Biochemical Genetics (2023) 62:18–39
https://doi.org/10.1007/s10528-023-10416-7

METHODOLOGY ARTICLE

Revisiting of Properties and Modified Polyethylenimine-Based Cancer Gene Delivery Systems

Nejad Mohammadi[1,2] · Nashmin Fayazi Hosseini[3] · Hossein Nemati[4] · Hemen Moradi-Sardareh[5,6] · Mohsen Nabi-Afjadi[7] · Gholam Ali Kardar[1,2]

Received: 23 April 2023 / Accepted: 6 June 2023 / Published online: 2 July 2023

Abstract

A new era of medical technology in cancer treatment is a directly specific modification of gene expression in tumor cells by nucleic acid delivery. Currently, the main challenge to achieving this goal is to find a non-toxic, safe, and effective strategy for gene transfer to cancer cells. Synthetic composites based on cationic polymers have historically been favored in bioengineering due to their ability to mimic bimolecular structures. Among them, polyethylenimines (PEIs) with superior properties such as a wide range of molecular weight and a flexible structure may propel the development of functional combinations in the biomedical and biomaterial fields. Here, in this review, we will focus on the recent progressions in the formulation optimization of PEI-based polyplex in gene delivery to treat cancer. Also, the effect of PEI's intrinsic characteristics such as structure, molecular weight, and positive charges which influence the gene delivery efficiency will be discussed.

✉ Mohsen Nabi-Afjadi
mohsennabi66@gmail.com

✉ Gholam Ali Kardar
gakardar@tums.ac.ir

1 Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

2 Immunology Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

3 Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

4 Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

5 Asadabad School of Medical Sciences, Asadabad, Iran

6 BioMad As Company, Oslo, Norway

7 Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Keywords Polyethylenimines (PEIs) · Polyplexe · Gene therapy · Cancer

Abbreviations

CPAM	Cationic polyacrylamides
CMC	Cell membrane complex
CPPS	Cell-penetrating peptides
CRT	Chimeric antigen receptor
EGF	Epidermal growth factor
GA	Glycyrrhetinic acid
GL	Glycyrrhizic acid
HA	Hyaluronic acid
PEG	Polyethylene
LPEIS	Linear Peis
KH	Lysine—Histidine
MnO2	Manganese dioxide
NLS	Nuclear localization signal peptide
EPM	PEI matrix
PDNA	Plasmid DNA
PTRAIL	Plasmid tumor necrosis factor-related apoptosis-inducing ligand
PDDA	Poly(dimethyldiallylammonium chloride)
PAE	Polyamide epichiorobydrin
PEI	Polyethyleneimine

Introduction

Oligonucleotide-based therapy is a promising novel approach to overcome some limitations of traditional cancer treatment methods (Dunbar et al. 2018). Since cancer remains one of the leading cause of death worldwide, discovering non-toxic and efficient treatment options have attracted a lot of attention (Zaimy et al. 2017; Shi et al. 2022). Today's advances in cancer gene therapy have led to several approved products on the global market for therapeutic usages including oncolytic viruses and Chimeric antigen receptor (CRT) t-cells as ex vivo genetically modified cell therapeutics (Dunbar et al. 2018; Zaimy et al. 2017). However, specific modification of gene expression in tumor cells is the ambition of nucleic acid therapies whether by delivering anti-tumor and immune-stimulatory genes with plasmid DNA (pDNA) or downregulation of gene expression-promoting cancer progression by degradation of messenger RNA. In general, there are three approaches to gene transfer, namely viral carriers, non-viral carriers, and methods based on physical means (Zaimy et al. 2017). Although viral vectors are significantly more efficient, their use has been challenged because of a high degree of manufacturing complexity, high costs, the limited size of loading genes, and safety concerns (Dunbar et al. 2018).

Physical methods of gene delivery such as microinjection, electroporation, needle injection, and gene gun, which are highly efficient in transferring genes through the passage of extracellular and intracellular barriers, have limited use due to low cell

viability and the cell senescence risk (Anguela and High 2019). Therefore, there has always been an attempt to find new non-viral strategies based on natural and synthetic substances to replace them (Anguela and High 2019; Mani et al. 2023). Non-viral nucleic acid vectors which have been frequently investigated in recent years are lipid/polymeric-based systems and inorganic materials-based systems (Dunbar et al. 2018).

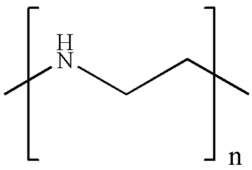
Polymers that are large compounds with numerous repeated units, can be broadly categorized into natural (such as chitosan and peptides) or synthetic polymers (such as polyethyleneimine (PEI)) (Freund 2014; Karami Fath et al. 2022a, 2022b). It has been a long time since polymeric materials act as a strong driving force in various industries (Chen et al. 2020). The outstanding and flexible mechanical properties of polymer materials have led to their wide use in the design and manufacture of biomedical products in various fields such as artificial organs, implants, dental materials, and drug delivery (Mani et al. 2023).

Polymeric micelles, with a core–shell structure, consisting of self-assembly of amphiphilic copolymers, are considered potential non-viral gene carriers instead of viral vectors (Dunbar et al. 2018). Cationic polymers are the most commonly used type of polymers for nucleic acid delivery (Anguela and High 2019). Polyplexes refer to overall positive charge complexes formed by strong electrostatic interactions between cationic polymers and polyanionic nucleic acids across cell membranes without damaging the cells and delivering genetic material to the *target site both* cell cytoplasm or nucleus (Chen et al. 2020*).* Polybrene, polylysine, cationic polyacrylamides (CPAM), poly(dimethyldiallylammonium chloride) (PDDA), polyamide epichlorohydrin (PAE), chitosan, and polyethyleneimines (PEIs) are common polycations that have frequently been applied in pharmaceutical and biological studies (Wang et al. 2020; Roca et al. 2022; Karpisheh et al. 2021). Outside the medical area, cationic polymers, as chelating agents to target heavy metals widely applied in plentiful applications including the paper industry, wastewater treatment, (Jahan and Zhang 2021) and petrochemical (Zhao et al. 2019). PEI-based polyplexes are well-known cationic polymers that are studied for in vitro and in vivo delivery of DNA, siRNA, and mRNA in different diseases (Adachi et al. 2021). In this review, we will review the properties of PEI and some of its formulation optimization for cancer gene therapy (Scheme 1).

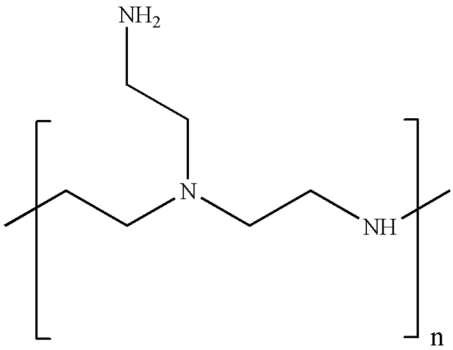
PEIs: Structure and Properties

PEIs are a well-defined group of the most extended and vastly developed polyplex (Höbel and Aigner 2010). As a cationic polymer, PEIs were introduced as the gold standard for non-viral gene transfection with high-gene transfection efficiency (Wu et al. 2019). In addition, gene delivery, the attractive structural features of PEIs, and their physical and chemical properties are the reasons for their wide uses in other industries such as the paper industry and water purification (Virgen-Ortiz et al. 2017; Shen et al. 2017). Today, one of the broad applications of PEI is a functional coating due to its strong tendency to neutralize and complex with negative and metal ions (Zou et al. 2019). Having high compatibility and processability on different

Scheme 1 Schematic diagram of the chemical structures of linear and branched PEI



Linear PEI



Branched PEI

substrates such as carbon, metals, resins, or textiles, PEIs have expanded their use in making highly innovative products (Wu et al. 2019). In 1995 by Boussif et al., PEI was the first reported as a non-viral cell transfection reagent that can be considered the first attempt to use polymer in vivo (Vermeulen et al. 2018). In recent years, PEI and modified PEI-derived biomaterials with different sizes and molecular weights, branching degrees, ionic strengths, and different zeta potentials have been used in gene delivery (Höbel and Aigner 2010).

PEIs are made of repeating units of amine groups and two aliphatic carbons. It has been investigated that the physicochemical, and also biological, properties of PEI-based construct are closely related to the level of polymerization, branching as well as charge density (Xun et al. 2018). For instance, PEI's high aqueous solubility depends on the rate of repeating ethylimine units (Singh et al. 2018). Based on their topology, PEIs are divided into two main types including linear and branched (Fig. 1) (Xu et al. 2018). The polymer synthesis method determines the ratio of different amino groups in the final structure which affects the physicochemical properties (Xun et al. 2018). Linear PEIs (LPEIs) have only primary and secondary amine groups in the terminal and the polymer backbone. The LPEIs' melting point is about 73–75 °C with the solid state at room temperature. On the other hand, branched PEIs (BPEIs) due to having three different types of amino groups (primary, secondary, and tertiary) are viscous liquids, without regard to their molecular weight (Zou et al. 2019). Despite LPEI homopolymers which are prepared by cationic ring-opening polymerization of cyclic imino-ethers followed by acid- or base-catalyzed

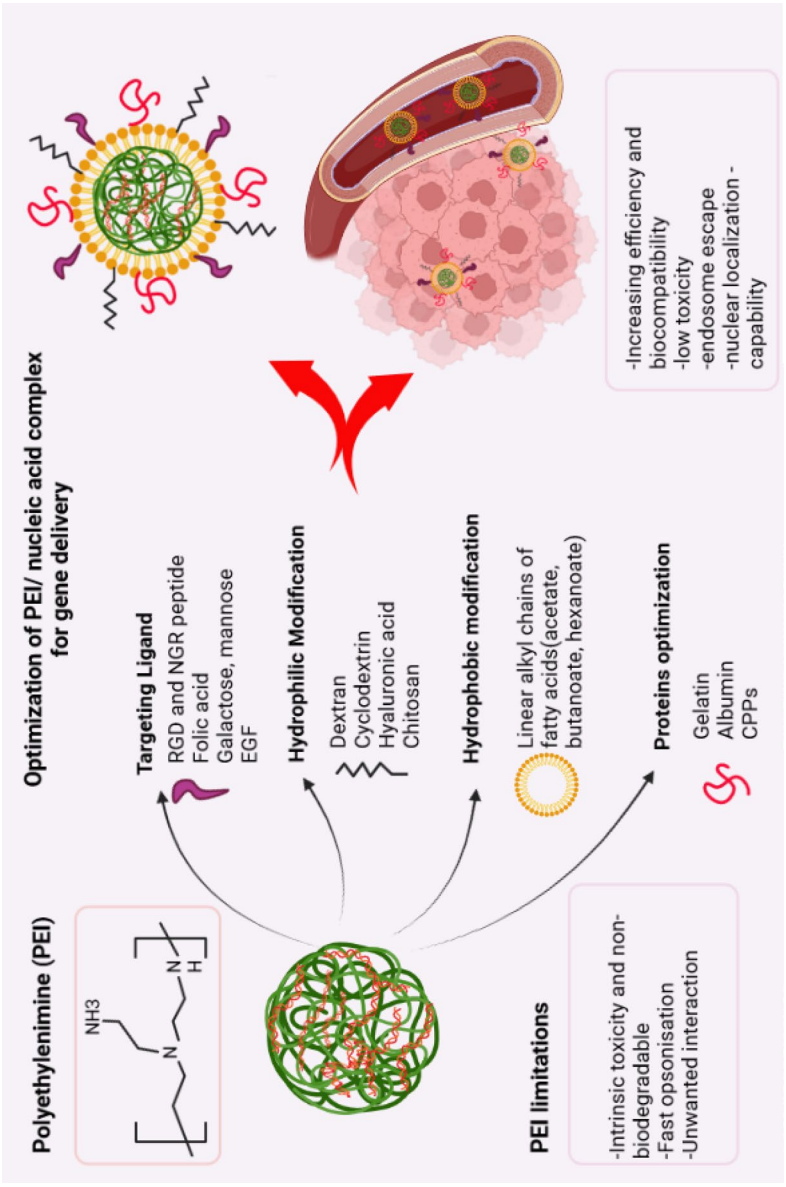


Fig. 1 An overview of the optimization of the PEI/nucleic acid complex for gene delivery

hydrolysis, BPEI can be prepared by chemical polymerization of ethyleneimine in the presence of both hydrochloric acid and sulfuric acid (Zhang et al. 2022). In addition, using different catalysts for the synthesis of broad molecular weight of BPEI (300–750,000 Da), and conducting an intramolecular dehydration reaction of ethanolamine with metal-based catalysts to prepare purified products on an industrial scale are other BPEI synthesis methods (Wang et al. 2020; Zou et al. 2019). Although both linear and branched structures are used for gene transfer, by comparison, the transfection efficiency of the liner PEI/DNA complex is much higher than the branched PEI/DNA complex, which is probably due to the large size of the liner PEI/DNA complex. This is because these large complexes precipitate more than small branched PEI/DNA complexes interacting more with the cell surface(Wang et al. 2020).

PEIs: as a Gene Carrier

In general, non-viral vectors based on cationic materials consider a great candidate for gene delivery in favor of their capacity to self-assembling with the DNA, transferring the high amount of nucleic acid and scalable production (Pandey and Sawant 2016). Among cationic polymers, PEIs complex with the nucleic acid molecules via electrostatic interactions and simplify their cytosolic delivery to the target cells via their positive core–shell structure (Xun et al. 2018). In addition to having high cationic charge density, other unique properties such as dynamic structure and flexible branching chains promote their widespread use for gene delivery (Wang et al. 2020). However, PEI is one of the most popular cationic polymers that have been extensively explored as a gene carrier for in vitro and in vivo applications, its toxicity, and biocompatibility are concerns for using it in gene therapy protocols (Li et al. 2022). Nevertheless, improving the practical properties that affected the biocompatibility and biodegradability of PEIs can be remedied by their precise modifications. In the PEI structure, having three types of amine groups including primary, secondary, and tertiary allow conjugate with functional groups such as hyaluronic acid, dextran, and polyethylene glycol to remove their toxicity (Park et al. 2022). Even they can be targeted to specific organs and cellular sites through targeting ligand attachment (Masoumzadeh 2021). Branched PEI with a molecular weight of 25 kDa (PEI25k) has been considered one of the gold standards of polymer-based gene vectors (Masoumzadeh 2021). These manipulations have led to the rise of several available commercial PEI-based polymers to use for gene delivery such as ExGen500 (Jahan and Zhang 2021) and jetPEI (Zhao et al. 2019). Meleshko and colleagues combined pDNA with linear PEI at the low molecular weight (8 kDa) to deliver vaccines. This marks the initial use of PEI as a carrier for an idiotypic DNA vaccine in human phase I clinical trials, which has been sanctioned by the regional regulatory authorities of the State Committee on Science and Technology of the Republic of Belarus (Meleshko et al. 2017). Table 1 presents the details of the clinical studies of PEI-based gene delivery systems for treating different cancer.

In general, the functional mechanism of PEIs, as gene transfer agents, lies in their chemical structure. For example, the charge density and molecular weight of PEI

Table 1 Clinical studies of PEI-based cancer nucleic acids delivery

NCT number	Study status	Cancer type	Interventions	Phase	Participation number	PEI type
NCT03739138	Terminated	Advanced solid tumors	MK-4621 and pembrolizumab	1	30	Linear (JetPEI™)
NCT01007240	Unknown	Carcinoma of head and neck	Silicon-quaternary ammonium	1	10	Linear PEI
NCT04049864	Recruiting	Relapsed neuroblastoma	DNA-Lenalidomide	1	12	Linear PEI
NCT04160065	Recruiting	Merkel cell carcinoma, cutaneous squamous cell carcinoma, non-melanoma skin cancers	IFx-Hu2.0	1	20	Linear PEI (jetPEI)
NCT04925713	Completed	Skin cancer	IFx-Hu2.0	1	5	Linear PEI (jetPEI)
NCT01274455	Completed	Pancreatic ductal adenocarcinoma	Gene therapy product CYL-02 = plasmid DNA	1	22	Linear PEI
NCT02806687	Active NOT_Recruiting	Pancreatic adenocarcinoma	Gene therapy product CYL-02 and gemcitabine	2	68	Linear PEI

crucially influence the properties and structure of PEIs used as gene carriers and affect both transfection efficiency and blood circulation time (Zakeri et al. 2018). Numerous primary and secondary amine groups on PEI reach their pKa value of nearly 11 which leads to playing as an alkaline polymer electrolyte in aqueous solutions (Wang et al. 2020). The mechanism is an ionic interaction between the high cationic charge of PEI and the anionic charge of DNA, which condenses DNA strands into nanosized particles and delivers targeted genetic material across the nucleus (Saeed et al. 2022). In fact, PEIs strong positive surface enhance the interaction with the negatively charged cell membrane facilitating endocytosis-mediated absorption and also increasing the stability of gene by protecting them against enzymatic degradation (Kriplani and Guarve 2022). In addition, the strong buffering capacity of PEI (buffering range from 5.1 to 7.4), which is directly related to the number and types of amino groups on the PEIs chain, enables it to bind a large number of protons (Wang et al. 2020). So, PEIs act as a "proton sponge" effect and lead to increase transport of protons into lysosomes (Faizullin et al. 2022). Increased influx of protons by V-ATPase pump leads to osmotic swelling and rupture of the endocytic vesicle that releases genetic material into the cytosol and evades from nuclease digestive enzymes (Kriplani and Guarve 2022). So, through inhibition of the lysosomal lysis, the buffering capacity of PEIs increases their transfection efficiency (Vermeulen et al. 2018). In summary, unique properties of PEI including high nucleic acid condensing ability, proton sponge effect, escape from endosomes, and nuclear localization capability led to the emergence of PEIs-based gene delivery systems to condense fragile biological materials into nano-scale particles in order to transport them in a more stable and integrated structure (Wang et al. 2020; Masoumzadeh 2021).

Although numerous inherited properties have given unique advantages to PEI. Some of these can be an obstacle to its application in biomaterials engineering (Adachi et al. 2021). For example, PEI faces the quandary toxicity related to high molecular weight, as well as its excessive cationic activity leading to unwanted interactions with the cell membrane and blood components, and immune response which follows the fast opsonization and low half-life of the polyplex (Long et al. 2017). Furthermore, the high cationic charge of PEI affects the size scale of polyplex (Wu et al. 2021).

In fact, the composition and degree of lining or branching structure molecular weight, size, and zeta potential of polyplexes are the main factors that identified the transfection ability of PEI-based delivery systems (Gao et al. 2015). The gold standard of non-viral gene delivery (25 kDa branched PEI) has high transfection efficiency and cytotoxicity at the same time (Wu et al. 2021). PEI with low molecular weight ($MW < 2000$) is an alternative with less toxicity, but due to low transfection efficiency, gene vectors are not fulfilled (Xun et al. 2018).

The main limiting factor of PEIs for gene transfer is their cytotoxicity by nature, which is directly related to the molecular weight, surface charge, and branched or liner structure of PEIs (Wang et al. 2022). Several studies have shown that lower molecular weight significantly reduces the level of cytotoxicity compared to higher molecular weight constructs (Cheraghi et al. 2017). A study showed that the PEI/nucleic acid complex is far less toxic than free PEI (Babaei et al. 2017). Also,

liposomal membrane degradation, and most likely cell membrane, were reported to be more severe by bPEI than lPEI (Xu et al. 2019). Comparing the cytotoxicity of the same concentrations of PE I800Da, PEI 25kD, and linear PEI 20kD showed branched PEIs are the most toxic group and the main reason is the high density of methylene charge in the branched PEIs structure (Freund 2014). Apoptotic-mediated cell death after intracellular stress and mitochondrial changes, as a result of the swelling and rupture of the endosome containing the polymer, is introduced as the main cell death pathway via PEIs (Mulenos et al. 2020). Also, mitochondrial depolarization, which activates caspase 9, was introduced as another pathway of PEI polyplex-induced cytotoxicity (Vaidyanathan et al. 2016). Also, a previous study showed that the toxicity of PEI can be up to necrosis depending on its molecular weight. When PEI is used as a robust mucosal adjuvant, it stimulates the production of pro-inflammatory cytokine and humoral immune responses (Monnery et al. 2017). Also, some adverse cellular responses such as stress response, oncogenic mutations, and apoptosis or necrosis have been reported when PEI is used in biomedicine or implants (Xun et al. 2018). Another study reported that high molecular weight and dispensable charged groups of PEIs make them collected in cells and lead to cell cytotoxicity (Monnery et al. 2017). Despite PEI being proven as an effective gene delivery system based on cationic polymers, it is not biodegradable and cannot be destroyed through biological catalysis (Wang et al. 2022). The reason for the critical importance of biodegradability in gene therapy is the need for frequent administration, as long-term exposure leads to accumulation and toxicity (Monnery et al. 2017). Another study showed that having a large number of positive charges greatly increases the possibility of unwanted connections and their aggregation with negatively charged molecules, thus, reducing the transfer efficiency (Berg et al. 2021). Also, non-specific interaction of the abundant cationic charges of PEIs can cause some undesirable results such as bio-aggregation (Wang et al. 2022). According to what aforementioned, although PEIs are considered one of the gold standard materials for gene delivery, they need optimization before in vivo application.

Formulation Optimization of PEI/ Nucleic Acid Complexes for Gene Delivery

Although PEIs have unique properties as gene carriers, in many cases, their engineering and modifying are necessary to provide desired properties such as biodegradability and biocompatibility (Cho et al. 2022). Because their molecular weight is directly related to toxicity, some PEIs derivatives with lower molecular weight have been reported to reduce the toxicity profile, but in most cases, these have also limited transfection efficiency (Mani et al. 2023). So, such applied strategies that may affect the crucial factors involved in gene delivery, such as binding capability toward DNA, uptake, and endosomal escape, should be balanced between transfection efficacy and eliminate their undesirable features (Yang et al. 2022a). For example, thiolated crosslinked PEIs retain prominent cell transfection, but the particle size of polyplex increases to about (> 1000 nm) (Ma et al. 2017). This large-sized polyplex not only has more difficulty entering cells, but if they are not quickly degraded

inside the cell, they can damage the cell through the large amounts of the positive charge they carry (Prabha et al. 2016). As a therapeutic delivery, the optimal size is proposed between 20 and 200 nm, which is large enough to prevent filtration and small enough to be absorbed through the cell membrane (Prabha et al. 2016). To overcome such *a troublesome issue,* recently several hybrid structures consisting of PEI with other functional groups have been evaluated. Increasing efficiency and biocompatibility, and decreasing toxicity have been reported as the results of various formulation optimization of PEI/ nucleic acid complexes which have been designed for the treatment of various cancers (Zakeri et al. 2018). Some of the most important functional groups that have been used to modify PEIs-based carriers are discussed below. Table 2 presents the details of some optimized combinations of PEI-based nucleic acid carriers to treat different cancer.

Hydrophobic and Hydrophilic Modification of PEI

Some of the formulation optimizations of PEI/nucleic acid have been made through grafting hydrophobic and hydrophilic biocompatible polymers whether physical or chemical binding (Lv et al. 2017a). Hydrophilic blocks including dextran, cyclodextrin, hyaluronic acid, chitosan, and polycarbonate are the most known candidates for modifying PEI to improve their biocompatibility and biodegradability (Hu et al. 2018). As well as, hyaluronic acid (HA) or polyethylene (PEG), dextran, cyclodextrins, and chitosan were widely used to reduce surface charge-dependent cytotoxicity (Wang et al. 2020).

PEGylation is a strategy to increase the blood circulation time of the PEI complex (Li et al. 2020). Assembling PEIs with bio-detachable anionic groups like PEG, relying on PEI's numerous functional amine groups, reduces aggregation and clearance, and also improves stability and biocompatibility through inhibiting PEI interactions with serum components. (Wang et al. 2022). The properties of PEG–PEI conjugates are closely related to the molecular weight of PEG and the degree of PEGylation. In other words, PEG density and chain length affect biocompatibility and biological performance (Li et al. 2020). Despite improving the circulation time, PEGylation can decrease the transfection efficiency by reducing the non-specific interactions between the polyplex and cells (Zhao et al. 2017). Modification of the periphery of PEI with PEG leads to decrease in the surface charge density of the polymers and disturbs cellular uptake, intracellular trafficking, and endosomal escape (Fang et al. 2017). This phenomenon, which is called "the PEG problem," leads to a decrease in gene expression (Cho et al. 2022) In a study, a concentrated PEI-lipid hybrid made of C15 epoxide-terminated lipids and PEI (0.6 kDa), which was formulated into nanoparticles using PEG-lipids, systematically delivered siRNA to lung endothelial and silenced the target genes at very low doses (Ulkoski et al. 2019). Also, a study carried out by Wang and his group presented PEG-grafted branched PEIs as a nano-scaled cationic carrier for treating retinoblastoma which can condense the genes effectively (Wang et al. 2022). In vitro experiments of the retinoblastoma cell line indicated that PEI-g-PEG-based non-viral gene vectors significantly decrease the cytotoxicity-related PEI, and also have clear endocytosis effects. As well as the

Table 2 Optimized combination of PEI-based nucleic acid systems to treatment different cancer

PEI type	Gene therapy	Combination	Cancer type	Efficacy	References
Branched PEI (1.8 K)	shGPX4 and shMTHFD2 plasmids	Thioketal-crosslinked fluorinated-PEI-hyaluronic acid (HA)	Skin cancers	Transfection efficiency, efficient uptake, lysosomal escape and safety	Yang et al. (2022b)
PEI (800 Da)	DNA	Iron oxide-PEI (800 Da)-chitosan	Breast cancer	Safely, DNA protection, extra biocompatibility, high transfection efficiency, and significantly lower cytotoxicity	Lin et al. (2022a)
PEI, M_w = 25 kDa and 10 kDa	siRNA	zwitterions- PEI_{10k} and PEI_{25k}	Cervical carcinoma and lung carcinoma	Reduced cytotoxicity and non-specific protein adsorption, enhanced endocytosis, and improved siRNA delivery efficiency	Liu et al. (2022)
Branched PEI 1.2 kDa	siOPA1	PEI-polymannitol, polyglycerol, and polylactitol	Lung cancer	Safe, protecte DNA cargo, easily released, improve cellular uptake, less cytotoxic compared with PEI (25 KDa)	Cho et al. (2022)
PEI (MW ~ 1800)	CRISPR/Cas9 system in both DNA (px458 plasmid) and ribonucleoprotein (RNP) forms	PEI-bovine serum albumin	Breast cancer	Safety and biocompatibility, no remarkable toxicity effects,high loading capacity, high transfection efficiency	Rahimi et al. (2022)
PEI (25 kDa) (Mw = 2000)	PD-L1-siRNA	PEI-stearic acid-EGFR short peptide	Non-small cell lung cancer (NSCLC)	Safe and highly efficient	Yang et al. (2022a)
Branched PEI (25 kDa)	Pro-apoptotic oligonucleotide (polyinosinic–polycytidylic acid sodium salt) (PIC)	PEI-iron oxide (SPION@bPEI) conjugated with Erbitux (Erb)	Cervical cancer	Non-toxic, cationic nature, and intrinsic luminescence	Khodadust et al. (2022)

Table 2 (continued)

PEI type	Gene therapy	Combination	Cancer type	Efficacy	References
Branched PEI (MW = 25 kDa)	Anti-EGFR1 siRNAs	Guanidinium ionic liquid (GuIL), PEI and PEGylated folic acid (FA-PEG)	Triple-negative breast cancer (TNBC)	Improve cellular uptake, escape endosomal	Cho et al. (2022)
Branched PEI (25 K)	Gene (plasmid)	Three DNA-aptamers (AS1411, WY-5a, and Sgs-8) conjugated to PEI	Prostate cancer	Increase the efficiency of plasmid delivery	Askarian et al. (2022)
PEI	siRNA and DNA delivery	Exosome-PEG-PEI and PD-L1 antibody (Exo-PEG-PEI-PD)	Lung cancer	Long circulation time and positive electricity, safety	Lin et al. (2022b)

expression of encoded functional proteins in the treated retinoblastoma cells shows effective gene transfection (Wang et al. 2022).

Chitosan is a non-toxic linear cationic polysaccharide that is one of the most popular polymers for grafting in PEI (Javan et al. 2018). One of the significant advantages of chitosan is the quick dissociation of polymer and nucleic acid polyplexes upon cellular internalization. Also, the PEI-chitosan complex leads to more efficient gene transfection by providing a stronger combination related to PEI while minimizing the negative impact of charge density. However, some research showed that the use of chitosan limits transfection efficiency, but the positive impacts such as low toxicity, biodegradability, and biocompatibility of the composition of chitosan/PEI, are the main reasons for its popularity. For example, PEIs/DNA coated with glycol chitosan exhibited significantly lower cytotoxicity compared to commercial transfection reagents, such as jetPRIME and Lipofectamine 2000 (Cheng et al. 2022). Chitosan nanoparticles grafted by histidine and PEI were used for gene delivery into the breast cancer cells (MCF-7). The results demonstrated that cell uptake of the nanoparticle-containing chitosan NPs significantly ($p < 0.05$) increased to nanoparticles without chitosan.

Dextran is another typical example of a biodegradable natural polysaccharide that is explored for gene delivery applications (Chen et al. 2020). It can reduce the toxicity of PEI and increase the stability of the PEI/ DNA complex in the presence of serum. Significant efficient transportation and low cytotoxicity of the dextran-grafted PEIs coated with iron oxide nanoparticles were reported by Gong et al., as the result of miRNA transfer into osteosarcoma (OS) cells and OS-bearing nude mice (Gong et al. 2020). Cyclodextrins, a family of cyclic oligosaccharides, are a successful hydrophilic polymer for gene delivery. Cyclodextrins in different forms (α-, β-, or γ-) are used in combination with PEIs to improve their gene delivery efficiency (Plesselova et al. 2021).

Modification of low-molecular-weight PEIs (600 KDa) with linear biodegradable polyesters is another effective strategy to ameliorate the degradable ability of PEI polymers (Thomas et al. 2019). In addition to improving the biocompatibility, the results of the studies showed that this new combined PEI complex also reduced the cytotoxicity of PEI polymer and increased the transfection efficiency several times compared to 25 kDa PEI (Steinman et al. 2019; Lv et al. 2017b). Novel carriers composed of PEI 600 conjugated with amino alcohol esters, diglycidyl adipate, diglycidyl succinate, and diglycidyl oxalate are some other promising biodegradable non-viral gene delivery systems (Lv et al. 2017b). In vitro, experimental results such as agarose gel retardation and MTT assays showed that facilitating the release of DNA and reducing the cytotoxicity of these new vectors compared to the gold standard PEI (25 kDa) has led to an effective increase in transfection efficiency (Liu et al. 2017*)*.

In addition to hydrophilic modification, having all three types of amine groups (primary, secondary, and tertiary) make PEIs suitable polymers for applying hydrophobic modifications to improve their biological activities (Linsha Mali et al. 2021). Linear alkyl chains of fatty acids including acetate, butanoate, hexanoate, myristate, and butyric anhydrides are the most commonly used hydrophobic modifying groups to PEIs (Linsha Mali et al. 2021). Other hydrophobic molecules that have been

used for PEI modification are cyclic hydrophobic molecules (such as cholesteryl chloroformate, and dexamethasone mesylate) and Pluronic conjugates ions with PEO–PPO–PEO Poloxamers (Rey-Rico and Cucchiarini 2018). Also, aliphatic lipid and alkane chains have been recommended to increase colloidal stability and biocompatibility (Ewe et al. 2017).

In general, hydrophobic modification of polyplex can influence the gene transfection efficiency by several mechanisms such as increasing the amount of the encapsulated genetic materials, enhancing uptake by the cell membrane, decreasing toxicity, alleviating inhibition associated with serum, and facilitating polycation/DNA complex dissociation. In addition, the efficiency of hydrophobically modified PEI was affected by various factors including the degree of substitution and position of the alkyl group and the chain length (Xue et al. 2021a). For example, 57% acetylation of primary amines in the PEI structure increases gene transfection efficiency while more acetylation led to reducing in transfection efficiency (Masoumzadeh 2021). Acylation keeps hidden the free amine groups of PEI reducing the natural PEI steric hindrance. Also, the conjugation of acetate, butanoate, and hexanoate to PEI with a degree of substitution of 25% creates maximum transfection efficiency (Pandey and Sawant 2016). A siRNA LMW PEI-based vector conjugated with PEIs, cholesterol, and PEG was specifically uptake through the caveolae-mediated pathway and promoted cellular uptake due to Caveolin/cholesterol complexes in the plasma membrane (Zhang et al. 2020). Similarly, cholesterol modification boosts the siRNA delivery efficacy of PIE-based CXCR4 antagonists (Wu et al. 2018). In a study, fluorination was used to reduce the cytotoxicity of PEI, and improve the nucleic acid delivery potency by increasing the hydrophobicity and lipophobicity of PEI (Xue et al. 2021b). In another study, coated magnetic nanocarriers with heptafluorobutyric-PEI-PEG were used as siRNA deliver for knockdown CXCR4 expression in cancer cells. Finally, a significant decrease in cytotoxicity and an increase in transfection efficiency by up to 90% were reported as the result (Adachi et al. 2021). Also, to limit the toxicity profile formation, the hybrid construction of low-molecular-weight PEI (0.8–1.8 kDa) with biodegradable cross-linking such as ester, imine, or disulfide bonds has been suggested. These designed contractions can significantly reduce toxicity while maintaining gene transfer efficiency compared to the gold standard version of PEI (25 kDa) (Ullah et al. 2020).

Peis-Proteins Optimized Formulation

Significant biocompatibility and biodegradability features of proteins make them a great functional group to modify PEIs-based non-viral gene delivery (Heifetz et al. 2016). Among them, gelatin and albumin are the most famous protein carriers that are used in two forms alone or in combination with other polymers such as PEI (Chamundeeswari et al. 2019). These biodegradable polymers, proteins, have a large folded structure with a natural origin that is able to improve some certain biological activities of PEI-mediated polyplex such as biodegradability, stability, and even targeting (Chamundeeswari et al. 2019). For example, PEI-coated-serum albumin nanoparticles lead to increase circulation half-lives and high gene capacity (Setua

2020). In another study, PEI-coated-serum albumin nanoparticles lead to increase circulation half-lives and high gene capacity (Setua 2020). Also, coating the PEI/DNA complex with anionic gelatin, for targeted gene transfer to matrix metalloproteinase-secreting cancer cells, not only improved the stability of nanoparticles by reducing the interaction with erythrocytes due to the decreased surface positive charge, but also gene transfection improved by increasing the interaction between the polyplex and surface cell (Ge et al. 2012).

Recently, some smart polymeric gene delivery systems have been designed by modifying the PEI chain with some Cell-Penetrating Peptides (CPPs) such as lysine-histidine63 (KH), Tat peptide sequence64, and polylysine (He et al. 2020). CPPs are short amphipathic peptides (containing 5–30 amino acid residues) with an inherent ability to cross the cell membrane through passive diffuse or endocytosis for delivering their cargo into cells (Torres-Vanegas et al. 2021). Since 1999, the incorporation of CPPs into NPs for targeted drug delivery and imaging has been the subject of many studies. For instance, Pan and colleagues constructed a mannosylated carrier containing low-molecular-weight PEI and CPP (Man-PEI5k–CPP) to deliver plasmid tumor necrosis factor-related apoptosis-inducing ligand (pTRAIL) to target colon overexpressed mannose receptors colorectal cancer cells. Co-targeting (tumor-targeting ligand and the CPP) of the Man-PEI5k–CPP/pTRAIL significantly increased the transfection efficiency and inhibition efficacy on HCT116 in vitro and in vivo (Pan et al. 2017). Another strategy for the modification of PEIs to prosper polymer-based gene delivery is amino acid modification. Although few studies have evaluated amino acid-modified PEI, results showed the success of these biomolecules through increasing transfection efficiency, and maintaining minimal toxicity. Lysine–histidine (KH) peptides, 6-bromohexanoic acid (alkyl), and various heterocyclic amines, glycolic acid, histidine, piperazine-2-carboxylic acid dihydrochloride, piperazine, pyridine, and imidazole rings were conjugated to the low molecular weight of PEI (10Kd and 25Kd) (Pandey and Sawant 2016; Zakeri et al. 2018). For example, PEG-modified l-arginine oligo(-alkylaminosiloxane) grafted with PEI to facilitate cellular uptake mediated by arginine and improve siRNA delivery transfection through ionic interactions between its positive charge and negative on the cell surface (Lu et al. 2019).

Recently, cell membrane complex (CMC) modulates adverse reactions and their side effects of a cationic polymer by shielding their positive charge, and also increasing gene transfection efficiency by imparting bioactive molecules that are necessary to specific cell behaviors (Chugh et al. 2021). In a study carried out by Fang et al., a novel gene-carrier system consisting of disulfide-crosslinked LMW-PEI and manganese dioxide (MnO2) nanosheets, and nuclear localization signal peptide (NLS) was designed. Also, the melanoma cell membrane was used to coat the nano-gene-carrier system. As a result, they reported that disulfide-crosslinked LMW-PEI led to increasing transfection efficiency and decreased toxicity, and also cancer cell membrane coating induced homologous targeting to gene carriers, and also promoted immune escape abilities (Fang et al. 2022). In another study by Liu et al., an extracellular matrix was used to coat the formulated PEI/DNA complexes for cancer gene therapy. Compared with PEI/DNA complexes, these tumor-targeted core–shell

structures showed lower cytotoxicity, homologous targeting, and high transfection efficiency (Liu et al. 2021a).

Exosomes are cell-derived bilayer nan membrane vesicles, that have recently been considered as a potential gene carrier (Uddin et al. 2022; Karami Fath et al. 2022c). The reason for choosing exosome, as an extracellular vesicle, for transferring therapeutic substances, especially nucleic acid, is due to its natural biological advantages. Some of these key features that make exosome better than other gene delivery systems include biodegradability, biocompatibility, no or low toxicity, low immunogenicity, ability to cross biological barriers, intrinsic cell tropism, ability to fuse with the cell membrane and escape from endosomes (Hosseini et al. 2022; Fayazi et al. 2021). In addition, unlike polyplexes, which bind very tightly to nucleic acid and have a weak release inside the cell, exosomes can effectively transport and release their cargo to the target cell through proteins and active groups on their surface (Jiang et al. 2017). Therefore, the use of exosomes to improve the transfection efficiency of PEI-based gene delivery system have been investigated in several studies. For example, an exosomal-based delivery system consisting of bovine milk-derived exosomes and PEI was evaluated by Mungala et al. (Munagala et al. 2021), to deliver KRAS siRNA and wild-type (WT) p53 pDNA. In vitro experiments showed that the exosomes and PEI matrix (EPM) were platforms able to protection of entrapped nucleic acid from enzymatic degradation, and also it was biocompatible with no adverse response (Munagala et al. 2021). In another study, exosome-PEG-PEI-maleimide was constructed and then loaded by siRNA. Also, the modified PD-L1 antibody was used to improve the targeting of tumor cells by the constructed gene carrier. As a result, the safety and toxicity associated with PEI were significantly reduced, as well as in vitro evaluations showed that the ability to detect tumor cells was greatly improved by the exosome-PEG-PEI-PD-L1 platform (Lin et al. 2022c).

Optimization Tumor Targeting PEI-based Polyplex by Ligand

While modification of PEI is mostly applied to improve the physicochemical properties and biocompatibility of this polyplex, distinct targeting ligands are applied with the purpose of specific transfer of polymers to the desired cell or tissue (Pang et al. 2019). Such modifications overcome much of the inefficiency of transfection due to the inability of the genetic material to enter the cell nucleus (Jiang et al. 2019). Different types of molecules have been used as ligands for specific targeting. In many studies, various targeting ligands such as polypeptide (RGD peptide and NGR peptide), (Ahmed 2017) folic acid (Liu et al. 2021b), galactose, mannose, epidermal growth factor (EGF) and some others have been used for functionalization cationic polymers improved receptor-mediated endocytosis (Lee et al. 2015; Hari et al. 2022). For example, in a study carried out by Cao et al., folic acid was used for targeting and reducing the cytotoxicity of PEi-mediated nucleic acid transfer (Cao et al. 2020). The hydrophobic modification of the PEI/DNA polyplex by folic acid results in cell-specific uptake and high transfection efficiency of approximately 100 fold than 1.8 K PEI-based polyplexes (Cao et al. 2020). Targeted delivery to

hepatocellular carcinoma via highly expressed glycyrrhetinic acid (GA) receptors on their surface was enhanced by conjugating PEI with glycyrrhizic acid (GL) or glycyrrhetinic acid (GA) PEI. These results confirmed that the PEI-GA-GL-mediated gene delivery increased GA receptor-mediated endocytosis, like the SV40 virus, via a caveola- and clathrin-independent pathway (Cao et al. 2019). Also, a modified polymyxin-PEI-nanoplexe was designed to enhance the targeted delivery of DNA cargo to megalin-expressing kidney cells. The result showed that targeting megalin, as an endocytic multi-ligand receptor, by polymyxin-PEI nanoparticle improved both safety and transfection efficiency (Oroojalian et al. 2017). Also, the melanoma cell membrane (Fang et al. 2022) and extracellular matrix (Liu et al. 2021a) were used to coat gene carriers to target cancer cells. As mentioned above, both modifications significantly increased homotypic targeting and led to effective internalization and increase transfection efficiency. In general, Fig. 1 summarizes the optimization of the PEI/nucleic acid complex for gene delivery.

Conclusion

For 20 years, the use of cationic polymers in medical science, design, and formation of biodegradable polymers has been the main challenge in making use of polymers as carriers and vectors. Polyethylenimine (PEI) is well known for its effective gene transfection caused by nanosized condensed complex with nucleic acids, acts as a proton sponge, escapes from endosomal vesicles, and has nuclear localization capability. However, intrinsic toxicity and non-biodegradable capability of PEI are the main challenges of this non-viral gene delivery system, but its modified derivatives by natural and synthetic materials that are biodegradable or reduced in size within the renal filtration threshold for rapid renal clearance, will lead to satisfying the demands of modern bioengineering. Moreover, in order to attain gene delivery that is more precise and effective, it is recommended to alter the process by targeting ligands that can identify specific markers in the intended regions. This modification will enable the delivery of encapsulated payloads through active targeting. In addition, incorporating special functionalized groups into the vectors to enhance their buffering capacity can help slow the acidification of endosomes, leading to increased cargo release and improved transfection efficiency. Overall, a combination of modifications to PEIs from various perspectives could be a preferred approach for future studies.

Acknowledgements Not applicable.

Authors Contribution The core idea of this study came from NM. GAK and MN-A directed the other authors. All authors wrote the manuscript. Final editing was done by GAK, HM-S and MN-A. MN-A and GAK supervised the manuscript.

Funding Not applicable.

Data Availability Not applicable

Declarations

Conflict of interest There are neither ethical nor financial conflicts of interest involved in the manuscript. The manuscript is not submitted for publication elsewhere.

Ethics Approval There is no involvement of human or animal in this study.

Consent for Publication Not applicable

References

Adachi H, Hengesbach M, Yu Y-T, Morais P (2021) From antisense RNA to RNA modification: therapeutic potential of RNA-based technologies. Biomedicines 9(5):550

Ahmed M (2017) Peptides, polypeptides and peptide–polymer hybrids as nucleic acid carriers. Biomaterials Science 5(11):2188–2211

Anguela XM, High KA (2019) Entering the modern era of gene therapy. Annu Rev Med 70:273–288

Askarian S, Nasab NK, Aghaee-Bakhtiari SH, Abadi MHJN, Oskuee RK: Investigating Efficacy of Three DNA-Aptamers in Targeted Plasmid Delivery to Human Prostate Cancer Cell Lines. *Molecular Biotechnology* 2022:1–11.

Babaei M, Eshghi H, Abnous K, Rahimizadeh M, Ramezani M (2017) Promising gene delivery system based on polyethylenimine-modified silica nanoparticles. Cancer Gene Ther 24(4):156–164

Cao M, Gao Y, Zhan M, Qiu N, Piao Y, Zhou Z, Shen Y (2019) Glycyrrhizin acid and glycyrrhetinic acid modified polyethyleneimine for targeted DNA delivery to hepatocellular carcinoma. Int J Mol Sci 20(20):5074

Cao M, Gao Y, Qiu N, Shen Y, Shen P (2020) Folic acid directly modified low molecular weight of polyethyleneimine for targeted pDNA delivery. J Drug Deliv Sci Technol 56:101522

Chamundeeswari M, Jeslin J, Verma ML (2019) Nanocarriers for drug delivery applications. Environ Chem Lett 17:849–865

Chen C-K, Huang P-K, Law W-C, Chu C-H, Chen N-T, Lo L-W (2020) Biodegradable polymers for gene-delivery applications. Int J Nanomed 15:2131

Cheng B, Ahn H-H, Nam H, Jiang Z, Gao FJ, Minn I, Pomper MG (2022) A unique core-shell structured, glycol chitosan-based nanoparticle achieves cancer-selective gene delivery with reduced off-target effects. Pharmaceutics 14(2):373

Cheraghi R, Alipour M, Nazari M, Hosseinkhani S: Optimization of conditions for gene delivery system based on PEI. *Nanomedicine Journal* 2017, 4(1).

Cho KS, Kim S, Chun HB, Cheon JH, Cho M-H, Lee AY, Arote RB (2022) Efficient gene transfection to lung cancer cells via Folate-PEI-Sorbitol gene transporter. PLoS ONE 17(5):e0266181

Chugh V, Vijaya Krishna K, Pandit A (2021) Cell membrane-coated mimics: a methodological approach for fabrication, characterization for therapeutic applications, and challenges for clinical translation. ACS Nano 15(11):17080–17123

Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M (2018) Gene therapy comes of age. Science 359(6372):4672

Ewe A, Panchal O, Pinnapireddy SR, Bakowsky U, Przybylski S, Temme A, Aigner A (2017) Liposome-polyethylenimine complexes (DPPC-PEI lipopolyplexes) for therapeutic siRNA delivery in vivo. Nanomedicine 13(1):209–218

Faizullin B, Gubaidullin A, Gerasimova T, Kashnik I, Brylev K, Kholin K, Nizameev I, Voloshina A, Sibgatullina G, Samigullin D (2022) "Proton sponge" effect and apoptotic cell death mechanism of Agx-Re6 nanocrystallites derived from the assembly of [{Re6S8}(OH) 6–n (H2O) n] n–4 with Ag+ ions. Colloids Surf, A 648:129312

Fang Y, Xue J, Gao S, Lu A, Yang D, Jiang H, He Y, Shi K (2017) Cleavable PEGylation: a strategy for overcoming the "PEG dilemma" in efficient drug delivery. Drug Delivery 24(2):22–32

Fang Z, Zhang M, Kang R, Cui M, Song M, Liu K (2022) A cancer cell membrane coated nanoparticles-based gene delivery system for enhancing cancer therapy. Int J Pharm 629:122415

Fayazi N, Sheykhhasan M, Soleimani Asl S, Najafi R (2021) Stem cell-derived exosomes: a new strategy of neurodegenerative disease treatment. Mol Neurobiol 58:3494–3514

Freund A: Complexation of Cationic Polymers with Nucleic Acids for Gene Delivery: The University of Manchester (United Kingdom); 2014.

Gao S, Tian H, Guo Y, Li Y, Guo Z, Zhu X, Chen X (2015) miRNA oligonucleotide and sponge for miRNA-21 inhibition mediated by PEI-PLL in breast cancer therapy. Acta Biomater 25:184–193

Ge J, Min S-H, Kim DM, Lee DC, Park KC, Yeom YI (2012) Selective gene delivery to cancer cells secreting matrix metalloproteinases using a gelatin/polyethylenimine/DNA complex. Biotechnol Bioprocess Eng 17(1):160–167

Gong M, Liu H, Sun N, Xie Y, Yan F, Cai L (2020) Polyethylenimine-dextran-coated magnetic nanoparticles loaded with miR-302b suppress osteosarcoma in vitro and in vivo. Nanomedicine 15(07):711–723

Hari SK, Gauba A, Shrivastava N, Tripathi RM, Jain SK, Pandey AK: Polymeric micelles and cancer therapy: An ingenious multimodal tumor-targeted drug delivery system. *Drug Delivery and Translational Research* 2022:1–29.

He J, Xu S, Mixson AJ (2020) The multifaceted histidine-based carriers for nucleic acid delivery: advances and challenges. Pharmaceutics 12(8):774

Heifetz A, Trani G, Aldeghi M, MacKinnon CH, McEwan PA, Brookfield FA, Chudyk EI, Bodkin M, Pei Z, Burch JD (2016) Fragment molecular orbital method applied to lead optimization of novel interleukin-2 inducible T-cell kinase (ITK) inhibitors. J Med Chem 59(9):4352–4363

Höbel S, Aigner A: Polyethylenimine (PEI)/siRNA-mediated gene knockdown in vitro and in vivo. In: *RNA Interference*. Springer; 2010: 283–297.

Hosseini NF, Amini R, Ramezani M, Saidijam M, Hashemi SM, Najafi R (2022) AS1411 aptamer-functionalized exosomes in the targeted delivery of doxorubicin in fighting colorectal cancer. Biomed Pharmacother 155:113690

Hu W-W, Yeh C-C, Tsai C-W (2018) The conjugation of indolicidin to polyethylenimine for enhanced gene delivery with reduced cytotoxicity. J Mater Chem B 6(36):5781–5794

Jahan I, Zhang L: Natural polymer-based electrospun nanofibrous membranes for wastewater treatment: A review. *Journal of Polymers and the Environment* 2021:1–21.

Javan B, Atyabi F, Shahbazi M (2018) Hypoxia-inducible bidirectional shRNA expression vector delivery using PEI/chitosan-TBA copolymers for colorectal Cancer gene therapy. Life Sci 202:140–151

Jiang L, Vader P, Schiffelers R (2017) Extracellular vesicles for nucleic acid delivery: progress and prospects for safe RNA-based gene therapy. Gene Ther 24(3):157–166

Jiang C, Chen J, Li Z, Wang Z, Zhang W, Liu J (2019) Recent advances in the development of polyethylenimine-based gene vectors for safe and efficient gene delivery. Expert Opin Drug Deliv 16(4):363–376

Karami Fath M, Azargoonjahromi A, Kiani A, Jalalifar F, Osati P, Akbari Oryani M, Shakeri F, Nasirzadeh F, Khalesi B, Nabi-Afjadi M (2022a) The role of epigenetic modifications in drug resistance and treatment of breast cancer. Cell Mol Biol Lett 27(1):1–25

Karami Fath M, Babakhaniyan K, Zokaei M, Yaghoubian A, Akbari S, Khorsandi M, Soofi A, Nabi-Afjadi M, Zalpoor H, Jalalifar F (2022b) Anti-cancer peptide-based therapeutic strategies in solid tumors. Cell Mol Biol Lett 27(1):33

Karami Fath M, Azami J, Jaafari N, Akbari Oryani M, Jafari N, Azargoonjahromi A, Nabi-Afjadi M, Payandeh Z, Zalpoor H, Shanehbandi D (2022c) Exosome application in treatment and diagnosis of B-cell disorders: leukemias, multiple sclerosis, and arthritis rheumatoid. Cell Mol Biol Lett 27(1):1–28

Karpisheh V, Afjadi JF, Afjadi MN, Haeri MS, Sough TSA, Asl SH, Edalati M, Atyabi F, Masjedi A, Hajizadeh F (2021) Inhibition of HIF-1α/EP4 axis by hyaluronate-trimethyl chitosan-SPION nanoparticles markedly suppresses the growth and development of cancer cells. Int J Biol Macromol 167:1006–1019

Khodadust R, Unal O, Acar HY (2022) Theranostic potential of self-luminescent branched polyethyleneimine-coated superparamagnetic iron oxide nanoparticles. Beilstein J Nanotechnol 13(1):82–95

Kriplani P, Guarve K (2022) Transdermal drug delivery: a step towards treatment of cancer. Recent Pat Anti-Cancer Drug Discov 17(3):253–267

Lee D, Lee YM, Kim J, Lee MK, Kim WJ (2015) Enhanced tumor-targeted gene delivery by bioreducible polyethylenimine tethering EGFR divalent ligands. Biomaterials Science 3(7):1096–1104

Li A, Qiu J, Zhou B, Xu B, Xiong Z, Hao X, Shi X, Cao X (2020) The gene transfection and endocytic uptake pathways mediated by PEGylated PEI-entrapped gold nanoparticles. Arab J Chem 13(1):2558–2567

Li J, Yu X, Shi X, Shen M (2022) Cancer nanomedicine based on polyethylenimine-mediated multifunctional nanosystems. Prog Mater Sci 124:100871

Lin G, Huang J, Zhang M, Chen S, Zhang M (2022a) Chitosan-crosslinked low molecular weight pei-conjugated iron oxide nanoparticle for safe and effective DNA delivery to breast cancer cells. Nanomaterials 12(4):584

Lin X, Lin L, Wu J, Jiang W, Wu J, Yang J, Chen C (2022c) A targeted siRNA-loaded PDL1-exosome and functional evaluation against lung cancer. Thoracic Cancer 13(11):1691–1702

Lin X, Lin L, Wu J, Jiang W, Wu J, Yang J, Chen C: A targeted siRNA-loaded PDL1-exosome and functional evaluation against lung cancer. *Thoracic Cancer* 2022b.

Linsha Mali A, Priya S, Rekha M (2021) Hydrophobic and hydrophilic modifications of polyethylenimine towards gene delivery applications. J Appl Polym Sci 138(45):51323

Liu Q, Su R-C, Yi W-J, Zhao Z-G (2017) Biodegradable poly (amino ester) with aromatic backbone as efficient nonviral gene delivery vectors. Molecules 22(4):566

Liu L, Chen Y, Liu C, Yan Y, Yang Z, Chen X, Liu G (2021a) Effect of extracellular matrix coating on cancer cell membrane-encapsulated polyethyleneimine/DNA complexes for efficient and targeted DNA delivery in vitro. Mol Pharm 18(7):2803–2822

Liu C, Xie Y, Li X, Yao X, Wang X, Wang M, Li Z, Cao F (2021b) Folic acid/peptides modified PLGA–PEI–PEG polymeric vectors as efficient gene delivery vehicles: synthesis, characterization and their biological performance. Mol Biotechnol 63:63–79

Liu F, Su H, Li M, Xie W, Yan Y, Shuai Q (2022) Zwitterionic modification of polyethyleneimine for efficient in vitro siRNA delivery. Int J Mol Sci 23(9):5014

Long Z, Zhang J, Shen Y, Zhou C, Liu M (2017) Polyethyleneimine grafted short halloysite nanotubes for gene delivery. Mater Sci Eng, C 81:224–235

Lu S, Morris VB, Labhasetwar V (2019) Effectiveness of small interfering RNA delivery via arginine-rich polyethylenimine-based polyplex in metastatic and doxorubicin-resistant breast cancer cells. J Pharmacol Exp Ther 370(3):902–910

Lv J, Hao X, Li Q, Akpanyung M, Nejjari A, Neve AL, Ren X, Feng Y, Shi C, Zhang W (2017a) Star-shaped copolymer grafted PEI and REDV as a gene carrier to improve migration of endothelial cells. Biomater Sci 5(3):511–522

Lv P, Zhou C, Zhao Y, Liao X, Yang B (2017b) Modified-epsilon-polylysine-grafted-PEI-β-cyclodextrin supramolecular carrier for gene delivery. Carbohyd Polym 168:103–111

Ma Z, Wang P, Xiong L, Pei Y (2017) Thiolate-protected gold nanoclusters: structural prediction and the understandings of electronic stability from first principles simulations. Wiley Interdiscip 7(4):e1315

Mani S, Jindal D, Singh M (2023) Gene therapy, a potential therapeutic tool for neurological and neuropsychiatric disorders: applications, challenges and future perspective. Curr Gene Ther 23(1):20–40

Masoumzadeh R (2021) Polyethyleneimine-based materials for gene therapy, bioimaging and drug delivery systems applications. Adv Appl NanoBio-Technol 2(1):13–16

Meleshko A, Petrovskaya N, Savelyeva N, Vashkevich K, Doronina S, Sachivko N (2017) Phase I clinical trial of idiotypic DNA vaccine administered as a complex with polyethylenimine to patients with B-cell lymphoma. Hum Vaccin Immunother 13(6):1398–1403

Monnery BD, Wright M, Cavill R, Hoogenboom R, Shaunak S, Steinke JH, Thanou M (2017) Cytotoxicity of polycations: Relationship of molecular weight and the hydrolytic theory of the mechanism of toxicity. Int J Pharm 521(1–2):249–258

Mulenos MR, Lujan H, Pitts LR, Sayes CM (2020) Silver nanoparticles agglomerate intracellularly depending on the stabilizing agent: implications for nanomedicine efficacy. Nanomaterials 10(10):1953

Munagala R, Aqil F, Jeyabalan J, Kandimalla R, Wallen M, Tyagi N, Wilcher S, Yan J, Schultz DJ, Spencer W (2021) Exosome-mediated delivery of RNA and DNA for gene therapy. Cancer Lett 505:58–72

Oroojalian F, Rezayan AH, Mehrnejad F, Nia AH, Shier WT, Abnous K, Ramezani M (2017) Efficient megalin targeted delivery to renal proximal tubular cells mediated by modified-polymyxin B-polyethylenimine based nano-gene-carriers. Mater Sci Eng 79:770–782

Pan Z, Kang X, Zeng Y, Zhang W, Peng H, Wang J, Huang W, Wang H, Shen Y, Huang Y (2017) A mannosylated PEI–CPP hybrid for TRAIL gene targeting delivery for colorectal cancer therapy. Polym Chem 8(35):5275–5285

Pandey AP, Sawant KK (2016) Polyethylenimine: a versatile, multifunctional non-viral vector for nucleic acid delivery. Mater Sci Eng, C 68:904–918

Pang L, Pei Y, Uzunalli G, Hyun H, Lyle LT, Yeo Y (2019) Surface modification of polymeric nanoparticles with M2pep peptide for drug delivery to tumor-associated macrophages. Pharm Res 36:1–12

Park S-C, Heo H, Jang M-K (2022) Polyethylenimine grafted-chitosan based Gambogic acid copolymers for targeting cancer cells overexpressing transferrin receptors. Carbohyd Polym 277:118755

Plesselova S, Garcia-Cerezo P, Blanco V, Reche-Perez FJ, Hernandez-Mateo F, Santoyo-Gonzalez F, Giron-Gonzalez MD, Salto-Gonzalez R (2021) Polyethylenimine–bisphosphonate–cyclodextrin ternary conjugates: supramolecular systems for the delivery of antineoplastic drugs. J Med Chem 64(16):12245–12260

Prabha S, Arya G, Chandra R, Ahmed B, Nimesh S (2016) Effect of size on biological properties of nanoparticles employed in gene delivery. Artif Cells Nanomed Biotechnol 44(1):83–91

Rahimi H, Zaboli KA, Thekkiniath J, Mousavi SH, Johari B, Hashemi MR, Nosrati H, Goldschneider D, Bernet A, Danafar H: BSA-PEI Nanoparticle Mediated Efficient Delivery of CRISPR/Cas9 into MDA-MB-231 Cells. *Molecular Biotechnology* 2022:1–12.

Rey-Rico A, Cucchiarini M (2018) PEO-PPO-PEO tri-block copolymers for gene delivery applications in human regenerative medicine—an overview. Int J Mol Sci 19(3):775

Roca S, Dhellemmes L, Leclercq L, Cottet H (2022) Polyelectrolyte Multilayers in Capillary Electrophoresis. ChemPlusChem 87(4):e202200028

Saeed MU, Hussain N, Bilal M: Biomaterials in Gene Delivery. In: *Functional Biomaterials.* Springer; 2022: 129–148.

Setua S: Development of Novel Therapeutic Strategies for Pancreatic Cancer Treatment. The University of Tennessee Health Science Center; 2020.

Shen X, Du H, Mullins RH, Kommalapati RR (2017) Polyethylenimine applications in carbon dioxide capture and separation: from theoretical study to experimental work. Energ Technol 5(6):822–833

Shi W, Li C, Wartmann T, Kahlert C, Du R, Perrakis A, Brunner T, Croner RS, Kahlert UD (2022) Sensory ion channel candidates inform on the clinical course of pancreatic cancer and present potential targets for repurposing of FDA-approved agents. J Pers Med 12(3):478

Singh D, Bedi N, Tiwary AK (2018) Enhancing solubility of poorly aqueous soluble drugs: critical appraisal of techniques. J Pharm Investig 48(5):509–526

Steinman NY, Starr RL, Brucks SD, Belay C, Meir R, Golenser J, Campos LM, Domb AJ (2019) Cyclopropenium-based biodegradable polymers. Macromolecules 52(9):3543–3550

Thomas T, Tajmir-Riahi H-A, Pillai C (2019) Biodegradable polymers for gene delivery. Molecules 24(20):3744

Torres-Vanegas JD, Cruz JC, Reyes LH (2021) Delivery systems for nucleic acids and proteins: Barriers, cell capture pathways and nanocarriers. Pharmaceutics 13(3):428

Uddin N, Binzel DW, Shu D, Fu T-M, Guo P: Targeted delivery of RNAi to cancer cells using RNA-ligand displaying exosome. *Acta Pharmaceutica Sinica B* 2022.

Ulkoski D, Bak A, Wilson JT, Krishnamurthy VR (2019) Recent advances in polymeric materials for the delivery of RNA therapeutics. Expert Opin Drug Deliv 16(11):1149–1167

Ullah I, Zhao J, Su B, Rukh S, Guo J (2020) Ren X-k, Xia S, Zhang W, Feng Y: Redox stimulus disulfide conjugated polyethyleneimine as a shuttle for gene transfer. J Mater Sci 31(12):1–12

Vaidyanathan S, Chen J, Orr BG, Banaszak Holl MM (2016) Cationic polymer intercalation into the lipid membrane enables intact polyplex DNA escape from endosomes for gene delivery. Mol Pharm 13(6):1967–1978

van den Berg AI, Yun C-O, Schiffelers RM, Hennink WE (2021) Polymeric delivery systems for nucleic acid therapeutics: Approaching the clinic. J Control Release 331:121–141

Vermeulen LM, De Smedt SC, Remaut K, Braeckmans K (2018) The proton sponge hypothesis: fable or fact? Eur J Pharm Biopharm 129:184–190

Virgen-Ortiz JJ, Dos Santos JC, Berenguer-Murcia Á, Barbosa O, Rodrigues RC, Fernandez-Lafuente R (2017) Polyethylenimine: a very useful ionic polymer in the design of immobilized enzyme biocatalysts. J Mater Chem B 5(36):7461–7490

Wang Z, Huang C, Lv H, Zhang M, Li X (2020) In silico analysis and high-risk pathogenic phenotype predictions of non-synonymous single nucleotide polymorphisms in human Crystallin beta A4 gene associated with congenital cataract. PLoS ONE 15(1):e0227859

Wang J, Wang H, Zhao P, Chen Z, Lin Q (2022) Hyperbranched-star PEI-g-PEG as a nonviral vector with efficient uptake and hypotoxicity for retinoblastoma gene therapy application. Colloid Interface Sci Commun 50:100647
Wu P, Luo X, Wu H, Yu F, Wang K, Sun M, Oupicky D (2018) Cholesterol modification enhances antimetastatic activity and sirna delivery efficacy of Poly (ethylenimine)-based CXCR4 antagonists. Macromol Biosci 18(11):1800234
Wu T, Liu C, Kong B, Sun J, Gong Y, Liu K, Xie J, Pei A, Cui Y (2019) Amidoxime-functionalized macroporous carbon self-refreshed electrode materials for rapid and high-capacity removal of heavy metal from water. ACS Cent Sci 5(4):719–726
Wu Y, Dong Z, Chen S, Yu X-Y, Tang S (2021) Intracellular delivery of proteins into living cells by low-molecular-weight polyethyleneimine. Int J Nanomed 16:4197
Xu X, Pejcic B, Heath C, Wood CD (2018) Carbon capture with polyethylenimine hydrogel beads (PEI HBs). Journal of Materials Chemistry A 6(43):21468–21474
Xu Y-B, Chen G-L, Guo M-Q (2019) Antioxidant and anti-inflammatory activities of the crude extracts of Moringa oleifera from Kenya and their correlations with flavonoids. Antioxidants 8(8):296
Xue L, Yan Y, Kos P, Chen X, Siegwart DJ (2021a) PEI fluorination reduces toxicity and promotes liver-targeted siRNA delivery. Drug Deliv Transl Res 11(1):255–260
Xue L, Yan Y, Kos P, Chen X, Siegwart DJ (2021b) PEI fluorination reduces toxicity and promotes liver-targeted siRNA delivery. Drug Deliv Transl Res 11:255–260
Xun M-M, Huang Z, Xiao Y-P, Liu Y-H, Zhang J, Zhang J-H, Yu X-Q (2018) Synthesis and properties of low-molecular-weight PEI-based lipopolymers for delivery of DNA. Polymers 10(10):1060
Yang S, Wong KH, Hua P, He C, Yu H, Shao D, Shi Z, Chen M (2022b) ROS-responsive fluorinated polyethyleneimine vector to co-deliver shMTHFD2 and shGPX4 plasmids induces ferroptosis and apoptosis for cancer therapy. Acta Biomater 140:492–505
Yang G, Zhou D, Dai Y, Li Y, Wu J, Liu Q, Deng X: Construction of PEI-EGFR-PD-L1-siRNA dual functional nano-vaccine and therapeutic efficacy evaluation for lung cancer. *Thoracic Cancer* 2022a.
Zaimy M, Saffarzadeh N, Mohammadi A, Pourghadamyari H, Izadi P, Sarli A, Moghaddam L, Paschepari S, Azizi H, Torkamandi S (2017) New methods in the diagnosis of cancer and gene therapy of cancer based on nanoparticles. Cancer Gene Ther 24(6):233–243
Zakeri A, Kouhbanani MAJ, Beheshtkhoo N, Beigi V, Mousavi SM, Hashemi SAR, Karimi Zade A, Amani AM, Savardashtaki A, Mirzaei E (2018) Polyethylenimine-based nanocarriers in co-delivery of drug and gene: a developing horizon. Nano Rev Experiments 9(1):1488497
Zhang H, Men K, Pan C, Gao Y, Li J, Lei S, Zhu G, Li R, Wei Y, Duan X (2020) Treatment of colon cancer by degradable rrPPC Nano-conjugates delivered STAT3 siRNA. Int J Nanomed 15:9875
Zhang W, Chen D, Wang X, Xie X (2022) Insight into the synthesis of branched polyethylenimines from 2-haloethylamine via a one-pot two-stage process. Polymer 255:125113
Zhao G, Long L, Zhang L, Peng M, Cui T, Wen X, Zhou X, Sun L, Che L (2017) Smart pH-sensitive nanoassemblies with cleavable PEGylation for tumor targeted drug delivery. Sci Rep 7(1):1–10
Zhao H, Zhang C, Qi D, Lü T, Zhang D (2019) One-Step synthesis of polyethylenimine-coated magnetic nanoparticles and its demulsification performance in surfactant-stabilized oil-in-water emulsion. J Dispersion Sci Technol 40(2):231–238
Zou Y, Li D, Shen M, Shi X (2019) Polyethylenimine-based nanogels for biomedical applications. Macromol Biosci 19(11):1900272