Polyethylenimine

Polyethylenimine (PEI) or polyaziridine is a polymer with repeating unit composed of the amine group and two carbon aliphatic CH2CH2 spacer. Linear polyethyleneimines contain all secondary amines, in contrast to branched PEIs which contain primary, secondary and tertiary amino groups. Totally branched, dendrimeric forms were also reported.

PEI is produced on industrial scale and finds many applications usually derived from its polycationic character.

Gene delivery holds therapeutic promise for the treatment of neurological diseases and spinal cord injury. Although several studies have investigated the use of non-viral vectors, such as polyethylenimine (PEI), their clinical value is limited by their cytotoxicity.

The delivery of nucleic acids such as DNA or siRNA still represents a major hurdle, especially with regard to possible therapeutic applications in vivo. Much attention has been focused on the development of non-viral gene delivery vectors, including liposomes or cationic polymers. Among them, polyethylenimines (PEIs) have been widely explored for the delivery of nucleic acids and show promising results. The combination of cationic polymers and liposomes (lipopolyplexes) for gene delivery may further improve their efficacy and biocompatibility, by combining the favourable properties of lipid systems (high stability, efficient cellular uptake, low cytotoxicity) and PEIs (nucleic acid condensation, facilitated endosomal release).

In a study, Ewe et al., systematically analyse various conditions for the preparation of liposome-polyethylenimine-based lipopolyplexes with regard to biological activity (DNA transfection efficacy, siRNA knockdown efficacy) and physicochemical properties (size, zeta potential, stability). This includes the exploration of lipopolyplex compositions containing different liposomes and different relevant branched or linear low-molecular-weight PEIs. We establish optimal parameters for lipopolyplex generation, based on various PEIs, N/P ratios, lipids, lipid/PEI ratios and preparation conditions. Importantly, we also demonstrate that certain lipopolyplexes retain their biological activity and physicochemical integrity upon prolonged storage, even at 37°C and/or in the presence of serum, thus providing formulations with considerably higher stability as compared to polyplexes. In they established optimal liposome-polyethylenimine lipopolyplexes that allow storage under ambient conditions. This is the basis and an essential prerequisite for novel, promising and easy-to-handle formulations for possible therapeutic applications [1)].

Ewe et al., published the first study to explore polyethylenimine-based lipopolyplexes comprising a low-molecular weight PEI and the phospholipid DPPC for therapeutic siRNA use. Lipopolyplex structures are analyzed by electron microscopy. Biological efficacies are demonstrated in vitro by cellular uptake, knockdown of the target oncogene survivin, and concomitant cell growth inhibition. Upon systemic administration in tumor-bearing mice, here performed by intraperitoneal (i.p.) injection, radioactive biodistribution assays show lipopolyplex-mediated delivery of intact siRNAs. Absence of blood serum parameter alterations, erythrocyte aggregation or immunostimulation, and the observation of animal well-being and stable body weight confirm biocompatibility. Exploring therapeutic efficacies in a preclinical model, a considerable inhibition of prostate carcinoma xenograft growth is achieved, paralleled by an ~65% survivin knockdown in the tumors. They, thus, demonstrate that PEI-based lipopolyplexes represent an efficient platform for therapeutic use of small RNAs [2)].

Ediriwickrema et al., synthesized a multi-layered polymer nanoparticle (MLNP), comprising of poly(lactic-co-glycolic acid) with surface polyethyleneimine and functional peptides, for targeted drug and gene delivery. They confirmed the particle's ability to inhibit tumor growth through synergistic action of the drug and gene product. MLNPs achieved transfection levels similar to lipofectamine, while maintaining minimal cytotoxicity. The particles delivered camptothecin (CPT), and plasmid encoding TNF related apoptosis inducing ligand (pTRAIL) (CT MLNPs), and synergistically inhibited growth of multiple cancer cells in vitro. The synergy of co-delivering CPT and pTRAIL via CT MLNPs was confirmed using the Chou-Talalay method: the combination index (CI) values at 50% inhibition ranged between 0.31 and 0.53 for all cell lines. Further, co-delivery with MLNPs resulted in a 3.1-15 fold reduction in CPT and 4.7-8.0 fold reduction in pTRAIL dosing. CT MLNPs obtained significant HCT116 growth inhibition in vivo compared to monotherapy. These results support our hypothesis that MLNPs can deliver both small molecules and genetic agents towards synergistically inhibiting tumor growth [3].

Polyethylenimine (PEI) is an effective vehicle for in vivo gene delivery in many tissues including brain. PEI mediates transgene expression in brain neurons and glia. To investigate whether PEI-mediated nerve growth factor (NGF) gene transfer protected axotomized septal cholinergic neurons, Wu et al., injected linear PEI (in vivo jetPEI, Qbiogene) complexed with a plasmid encoding for mouse NGF (PEI/pNGF-W) into the rat septum. PEI complexed with a plasmid encoding for green fluorescent protein (PEI/pGFP) was used as the control. PEI-mediated gene expression was predominantly neuronal. Fimbria-fornix transections (FFTs), conducted 1 day after rats were injected with control vector, resulted in a 70% loss of septal cholinergic neurons. In contrast, PEI/pNGF-W injection prior to FFTs attenuated the loss of septal cholinergic neurons. This is the first study, that shows the neuroprotective effects induced by PEI-mediated trophic factor gene transfer in brain [4].

Platinum coils were prepared by successive deposition of cationic polyethyleneimine and anionic heparin, and VEGF was immobilized through affinity interaction with heparin. Unmodified, heparin-coated, or rhVEGF-immobilized platinum coil segments were inserted into the ligated external carotid arteries at the bifurcation of the common carotid artery (CCA) of adult female rats. The bifurcation segments of the CCA were harvested 2 weeks after the coil placement. rhVEGF-immobilized coils showed significantly greater endothelial formation at the aneurysm orifice and cell infiltration in the aneurysm body compared with the unmodified and heparin-coated coils. The percentage of sac occlusion was significantly greater in the rhVEGF-immobilized group (77.53 +/- 27.58%) than in the heparin-coated group (44.81 +/- 38.30%) and unmodified group (34.99 +/- 28.15%). Scanning electron microscopy showed a tendency for more fibrotic and cellular collections on the coil surface and more tissue mass filling in the coil lumen in the rhVEGF-immobilized group. Platinum microcoils coated with immobilized rhVEGF may be effective for the obliteration of aneurysms [5].

Axon regeneration

Nucleic acid-based therapy is a promising strategy to deliver bioactive molecules capable of promoting axon regeneration. Branched polyethylenimine (bPEI: 25kDa) is one of the most widely studied nonviral vectors, but its clinical application has been limited due to its cytotoxicity and low

transfection efficiency in the presence of serum proteins.

In a study, Gwak et al., synthesized cationic amphiphilic copolymers, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP), by grafting low molecular weight PLGA (4kDa) to bPEI (25kDa) at approximately a 3:1 ratio as an efficient nonviral vector.

They show that PgP micelle is capable of efficiently transfecting plasmid DNA (pDNA) and siRNA in the presence of 10% serum in neuroglioma (C6) cells, neuroblastoma (B35) cells, and primary E8 chick forebrain neurons (CFN) with pDNA transfection efficiencies of 58.8%, 75.1%, and 8.1%, respectively. They also show that PgP provides high-level transgene expression in the rat spinal cord in vivo that is substantially greater than that attained with bPEI. The combination of improved transfection and reduced cytotoxicity in vitro in the presence of serum and in vivo transfection of neural cells relative to conventional bPEI suggests that PgP may be a promising nonviral vector for therapeutic nucleic acid delivery for neural regeneration.

Gwak et al., report cationic amphiphilic copolymers, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP) that are capable of efficiently transfecting reporter genes and siRNA both in the presence of 10% serum in vitro and in the rat spinal cord in vivo. The combination of improved transfection and reduced cytotoxicity in the presence of serum as well as transfection of neural cells in vivo suggests PgP may be a promising nucleic acid carrier for CNS gene delivery [6].

In a study, Gwak et al., evaluated the ability of PgP to deliver siRNA targeting RhoA, a critical signaling pathway activated by multiple extracellular inhibitors of Axon regeneration. After generation of rat compression SCI model, PgP/siRhoA polyplexes were locally injected into the lesion site. Relative to untreated injury only, PgP/siRhoA polyplexes significantly reduced RhoA mRNA and protein expression for up to 4 weeks post-injury. Histological analysis at 4 weeks post-injury showed that RhoA knockdown was accompanied by reduced apoptosis, cavity size, and astrogliosis and increased Axon regeneration within the lesion site. These studies demonstrate that PgP is an efficient non-viral delivery carrier for therapeutic siRhoA to the injured spinal cord and may be a promising platform for the development of combinatorial TNA/drug therapy [7].

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