Polyethylenimine In Medicinal Chemistry

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Abstract: Polyethylenimine (PEI), an organic branched or linear polyamine polymer, has been successfully used in the past for DNA complexation and transfection in vitro and in vivo into several cell lines and tissues. PEI was also applied in different fields from gene therapy and several studies have emphasized the importance of this polymer in medicinal chemistry. In this brief critical review the uses and applications of this versatile polymeric molecule will be discussed.

Keywords: Polyethylenimine, polymer, DNA, transfection, gene therapy, drug delivery, medicinal chemistry, biomedical applications.

1. INTRODUCTION

Polyethylenimines (PEIs) are highly basic and positively charged aliphatic polymers, containing primary, secondary and tertiary amino groups in a 1:2:1 ratio. Every third atom of the polymeric backbone is therefore an amino nitrogen that may undergo protonation. As the polymer contains repeating units of ethylamine, PEIs are also highly watersoluble. PEIs are available in both linear and branched forms with molecular weights ranging from 700 Da to 1000 kDa.

PEIs have been extensively studied as a vehicle for non-viral gene delivery and therapy. Since its introduction in 1995 [1], PEI (Fig. 1) has been considered the gold standard for polymer-based gene carriers because of the excellent transfection efficiencies of its polyplexes (complex of nucleic acid and polymer) in both in vitro and in vivo models [2]. Polycation-mediated gene delivery is based on electrostatic interactions between the positively charged polymer and the negatively charged phosphate groups of DNA. In aqueous solution, PEI condenses DNA and the resulting PEI/DNA complexes, carrying a net positive surface charge, can interact with the negatively charged cell membrane and readily internalized into cells [3]. PEI retains a substantial buffer capacity at virtually any pH and it has been hypothesized that this simple molecular property is related to the efficiency of the complex multistage process of transfection. As a matter of fact, the ‘proton sponge’ nature of PEI is thought to lead to buffering inside endosomes. The proton influx into the endosome, along with that of counter-ions (generally chloride anions), maintains the overall charge neutrality even if an increase of ionic strength inside the endosome is expected. This effect generates an osmotic swelling and the consequent physical rupture of the endosome, resulting in the escape of the vector from the degradative lysosomal compartment. The proton sponge hypothesis has been a subject of debate, speculation and research without reaching a general consensus about the real mechanism involved [4-7].

However, it has been shown that both the efficacy and toxicity of PEI are strongly correlated with its molecular weight (MW) as well as its structure (branched or linear: b-PEI or l-PEI, respectively). Efficacy and adverse reactions seem thereby to be strongly associated. A good compromise between efficiency and toxicity was found for branched PEI with a molecular weight of 25 kDa. It is for this reason that PEI 25 kDa was the most widely used in several applications.

Fig. (1). Chemical structure of branched polyethylenimine (PEI).

For a long time, PEI has been also used in non pharmaceutical processes, including water purification, paper and shampoo manufacturing. It has been also reported that PEI is relatively safe for internal use in animals and humans [8]. PEI is widely used to flocculate cellular contaminants, nucleic acids, lipids and debris from cellular homogenates to facilitate purification of soluble proteins [9-11]. Enzymatic reactions in bioprocesses constitute another field in which PEI was used: as an immobilizing agent for biocatalysts [12], as a soluble carrier of enzymes [13] or in the formation of macrocyclic metal complexes mimicking metalloenzymes [14]. PEI is also a common ingredient in a variety of formulations ranging from washing agents to packaging materials.

Several papers reported the use of PEI in the field of medicinal chemistry and in this brief critical review, examples appeared in the last decade were considered and discussed (Table 1).

PEI’s application fields may be divided according to its use in:

1.1. Use of PEI as a drug.

1.2. Use of PEI for delivery of small drugs, and for the photo dynamic therapy (PDT).

1.3. Use of PEI for antimicrobial coating.

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1.4. Use of PEI for the preparation of nanosized delivery vectors.

Polyethylenimines in dendritic core-shell architectures encapsulating drugs. [38]
Polyethylenimines in dendritic core-shell architectures derivatized with poly(ethylene glycol) chains [39]
Polyethylenimines derivatized with different fatty acids in dendritic core-shell architectures [40-41]
nanosized cationic hydrogels for drug delivery [42]
Polyethylenimine and poly(D,L-lactide-co-glycolide) in micelle-like polymer aggregates preparation [43]
Polyethylenimines for nanostructured delivery systems for proteins [44-46,48]
Polyethylenimines for nanoparticle-mediated nuclear drug delivery [47]

1.5. Use of PEI for non-invasive optical imaging devices.

Polyethylenimines for quantum dots coating [49]
Polyethylenimines for non-invasive optical imaging (Near Infrared, NIR) assessment of caspases’ activity in vitro [50]
Protein-phosphorylation-responsive, cell-permeable, and biocompatible polyethylenimines [51]
Conjugation of polyethylenimine with a NIR-dye to obtain multifunctional delivery vectors for DNA delivery in vivo [52]
An important biological function of PEI was reported by Chu et al., showing that PEI readily blocks fibrin formation, thus exhibiting anticoagulant activity [17]. This study demonstrated that even at a nanomolar concentration, PEI significantly blocks thrombin-catalyzed fibrin formation in vitro, accounting for its anticoagulant property. This competitive inhibition was independent on the concentration of fibrinogen (FBG), thrombin, or NaCl. PEI showed no effect on thrombin amidolytic activity, suggesting that the blockade of thrombin interaction with FBG could explain the inhibition on fibrin formation. PEI also drastically depressed rabbit brain thromboplastin procoagulation, as assessed by a single-stage clotting assay using human plasma. In a THP-1 monocytic hypercoagulation cell line, a 4-hours exposure to bactericidal endotoxin or Ca$^{2+}$ ionophore A23187, resulted in a 5- or 10-fold enhancement in monocytic tissue factor (mTF) procoagulation, respectively. Monocytic TF hypercoagulation was offset by PEI included in the assay mixture, that is able to arrest mTF hypercoagulation with an IC$_{50}$ = 1.2 nM.

The antibacterial properties of PEIs have been investigated in details and was applied in the development of coated materials (see also further). Helander [18] studied the effect of PEI on the permeability properties of the Gram-negative bacterial outer membrane (OM) using *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* as target organisms. The OM is present in all Gram-negative bacteria, and due to the presence of lipopolysaccharide (LPS) in the outer leaflet of the membrane, it forms a permeability barrier against hydrophobic substances and macromolecules. For this reason, Gram-negative bacteria exhibit higher resistance to detergents and hydrophobic antibiotics than do Gram-positive bacteria. Sensitization of Gram-negative bacteria to hydrophobic antibiotics is a property shared by all polycationic permeabilizing agent, which either release considerable amounts of LPS from the OM or bind to LPS without releasing it into the medium. Due to the polycationic nature of PEI, it could be expected that this polymer may act as an efficient OM-permeabilizing agent. As expected, even at a concentration lower than 20 μg/ml PEI increased the bacterial uptake of 1-N-phenylglycylglycylalanylarginine, a hydrophobic fluorescent probe, indicating an increased hydrophobic permeation of the outer membrane. PEI also increased the susceptibility of bacteria toward other hydrophobic antibiotics like clindamycin, erythromycin, fucidin, novobiocin and rifampicin, without being bactericidal itself. Moreover, PEI is able to sensitize the bacteria to the lytic action of the anionic detergent SDS when bacteria are opportunist pre-treated with the polymer. No sensitization to lysozyme was observed with PEI, in agreement with similar findings on the inability of other polycationic permeabilizers. All these results show that PEI is able not only to disorganize the OM in a transient way but also in an irreversible manner, most likely by intercalating in the LPS layer similarly to polymyxin B nonapeptide.

Recently, Gao [19] prepared and characterized quaternarized polyethyleneimine (QPEI) and investigated their antibacterial properties mainly by using *Escherichia coli* as model bacterium and the colony count method. The experiment shown that QPEI has outstanding activity due to the antibacterial groups on the macromolecular chains. The antibacterial ratio reached 100% for bacterium suspensions of 10$^9$ CFU/ml with a polymer concentration of 15 mg/l for a contact time of 4 min. The cationic degree influences the antibacterial ability of QPEI greatly, and the higher the cationic degree, the stronger the antibacterial activity. The experimental data indicated that the pI of the *E. coli* protein is probably 4.5. When pH > 4.5, the antibacterial activity of QPEI increased with increasing pH, and when pH > 6 the antibacterial ratio reached a maximum and remained nearly constant. The enzyme activity measurements revealed that the antibacterial action of QPEI was essentially due to a sterilization process. Similarly to the small quaternary ammonium salt, QPEI causes cell death by disrupting cell membranes and releasing the intracellular contents.

As a permeabilizing agent, the mechanism of polyethyleneimine antibacterial activity most likely consists in the osmotic swelling after its internalization and membrane disruption. PEI is an efficient proton-sponge and after internalization into endosome compartments, an influx of cations takes place to balance that of H$^+$. Protons and counterions definitely determine a swelling of the polymer and the collapse of the endosome structure. After this event drugs and polymer are released into the cytosol where they can exert their specific effect.

### 1.2. Use of PEI for Delivery of Small Drugs and for the Photo Dynamic Therapy (PDT)

Setty [20] studied the effect of coating alginate beads with polycations for controlled drug delivery of low molecular weight therapeutic agents. As polycation, PEI was selected for its several advantageous properties (hydrophilicity, biocompatibility and thermal stability) and furosemide was chosen as a model water-insoluble drug. The furosemide-loaded calcium alginate (ALG), calcium alginate-polyethyleneimine (ALG-PEI) and alginate-coated ALG-PEI (ALG-PEI-ALG) beads by ionotropic/polyelectrolyte complexation method to achieve controlled release of the drug were prepared. Drug-loading efficiency (DLE) of ALG beads varied from 97.16% to 100.49%, and variation in formulation factors (incubation, CaCl$_2$ concentration, initial drug load) was found not to influence the DLE of ALG beads. Anyway, release of furosemide from ALG beads in simulated intestinal fluid (SIF, phosphate buffer solution of pH 6.8) was rapid and complete in 2.5 hours, irrespective of the variation in formulation factors. The drug release was accompanied with a rapid swelling and erosion/disintegration of ALG beads and this phenomenon has been considered as a major disadvantage of ALG beads in sustaining drug release in SIF. PEI treatment of ALG beads, however, prolonged the drug release considerably. Increase in both PEI concentration and exposure time decreased DLE. Release of furosemide from ALG-PEI beads was prolonged considerably compared with that from ALG beads. Ionic interaction between alginate and PEI led to the formation of polyelectrolyte complex membrane, the thickness of which was dependent on the conditions of PEI treatment (PEI concentration and exposure time). The membrane acted as a physical barrier to drug release from ALG-PEI beads. The coating of ALG-PEI beads further prolonged the release of the drug by increasing membrane thickness and reducing swelling of the beads possibly by blocking the surface pores. Furthermore, it
was shown that the encapsulated drug was not degraded by PEI treatment.

The hypothesis that PEIs could enhance nasal absorption of the negatively charged drug was tested by Ashan and coworkers [21]. They reasoned that since DNA and low molecular weight heparins (LMWHs) have similar charge distribution properties (LMWHs are negatively charged oligosaccharides used in the treatment of deep vein thrombosis and pulmonary embolism), PEI should also be able to form a complex with LMWH via electrostatic interactions. If so, this should neutralize the drug’s surface charge and facilitate its absorption via mucosal routes. Therefore, they designed a study to test this hypothesis. They found that PEI can enhance nasal absorption of enoxaparin, a LMWH, and that such enhancement occurs through neutralization of the negatively charged glycosaminoglycan unit of the drug. In this regard, enoxaparin was formulated with PEIs of different molecular weights and the efficacy of PEI in enhancing nasal absorption of LMWH was tested in a rodent model. It was shown that PEIs neutralize the negative surface charge of LMWHs. This neutralization may weaken or diminish the coulombic repulsion effect between the negatively charged cell membrane and the drug and consequently increase drug absorption across the epithelium. Otherwise, PEI could competitively bind with the negatively charged molecules of the cell surface and subsequently release the drug after endocytosis, as it is widely believed to happen in PEI-enhanced DNA transfection. The efficacy of PEIs in enhancing the bioavailability of nasally administered LMWH, was studied by administering LMWH formulated with PEI, into the nose of anesthetized rats and monitoring the drug absorption. They found that the efficiency may be ranked as PEI-1000 kDa > PEI-750 kDa > PEI-25 kDa. When PEI-1000 kDa was used at a concentration of 0.25%, there was a 4-fold increase in both the absolute and relative bioavailabilities of LMWH compared to the control formulation. Thus, the authors concluded that polyethylenimines can be used as potential carriers for nasally administered LMWHs after nasal administration.

Hamblin’s research group has been involved in the use of photo dynamic therapy (PDT) as a possible treatment for localized infections [22]. They showed that covalent conjugates (Fig. 2) between PEI and chlorin(e6) (ce6) can be used as a potent broad-spectrum antimicrobial photo sensitizers (PS) resistant to protease degradation and therefore constituting an alternative to the previously described poly-L-lysine chlorin(e6) (pL-ce6) conjugates [23]. They prepared a novel set of second-generation polycationic conjugates between chlorin(e6) and three molecular forms of polyethylenimine (PEI): a small linear, a small cross-linked, and a large cross-linked molecule. The conjugates were synthesized, characterized and tested for their ability to kill a panel of pathogenic microorganisms, the gram-positive Staphylococcus aureus and Streptococcus pyogenes, the gram negative Escherichia coli and Pseudomonas aeruginosa, and the yeast Candida albicans, after exposure to low levels of red light. The large cross-linked molecule efficiently killed all organisms, while the linear conjugate killed gram-positive bacteria and C. albicans. The small cross-linked conjugate was the least efficient antimicrobial PS and its remarkably low activity could not be explained by reduced photochemical quantum yield or reduced cellular uptake. In contrast to polylysine conjugates, the PEI conjugates were resistant to degradation by proteases such as trypsin that hydrolyze lysine-lysine peptide bonds. In fact, this macromolecular vehicle does not contain peptide bonds and is therefore resistant
to protease degradation. The advantage of protease stability combined with the ready availability of PEI suggested that these molecules may be superior to polylysine-PS conjugates for photodynamic therapy of localized infections.

PEI was also studied in cancer antiangiogenic photodynamic therapy mediated by polycation liposomes by Oku’s research group [24, 25]. These studies indicated that antiangiogenic photodynamic therapy (PDT), consisting in a laser irradiation at 15 min post-injection of photosensitizer in vivo, is effective for cancer treatment, and a photosensitizer, benzoporphyrin derivative monoacid ring A (BPD-MA), encapsulated in polycation liposomes (PCLs), liposomes modified with cetylated polyethylenimine (cetyl-PEI), is more effective than BPD-MA encapsulated in non-modified liposomes.

1.3. Use of PEI for Antimicrobial Coating

In several papers, PEIs are used as such or in the presence of copolymers as bactericidal coating materials.

Bourgeois [26] used PEI to build a specific delivery system for β-lactamases. The aim of that study was to provide a “proof of concept” of colon delivery of β-lactamases by pectin beads aiming to degrade residual β-lactam antibiotics, in order to prevent the emergence of resistant bacterial strains. Pectin is almost totally degraded by pectinolytic enzymes produced by colon microflora, but it is not digested by gastric or intestinal enzymes. In addition, pectin beads could efficiently protect β-lactamases from degradation by proteases contained in the upper gastrointestinal tract. The specific delivery system for β-lactamases was composed of a core of calcium pectinate bead, cross-linked at its surface with PEI [27]. Pectin beads were prepared according to ionotropic gelation method using CaCl₂ as a gelling agent. Particles were then washed and soaked in PEI. Beads thus obtained were solid, with an ovoid shape and an internal matrix-like structure. Instantaneous gelation of pectin allowed an easy encapsulation of β-lactamases in Ca-pectinate beads with an efficiency of 86.5%. PEI improved the stability of Ca-pectinate beads, protecting them from water penetration by cross-linking the free carboxylic functions of the Ca-pectinate network. The cross-linking step do not influence shape, size and efficiency of encapsulation of β-lactamases in beads. Thus, PEI made Ca-pectinate beads resistant to the denaturing effect of upper intestine conditions, allowing to delay β-lactamases release. In vitro studies showed that β-lactamases were released from pectin beads in a suitable colonic medium due to the action of pectinolytic enzymes. When ampicillin was added to this medium, the release of β-lactamases induced, as expected, the antibiotic inactivation. Finally, after oral administration of loaded-beads to CD1 mice, β-lactamases were retrieved in high concentrations in faeces.

Also Klibanov et al. published several papers on this topic [28-32]. They faced the problem to develop non-leaching, permanent, sterile surface materials, to be used in hospitals and community settings, by covalently functionalizing their surface with an antimicrobial compound. They found that covalent attachment of long-chained, moderately hydrophobic polycations (such as quaternarized N-alkyl-PEI) to surfaces of solid objects (Fig. 3) renders the latter permanently bactericidal, killing a broad range of pathogens, gram positive and gram negative bacteria, as well as fungi [25].

Concerning the mechanism, flexible polymers apparently cross the microbial cell envelope, delivering the active moiety into the membrane and killing the pathogen. Only long-chained, moderately hydrophobic immobilized polycations exhibited microbicidal activity. The immobilized polycations were found to be unique and apparently with no analogues in nature. They are not subject to existing mechanisms of resis-

![Fig. (3). The treatment by covalent attachment of long-chained hydrophobic quaternarized N-alkyl-PEI to surfaces renders the latter permanently bactericidal.](image-url)
tance, such as multi-drug resistance pumps or multi-drug tolerant cells, and no resistance develops upon repeated exposure to the polymer.

In a follow-up work, Klibanov replaced the surface-specific, multistep immobilization techniques with a single-step general procedure, similar to common painting [26]. Glass or polyethylene slides were briefly dipped into organic solutions of certain optimally hydrophobic N-alkyl-PEI (where PEI stands for branched 750-kDa polyethylenimine) polycations, followed by solvent evaporation. The resultant polycation-coated slides were able to kill on contact all of the encountered bacterial cells. This biocide effect was found not to be caused by N-alkyl-PEI molecules leached from the surface. Further examination of the mechanism of this bactericidal action suggested that the surface-deposited N-alkyl-PEI kills bacteria by rupturing their cellular membranes. This conclusion was further supported by studies in which the molecular weight of PEI and the hydrophobicity of the alkyl moiety were varied.

The work moved further: reasoning that influenza virus belongs to a class of enveloped viruses and is than protected from the outside by a lipid membrane, it was thought that the aforementioned hydrophobic polycations might damage it as well, thereby inactivating the virus [27]. In fact, painting a glass slide with branched or linear N,N-dodecyl methylpolyethylenimines (PEIs) and certain other hydrophobic PEI derivatives enables it to kill even influenza virus with essentially a 100% efficiency within minutes. For most of the coating poly-ions, this virucidal action is shown to be on contact, i.e., solely by the polymeric chains anchored to the slide surface; for others, a contribution of the poly-ion leaching from the painted surface was supposed to increase the efficiency.

Domb and coworkers applied PEIs to confer antibacterial properties to the composite resin materials widely used in the dental clinic for replacement of hard tissues [33]. They wanted to test the hypothesis that the insoluble crosslinked quaternary ammonium polyethylenimine (PEI) nanoparticles in composite resin restorative materials have a stable and long-lasting antibacterial effect against oral bacteria, *Streptococcus mutans*, without affecting the flexural strength of the commercial materials. Nanoparticles with N-octyl,N,N-dimethyl ammonium groups were reproducibly prepared from PEI by crosslinking with 1,5-dibromopentane, followed by alkylation with bromoctane and quaternarization with methyl iodide. Antimicrobial assays using *S. mutants* showed that these PEI nanoparticles when incorporated in dental composite resins at low concentration exhibited a strong antibacterial effect against the tested bacteria regardless of the commercial composite resin to which they were added. They could exclude that the antibacterial activity was due merely to bioactive components released to the medium. Furthermore results indicated that the addition of a small amount (1%) of the PEI nanoparticles did not affect the mechanical properties of the restoration composites. Composite resin materials incorporated with PEI nanoparticles maintained antibacterial properties over 1 month without leaching out and displayed no alteration of the original mechanical properties. Results shown that for composite resin restorations, incorporation of antibacterial nanoparticles may prevent biofilm formation and secondary caries.

Moeller has reported a new approach for the preparation of amphiphilic antimicrobial polymers (Fig. 4) based on a one-step multi-functionalization of PEI with derivatized cyclic carbonates [34, 35]. The aim of this work was to prepare and characterize water-soluble polymers with a strong affinity to lipid membranes. The rationale was to substitute a water-soluble hyperbranched macromolecule (i.e. PEI) by alkyl chains and ammonium groups, in such a way that the water solubility was preserved but with the polymers able to adsorb to lipid membranes at the same time. These amphiphilic molecules could be of interest for the preparation of new antimicrobial polymers and bactericidal or bacteria-repellent surfaces, in order to provide solutions for one of the biggest problems of modern medicine. Depending on their hydrophilic/hydrophobic balance, the obtained polymers could be used as water-soluble disinfectants and for antimicrobial coating materials. Primary amine groups of branched PEI were functionalized with quaternary ammonium groups, alkyl chains of different length, allylic and benzyl groups in a one-step reaction, using a carbonate coupler. The bactericidal properties of some of the amphiphilic polymers against Gram-negative and Gram-positive bacteria were investigated in solution regarding the effect of (i) the length of the alkyl chains, (ii) the hydrophilic/hydrophobic balance, and (iii) the kind of spacer between the cationic moiety and the polymer. Minimal inhibitory concentrations (a log 4 reduction of bacterial growth) against *Escherichia coli* and *Bacillus subtilis* were determined in the range of 0.3-0.4 mg/mL and 0.03-0.04 mg/mL for water-soluble polymers. Glass slides coated with functionalized PEIs showed a reduc-

![Fig. (4).](image-url) The preparation of amphiphilic antimicrobial polymers based on a one-step multi-functionalization of PEI with derivatized cyclic carbonates.
tion of colony forming units of at least 95%, up to 99.9%, against *E. coli* and *B. subtilis*. However, it was observed that polymers leached out of the coating. This suggested the authors to consider an improvement of the cross-linking method or the development of a covalent bond between the polymer and the surface.

PEI has been used in the construction of polyelectrolyte multilayer films that are able to coat any type of surface (e.g., metals and plastics) without shape limitation (e.g., planar, spherical, or curved). This layer-by-layer technique was recently developed with the aim of using it for different biological applications in the fields of biosensors, cell signaling control, and anti-adhesive surfaces. Egles [36] carried out studies concerning biofunctionalized interfaces, able to protect against infection of implanted materials by bacteria, one of the most serious complications following prosthetic surgery. They developed a new strategy based on the insertion of an antimicrobial peptide (defensin from *Anopheles gambiae* mosquitoes) into films built by the alternate deposition of polyanions and polycations, with the aim to isolate the peptides while retaining their bioactivities. Polyethyleneimine (PEI), poly(sodium 4-styrenesulfonate) (PSS), poly(allylamine hydrochloride) (PAH), poly(L-glutamic acid) (PGA), and poly(L-lysine) (PLL) were used to build the films. Multilayer films were prepared either on 12-mm glass coverslips or in 96-well plastic plates. PEI-(PSS-PAH) 2-(PGA-PLL)2-PGA films had a thickness of about 40 nm. Defensin peptides were embedded in the multilayer structure by adsorption during film construction. Noticeably, the biofunctionalization could be achieved only when positively charged poly(L-lysine) was the outermost layer of the film, likely because the close interaction of the bacteria with the positively charged ends of the films allows defensin to interact with the bacterial membrane structure. Antimicrobial assays were performed with two strains: *Micrococcus luteus* (a gram-positive bacterium) and *Escherichia coli* D22 (a gram-negative bacterium). The inhibition of *E. coli* D22 growth at the surface of defensin-functionalized films was found to be 98% when 10 antimicrobial peptide layers were inserted in the film architecture. Thus, the combination of good biocompatibility found for polyelectrolyte multilayer films and their high degree of stability, together with the possibility of varying the number of adsorbed active proteins or peptides and their amounts, could lead to biomedical applications ranging from the protection of several tools used for medical applications, such as catheter, needles, surgical tools, and tubes, to all types of materials that come into contact with wounds for a restricted period of time.

The research of Ji and coworkers advanced in the same field [37]. They explored the possibility to form albumin multilayers as a coating for biomedical 316L stainless steel, with the goal of developing a fast, easy processing and shape-independent method for non-thrombogenic coating. The 316L stainless steel is widely used in coronary stents, but the exposure to flowing blood of the metallic stents can result in thrombus formation. A confluent layer of albumin is believed to be both anti-thrombogenic and anti-infective. The “electrostatic self assembly” (ESA) method, which is based on the alternating physisorption of oppositely charged polyelectrolytes, represents a new, alternative solution for biomaterial. The buildup is easy and the procedure can be adapted to almost any type of surface. Moreover, the method is still valid whatever is the shape of the solid. Multilayer films consisting of polyethylenimine (PEI) and albumin were successfully prepared on biomedical 316L stainless steel surface, they were found stable in Tris--HCl (pH 7.35) buffer solution for 21 days, whereas less than 10% albumin was eluted by citrate phosphate buffered saline in 45 days. Static platelet adhesion experiments indicated that the PEI/albumin deposited on stainless steel could resist platelet adhesion effectively. Such an easy processing and shape-independent method may have good potential for surface modification of cardiovascular devices.

### 1.4. Use of PEI for the Preparation of Nanosized Delivery Vectors

In several papers, PEI takes part to the composition of nanoparticles used for drug delivery. The advantages of using nanoparticles for drug delivery result from their small size, that allow for the penetration through even small capillaries up to cytoplasm, allowing also an efficient drug accumulation at the target sites in the body. Furthermore, the use of biodegradable materials for nanoparticles preparation allows for sustained drug release within the target site over a period of days or even weeks after injection.

So, Haag and coworkers used functionalized hyperbranched PEI to build nanosized dendritic core-shell architectures (Fig. 5) able to encapsulate guest molecules potentially useful as drug delivery systems [38]. To this scope, the release of the encapsulated species occurred as a result of the pH drop in tumor and infected tissues (pH 5-6). It is well
known that imine bonds are sensitive to pH changes in a pH range of 5–7, thus they attached two different carboxyl compounds to the terminal amino groups of hyperbranched PEI leading to the formation of imine compounds in high yields (70–90%) and in multigram quantities. The transport capacities of these molecular nanocarriers were first determined using the congo red dye as an easily detectable polar model compound, used as pH indicator and as a neuroprotective drug. Results showed that a minimum core size (ca. 3000 g mol⁻¹) and a highly branched architecture are required for successful encapsulation of the guest molecules similarly to what found for other dendritic core-shell architectures. For efficient transport the degree of alkylation should be about 45–50% and the alkyl chains should have a minimum length (>C10). The transport properties of these nanocarriers were tested with different guests. Many other organic dyes, such as bromophenol blue, methyl orange, methyl red, and fluorescein, all of which contain polar anionic sulfonate or carboxylate groups and sodium counterions, were readily encapsulated and transported. In contrast, cationic dyes, such as the triphenylmethyl-based malachite green with an oxalate counterion, were not transported at all. The complexation of an antitumor drug (mercaptopurine), several oligonucleotides, as well as bacteriostatic silver compounds have been studied for the potential use of these nanocarriers in drug and gene delivery. Successful encapsulation and transport were observed in all cases by the PEI-based nanocarriers.

The higher selectivity for large anionic guest molecules can be explained by the strong interaction of such species with the polar groups in the core of these dendritic macro-molecules. Using several buffer solutions, it was found that the imine-based nanocarriers are very sensitive to an external drop in the pH value: the hydrolysis of the shell and the release of the encapsulated guest congo red occurs spontaneously at pH<7, whereas it is stable over several weeks at neutral pH opening the possibility of selectively release the encapsulated guest molecules in a physiologically relevant pH range. In a further work [39] they described the synthesis of readily accessible dendritic core-shell architectures with biocompatible poly(ethylene glycol) chains. Although the toxicity of PEI is relatively high, it has recently been demonstrated that polyethylenglycol (PEG) modification of these structures can reduce their toxicity dramatically, even for in vivo applications. PEIs can be modified with different shells, such as polyamidoamine (PAMAM) or polyethylene glycol to build water-soluble core-shell architectures with the core. Surprisingly, these shell-coated nanocarriers exhibited much higher transport capacities than the unfunctionalized PEI, which suggests that the core-shell architectures can further stabilize the host-guest system. The stability of the nanocarriers was evaluated with three different buffer solutions (pH 5, 7 and 8), showing that pH labile shell can be cleaved by lowering the pH to a value of 5–6. The pH-sensitivity is in the same range as observed in malignant tissues (tumor, infection) or endosomes and hence could be used as a trigger to release the encapsulated drugs from these nanocarriers.

In another approach, dendritic core-shell architectures were synthesized by simple melt reactions of polyethyleneimine (PEI) with different fatty acids [40]. The degree of functionalization of the PEI amides could be simply controlled by the stoichiometrical amounts of acid and PEI applied in bulk. These values were used to calculate the respective molecular weight. The structure-activity relationship between the guest and the host molecules was examined with various dyes and drug molecules. It was found that the transport capacity rises with decreasing concentration of the polymer in solution resulting in a maximum transport of around 3 g dye/g polymer or up to 150 guest molecules per dendritic core-shell architecture. Larger aggregates were formed at higher concentration resulting in a decreased number of encapsulated guest molecules due to more polymer-polymer interaction. The maximum encapsulation of the guest molecule is achieved at a pH between 5 and 8. The absence of water lead to a decreased number of encapsulated guest molecules, due to the formation of small aggregates.

Thünemann and coworkers [41] developed nanoparticle systems based on PEI as carriers for hydrophobic drugs. In these systems, the polymer caused the complexation of a fatty acid as well as the stabilization of the particles. Thus, PEI was used for the complexation of dodecanonic acid (C12) resulting in a poly(ethylene imine) dodecanoate complex (PEI–C12) with a stoichiometry of amino functions to carboxylic acid functions 2:1, a lamellar nanostructure and a repeating unit of 2.9 nm at room temperature. PEI–C12 was doped with coenzyme Q10 and the hormone triiodothyronine as typical hydrophobic and pharmacological active compounds, respectively. The PEI–C12 was shown to act as a guest matrix that dissolves the above mentioned molecules up to 20% (w/w) and 15% (w/w), respectively, forming homogeneous structures stable at room temperatures for at least 3 months.

Kabanov has recently reviewed the progress in the field of nanosized cationic hydrogels for drug delivery summarizing preparation methods, properties and interactions with cells [42]. These hydrogels belong to the family of nanoscale materials based on dispersed networks of cross-linked ionic and nonionic hydrophilic polymers. This work was focused on the nanosized cationic network of cross-linked poly(ethylene oxide) (PEO) and polyethyleneimine (PEI), PEO-cl-PEI nanogels. These appeared to be promising and versatile systems for drug delivery. The synthesis of micro- and nano-gels was carried out following different approaches (emulsion polymerization or copolymerization at elevated temperature using rapidly stirred solutions, addition or step-growth polymerization of the polyfunctional monomers in solution using a wide range of cross-linking systems). By varying the reaction conditions, including the type and dispersity of the media, addition of surfactants, temperature, and ratio of reagents the size of the particles could be controlled. In particular, PEO-cl-PEI nanogels were synthesized by cross-linking of branched PEI (25 kDa) with bis-activated PEO (8 kDa) molecules. Fine dispersed systems were obtained when the cross-linking reaction was performed by a modified solvent emulsification/evaporation method, leading to the formation of particles of 30 to 300 nm size. Formation of polyion-complexes leads to the collapse of the dispersed gel particles (Fig. 6). However, the complexes form stable aqueous dispersions due to the stabilizing effect of the PEO chain. These systems allow for immobilization of negatively charged biologically active compounds such as retinoic acid, indomethacin and oligonucleotides (bound to polycation
chains) or hydrophobic molecules (incorporated into nonpolar regions of polyion-surfactant complexes), leading to the formation of nanocomposite materials in which the hydrophobic regions from polyion-complexes are joined by the hydrophilic PEO chains. The average hydrodynamic diameters observed for the loaded PEO-cl-PEI nanogel (size within 100 nm) fitted well within the preferred size range for drug delivery systems. In fact, particles with diameters of less than 5–10 nm are rapidly removed through extravasation and renal clearance, while larger particles (ranging from ca. 10 to 70 nm) are still small enough to penetrate even the very small capillaries within the body tissues, and therefore may offer the most effective distribution in certain tissues. To allow for the targeted delivery of nanogels in the body, the surface of the nanogel particles have been modified with various biospecific ligands, polypeptide ligands to enhance receptor-mediated delivery. The advantages of these systems include simplicity of formulation with the drugs, high loading capacity and stability of the resulting formulation in dispersion. These systems allow for the immobilization of biologically active compounds of diverse structure including charged drugs, low molecular mass hydrophobic molecules and biopolymers. Furthermore, nanogels can be chemically modified to incorporate various ligands for targeted drug delivery. The in vitro studies suggest that nanogels can be used for efficient delivery of biopharmaceuticals in cells as well as for increasing drug delivery across cellular barriers.

Nam [43] proposed new micelle-like polymer aggregates prepared from oligomeric polyethylenimine and poly(D,L-lactide-co-glycolide) (PEI–PLGA) di-block copolymers, and investigated their micellar characteristics in aqueous media. PEI–PLGA were synthesized by directly coupling PLGA with a carboxy-terminal group of PEI. The block copolymers were prepared by varying the length of the hydrophobic PLGA block ($M_n = 6, 10$, and $21 \text{ K}$), while that of the hydrophilic PEI block ($M_n = 423$) was fixed. PEI–PLGA block copolymers were found to be self-assembled in water by using a PLGA segment as a hydrophobic aggregate block and a PEI segment as a hydrophilic corona-forming block. The block copolymers formed micelle-like aggregates with critical association concentration (CAC) in the range of $1.54–2.57 \times 10^{-3} \text{ g/l}$ in water. It was found that the size and CAC of the aggregates depended on the hydrophobic block length: as the hydrophobic PLGA block length became higher, lower CAC values were obtained. The dependency of the aggregate size and morphology on interactive small molecules (e.g., ions) could provide a key parameter to control aggregate structures by simply changing the medium composition during the manufacturing process. The cellular uptake behavior of PEI–PLGA aggregates was compared with that of plain PLGA showing that PEI–PLGA aggregates were readily adsorbed onto the cell surfaces and translocated into the cytoplasm, implying their versatile applicability as a drug carrier.

A couple of papers by Middaugh [44] and Yamada [45] reported the use of PEIs for protein transduction. Middaugh et al. developed a nanoparticle delivery systems (Fig. 7) which have the potential to deliver proteins, by improving their stability, increasing the duration of their therapeutic effect as well as permitting administration through non-parenteral routes [40]. In fact, a major problem in proteins administration is that they are often marginally stable and consequently easily damaged during their formulation as drugs. The authors described nanoparticles made by polyethylenimine (PEI) and dextran sulfate (DS) under aqueous conditions and stabilized by crosslinking with $\text{Zn}^{2+}$ ions. The oppositely charged polymers self-assemble through phase separation and form nanoparticles at room temperature. Thus, this technique is in principle applicable to a broad range of labile drugs and bioactive macromolecules including proteins and has been applied to low molecular weight small molecule drugs [46]. In this case the author considered the possibility of incorporating a protein into this nanoparticle system while maintaining the protein’s conformation and stability, using insulin as a model protein drug since its structure, stability and physicochemical characteristics have been extensively studied and it has a wide therapeutic utility. It was found that the pH of PEI solutions, the weight ratio of the two polymers, and zinc sulfate concentration play significant roles in controlling particle size. Spherical particles of 250 nm mean diameter were produced under optimal conditions with a zeta potential of approximately $+30\text{mV}$. The association of insulin with the particles appears to be an efficient process. Most of the formulations studied demonstrated an entrapment efficiency of 80–90%. Furthermore, results suggest that there was no significant insulin degradation upon incorporating insulin into PEI–DS particles and no significant conformational changes compared to free insulin under optimized formulation conditions. Rapid release characteristics were observed in in vitro dissolution studies: in
most formulations, insulin was completely released from nanoparticles within 5 min, independently of the PEI solution’s pH and the polymer ratio. Biological activity in streptozotocin-induced diabetic rats, however, exhibited a prolonged hypoglycemic effect.

Shen and coworkers [47] were interested in nanoparticle-mediated nuclear drug delivery and PEI nanoparticles are well suited to this scope, considering their wide use in gene delivery. However, PEI is a highly positive charged molecule at physiological pH, and may elicit some adverse reaction in vivo. Thus, they reported PEI nanoparticles with a negative-to-positive charge-reversal triggered by the solid tumor acidity (pH<7) or lysosomal (pH 4–5) for nuclear drug delivery. To this scope, polycaprolactone (Mn=3800)-block-PEI(Mn=1800) (PCL-PEI) was synthesized and its PEI blocks reacted with 1,2-cyclohexanedicarboxylic anhydride to convert the primary and secondary amines into correspondent amides (PCL-PEI/amide). The PEI block with 20% of its primary and secondary amines converted into their amides was found optimal in terms of the charge-reversal kinetics of the resulting nanoparticles. Folic acid (FA) moieties were also conjugated to the PEI block to form PCL-b-PEI/amide-FA for folate-receptor targeting (Fig. 7). The PCL-PEI/amide-FA formed nanoparticles of about 210 nm in diameter in water. The nanoparticles were about 120 nm in diameter if loaded with 14.6 wt% doxorubicin (DOX). The hydrolysis kinetics of the amides in the PCL-PEI/amide was tested at several pH values (the amides were hydrolyzed to more than 75% and 50% at pH values of 5.0 and 6.0, respectively, after 24 hours) and the charge reversal of the PCL-PEI/amide micelles was confirmed by measuring their \( \zeta \) potentials at different acidities corroborating the hypothesis that amides with \( \beta \)-carboxylic acids can hydrolyze in acidic conditions to regenerate the amines, giving rise to a negative-to-positive charge reversal. In vitro experiment showed that targeted charge-reversal nanoparticles TCRNs/DOX are more effective in killing SKOV-3 cancer cells than the free doxorubicin.

Finally, Yamada and coworkers [48] reported that cationized-proteins covalently modified with PEI (direct PEI-cationization) efficiently enter into cells. They investigated if a protein could be delivered into cells just by mixing the protein with a PEI-cationized carrier protein having a specific affinity (indirect PEI-cationization) (Fig. 8). PEI-cationized avidin (PEI-avidin), streptavidin (PEI-streptavidin), and protein G (PEI-protein G) were prepared by a carbodiimide reaction and the delivery of biotinylated proteins and antibodies into living cells was investigated.

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**Fig. (7).** The structure of the targeted charge-reversal nanoparticle (TCRN) and its pH-triggered charge reversal.

**Fig. (8).** Protein cationized directly with PEI by carbodiimide reaction (A). Biotinylated protein complexed with PEI-avidin or PEI-streptavidin (B). Antibody bound to PEI-protein G (C).
Results showed that PEI-avidin (and/or PEI-streptavidin) is able to convey very efficiently a biotinylated GFP (green fluorescent protein) into various mammalian cells. A GFP variant containing a nuclear localization signal was found to enter in the nucleus. These results, indicate that indirect PEI-cationization using non-covalent interaction could be as effective as the direct PEI-cationization for the transduction of proteins into living cells and for expression of their functions in the cytosol.

1.5. Use of PEI for Non-Invasive Optical Imaging Devices

Nie [49] used PEI to coat the surface of quantum dots (QD). The main difficulty of using QDs for cell-labeling resides in their delivery into the cytoplasm. However, PEI is not only able to move across cell membranes through rapid endocytosis but is also able to disrupt intracellular organelles through a “proton sponge” effect (see Introduction). Hyperbranched copolymer ligands such as polyethylene glycol (PEG) grafted polyethylenimine (PEI-g-PEG) were found to encapsulate and solubilize luminescent quantum dots through direct ligand-exchange reactions (Fig. 9). The grafted PEG segment was found essential for reducing the cytotoxicity of PEI as well as for improving the overall nanoparticle stability and biocompatibility. In comparison with previous QDs encapsulated with amphiphilic polymers, the cell-penetrating QDs were smaller in size and considerably more stable in acidic environments. Cellular uptake and imaging studies revealed that QDs coated with PEI-g-PEG2 are rapidly endocytosed and are initially stored in vesicles, then a slow endosomal escape and the release into the cytoplasm was observed.

PEI is also an important polymer for non-invasive optical imaging devices (Near Infrared, NIR) enabling the assessment of several cellular functions like caspases’ activity in vitro [50]. The cell-permeable branched polyethyleneimine (25 kDa), was modified with deoxycholic acid (DOCA) hydroxysuccinimide ester, resulting in PEI-DOCA nanoparticles (Fig. 10). After attaching the effector caspase-specific near-infrared (NIR) fluorescence probe, Cy5.5-DEVD, to amphiphilic bile acid-modified polymer backbone, this polymeric nanoparticle system can be easily controlled with the optical imaging technique. The imaging-probe entry into cells is an important area in apoptosis imaging because the caspases’ reaction occurs in the cytoplasm. Thus, the tracking of the fluorescein isothiocyanate (FITC)-labeled Cy5.5-DEVD26-PEI-DOCA20 nanoparticles in HeLa cells allowed for the monitoring of both caspase-3 and caspase-7 activity. Therefore, this polymeric nanoparticles can be used to measure apoptosis in cell-based high-throughput screens for inhibitors or inducers of apoptosis.

The use of PEI was also described in a recent paper where protein-phosphorylation-responsive, cell-permeable, and biocompatible polymeric nanoparticles for visualizing protein phosphorylation by protein kinase A (PKA) were reported (Fig. 11) [51]. Protein kinase A is one of the best studied and most important kinases in single cells. The polymeric nanoparticles possess a PKA specific peptide motif (Leu-Arg-Arg-Ala-Ser-Leu-Gly, termed kemptide) and are easily prepared by the self-assembly of a polyion-induced complex (PIC) composed of both positively and
negatively charged polyelectrolytes, as previously reported. To produce a phosphorylation-responsive polyelectrolyte, a positively charged polymer conjugate by chemical coupling of kemptide and Cy5.5 to poly(ethyleneimine) (25 kDa) was synthesized. Upon protein phosphorylation, PIC nanoparticles dissolve because negatively charged phosphate groups are incorporated into the serine residue of kemptide, resulting in polyelectrolyte solubilization. This new cellular imaging system allows to explore protein kinase activities in various single living cells that express protein kinases. The technique may also be applied to high-throughput cell-based drug-screening systems targeting protein kinases.

In our recent work [52] the conjugation of PEI with a near infrared (NIR) dye is aimed to obtain a multifunctional delivery vector whose localization can be monitored in vivo with non invasive techniques and that may serve to identify potential transfection sites and assess its efficiency to deliver DNA. To obtain the NIR-PEI conjugate, the indocyanine dye IR-820 was employed. The heterocyclic nitrogen atoms of IR-820 bear two alkyl-sulfonate groups that improve photosensitivity and provide a sphere of solvation in water, preventing this dye from aggregation. The NIR dye-polymer conjugate (IR820-PEI) is highly soluble in water, absorbs at 665 nm and emits at 780 nm displaying a large Stokes’ shift (115 nm). These characteristics make this system more suitable for use as fluorescence probes than do common cyanine dyes. This multifunctional polymeric molecule enabled to monitor the DNA delivery in vivo with optical imaging techniques.

Even if other devices are actually available in several cellular and molecular research fields, the versatility of polyethylenimine and its derivatives not only resides in the ability to complex DNA [61], siRNA, miRNA, PNA [53] and other molecules and deliver them in vitro and in vivo, but also in the possibility to obtain multifunctional delivery systems with improved properties (i.e.; superparamagnetic) following straightforward functionalization reactions. An example of PEI-based vectors bound to iron oxide nanoparticles having superparamagnetic properties was recently reported [54, 55]. Finally, several efforts have been made to render this polymeric molecule “tissue-specific” by coupling it with suitable monoclonal antibodies obtaining targeted DNA delivery systems. Therefore, polyethylenimine and its numerous derivatives will pave the way to great promises in a near future, both in basic sciences and biomedical applications.

2. CONCLUSIONS

This critical review was aimed to discuss the versatility of polyethylenimine as a powerful molecule in several medicinal chemistry applications ranging from drug delivery (other than gene therapy), use of PEI per se, or as a versatile moiety for imaging devices or other multifunctional systems.

The reviewed works indicate that PEI is a versatile molecule. PEI may be successfully employed in a large variety of biomedical applications. However, some intrinsic characteristics limit the use of this polymer as is. In particular, in vitro studies have demonstrated that PEI (branched, 25 kDa) may be cytotoxic most likely due to lack of biodegradability [56, 57]. This could be attributed to non-degradable methylene backbone. Moreover, recently PEI and poly(L-lysine) were reported to behave as apoptotic agents [58, 59].

Despite these limitations, suitable chemical derivatization and functionalization may diminish the cytotoxic effect of these polyanime polymers [60]. Other PEI hydrophobic
functionalizations have been demonstrated to diminish the transfection efficiency of slightly derivatized polymers but their ability to deliver other molecules like siRNA, miRNAs, PNA, and other drugs remained unaltered [61].

All of these results clearly indicate that more studies are needed to completely understand the mechanisms of actions, the suitable dosage for eliminating side effects, and study all the potential biomedical and clinical application of such a versatile polymer.

REFERENCES


