene assay, showed that conjugation of Gadoteric acid did not cause any negative effect on LPEI transfection ability in comparison with unlabeled LPEI.

In another part of this work, polyplexes based on different types of polyethylenimine, i.e. linear (LPEI), branched (BPEI) and disulfide crosslinked (c-LPEI), were tested for siRNA delivery with the aim to find optimal polyplex- and incubation parameters for efficient siRNA delivery, resulting in knockdown of the targeted gene (gene for Firefly luciferase in this case). The efficiency of siRNA delivery was investigated on cells stably expressing Firefly luciferase gene and estimated based on decrease in the luciferase activity as determined by Firefly luciferase reporter gene assay. BPEI-based polyplexes were the most efficient in siRNA delivery resulting in knockdown, enabling us also to define optimal concentration and some polyplex and incubation parameters.

Last type of nanoparticles tested for siRNA delivery were Layer-by-Layer assembled gold nanoparticles composed of different layers including BPEI or c-LPEI. Pilot *in vitro* testing of these nanoparticles, also evaluated by Firefly luciferase reporter gene assay, resulted in specifying of range of concentrations in terms of toxicity and potential knockdown efficiency for future experiments.