Lipoplexes and Polyplexes for Targeted Gene Delivery

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1 Introduction

Targeted gene delivery is a scientific approach with numerous advantages in the fields of bio- and personalized medicine [1–10]. The success of targeted gene therapy relies on gene transfer to cells and subcellular organelles and effective transgene expression. The in vivo effectiveness is strongly dependent on the delivery system, the physicochemical properties of the gene, the route of administration, and the target organelles [1–10]. Firstly, different chemical modifications have been used to alter the pharmacodynamic and pharmacokinetic behavior of nucleic acids and to achieve targeting to cells and issues. This scientific approach presented many limitations. For this reason, the nonviral delivery vectors and the nanosystems were the ideal carriers for the improvement of the stability of nucleic acids. According to the literature, up to 2016, "*Nonviral gene therapy has maintained its position as an*

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approach for treating cancer. This is reflected by the fact that more than 17% of all gene therapy trials employ nonviral approaches. Thus, nonviral vectors have emerged as a clinical alternative to viral vectors for the appropriate expression and delivery of therapeutic genes" [7]. Nowadays, this percentage has increased by more than 30% [10]. Additionally, they can ameliorate their administration, distribution, metabolism, and Excretion (ADME) profile of them because they can achieve cellular and tissue targeting. Furthermore, these nanosystems that have been modified by proteins and/or peptides are also able to facilitate nuclear translocation and enhance the efficacy of gene expression [5]. The overcome of intracellular barriers and the sustained targeted expression can also be designed by the nanosystems [3–8].

Delivery systems based on nanomaterials have been already used as nanomedicines and nanovaccines, as marketed products, and they exhibit higher loading capacity and ease of fabrication [9, 10]. Formulation scientists can manipulate their characteristics (i.e., surface charge, size, etc.) and the method of their preparation to achieve the ideal properties in vitro and in vivo. Namely, a positive surface charge allows carriers to interact electrostatically with anionic nucleic acids and antigens. In fact, cationic lipids and polymers can be utilized as carriers in gene therapy, especially for cancer treatment [1–10]. Except for cancer treatment, gene therapy is also widely utilized in ocular gene delivery and cardiovascular diseases. For gene therapy, lipid and polymer gene vectors condense negatively charged oligonucleotides and form a well-organized complex, also called lipoplex and polyplex, respectively. In general, these complexes because of Coulomb attractions and ion-pair mechanism interact with plasma and endosomal membranes leading to rapid cellular uptake, endosomal escape, and as a result gene silencing efficacy. However, it is well-known that cationic vectors exhibit extensive cytotoxicity and rapid clearance from the bloodstream, also called as "polycation dilemma" due to positive surface charge, which induces reactive oxygen species (ROS) formation and rather significant interactions with blood cell membranes and proteins [5–10]. There has been extensive research to solve these problems and a prominent solution is a PEGylation, although there are limitations to this approach, such as the "PEG dilemma," and efforts are needed to overcome them [9, 10].

This chapter aims to present the technology and the applications of lipoplexes and polyplexes in the field of targeted gene delivery. Special attention will be given to the mechanisms by which lipoplexes and polyplexes are used for the delivery and the release of the complexed nucleic acids. Several examples from the recent literature will be discussed.

2 The Technology of Lipoplexes

In the late 1990s, Radler et al. described the complexation process of cationic liposomes with DNA [11]. The formation of multilamellar lipid/DNA complexes was observed by several techniques and was fully characterized regarding their structure

and their properties. The mechanism of lipoplex formation is well established in the literature [11–15]. The negatively charged nucleic acids are attached to positively charged lipid molecules. This formation mechanism generally produces multilamellar liposomal structures [11–15]. The thickness of the lipid bilayer is around 4 nm and is spaced 2 nm apart from each other lipid bilayer by the negatively RNA/DNA biomacromolecules. These complexes opened a new horizon for gene delivery in several biomedical areas [11–15].

The main lipids that are used for the formation of lipoplexes are the ones with quaternary ammonium function, i.e., 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) or N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl-ammonium methyl sulfate (DOTMA). DOTAP is also characterized as pH-sensitive lipid and is used for lysosome delivery. Synthetic lipids are also used for the preparation of lipoplexes [11–20]. For example, Sato et al. synthesized a cationic pH-responsive lipid to formulate a multifunctional envelope-type nanodevice for gene delivery and targeting useful in cancer immunotherapy [15]. Cholesterol and other "helper" lipids are also components of the lipoplexes [16]. Their role is important because lipoplexes possessing cholesterol domains exhibit higher transfection efficiency in systematic circulation. Generally, the "rich" cholesterol domains in the lipid-DNA complex do not bind serum proteins such as albumin, and this may enable these moieties to enhance transfection efficiency by ameliorating membrane interactions and the final fusion [16, 17]. Cholesterol and the "helper" lipids influence the physicochemical and biological characteristics of the lipoplexes and for this reason, are extensively used for the development of lipoplexes [15–17].

From the technological point of view, several factors are crucial for the design and delivery of lipoplexes [18–26]. Firstly, the lipid/nucleic acid ratio and the chemical treatment of lipid before the complexation process with the nucleic acids are the main preformulation studies that should be done before choosing the formulation protocol [18]. Several studies in the literature showed that different lipids exhibit different transfection efficiency of DNA [18]. According to the literature findings, the chemical structure and the concentration of salts and biomolecules (i.e., serum components) present in the lipid/nucleic acid complexation medium are also crucial preformulation parameters [12, 18]. Except the lipid composition, the size, the size distribution, the fluidity, and the lamellarity of cationic liposomes affect the transfection efficiency of lipoplexes [19]. For example, Ramenzani et al. developed liposomes using several types of cationic lipids like 1,2-dioleoyl-3-trim ethylammonium-propane (DOTAP) or 3-beta-[N-(N′N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-CHOL) in combination with other (helper) lipids including 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), egg L-alpha-phosphatidylcholine (EPC), and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) [19]. The different formulations caused lipoplexes with different sizes, lamellarity, and consequently different transfection efficiency of the attached nucleic acid [19]. According to Koynova [20], "*the phase evolution of lipoplex lipids upon interaction and mixing with membrane lipids appears to be decisive for transfection success: specifically, lamellar lipoplex formulations, which were readily susceptible*

to undergoing lamellar-nonlamellar phase transition upon mixing with cellular lipids and were found rather consistently associated with superior transfection potency, presumably as a result of facilitated DNA release." Furthermore, the formation of cubic phases of lipoplexes composed of two novel cationic lipids (O-alkyl-phosphatidylcholines, 1,2-dioleoyl- sn-glycero-3-hexylphosphocholine (C6-DOPC) and 1,2-dierucoyl- sn-glycero-3-ethylphosphocholine (di22:1-EPC)) showed that the transfection efficiency is also related to lipoplex microstructure and microfluidity [21]. On the other hand, it seems therefore that the lipoplex structures have not any influence on transfection efficiency and cytotoxicity in the experimental procedure followed by Congiu et al. [22]. It should be highlighted that according to other authors, the morphologies of lipoplexes should be evaluated at two levels, size and self-assembled structures of lipoplexes, and understanding these aspects would be very important for formulation scientists to develop innovative systems with high transfection efficiency and low toxicity [23]. Regarding the formulation protocols, thin-film hydration method, ethanol injection, and a modified ethanol injection method have been used for the preparation of lipoplexes with DNA [23–25]. The selection of lipids is also crucial for the selection of the preparation protocol [23–25].

The aforementioned parameters, i.e., structure, size, lamellarity, and method of preparation, are important for the serum protein attraction, the toxicity, and the transfection efficiency [26–28]. Additionally, the coating of the lipoplex surface with hydrophilic polymers like polyethylene glycol (PEG) is a strategy to ameliorate the stability of the prepared complexes and the selectivity of cellular targeting [29–31]. In the case of PEG or PEGylated lipid utilization, the biodistribution of the complexes is also ameliorated because the blood concentration is elaborated and the targeting of specific tissues is achieved [29–31]. Lipoplexes can be administrated by different routes including the i.v. delivery, nasal route for brain delivery, intramuscular, etc. [11–31].

On the other hand, there are some limitations in the use of lipoplexes that the formulation scientists should overcome for their effective utilization in gene delivery and targeting [32–36]. Firstly, the toxicity of cationic lipids should be considered in several studies, i.e., evaluation of toxic effects in physiological cell lines and long-term adverse reactions (mutations, etc.) [32–36]. Additionally, the transfection efficiency of lipoplexes is low in comparison to other engineered nanoparticles and viral vectors. Some authors believe that the deeper study of interactions of the lipoplexes with the cellular membranes is the crucial step to understanding the toxicity and the low transfection efficiency of the lipoplexes [32–36]. Marchini et al. proposed the formation of multicomponent lipoplexes to overcome the low transfection efficiency of lipoplexes [36]. This kind of complex exhibited a distinctive ability of endosomal escape and release DNA into the nucleus [36].

3 The Technology of Polyplexes

Polyplexes are the complexes between polymers and nucleic acids (DNA/RNA). Several parameters ranging from the structure of polymers to the physicochemical characteristics of the resulting complexes are crucial for the design and development of polyplexes. Tang and Szoka [37] studied the ability of four cationic polymers to interact with DNA, forming polyplexes and delivering their cargo to cultured cells. The polymers that they used exhibited different chemical structures including polylysine, intact polyamidoamine dendrimer, fractured polyamidoamine dendrimer, and polyethyleneimine. All these cationic polymers interact via electrostatic attractive interactions with DNA forming a unit structure with nanoscopic size and variable morphology. On the other hand, the morphology of the resulted complexes was found to be strongly dependent on the structure/architecture of the polymer [37]. The charge density of chitosan, a biopolymer, and the number of charges per chain were found to be the crucial factors for the morphology and colloidal stability of its DNA complexes [38]. The presence of surfactant is also important for the control of size and colloidal stability of the resulting polyplexes [39]. The branched and linear polyethyleneimines, poly[N-ethyl-4-vinyl pyridinium bromide], polyamidoamine dendrimer, poly(propyleneimine) dendrimer, and a conjugate of Pluronic P123 and polyethyleneimine (P123-g-PEI(2K)) also block copolymers that have been studied for gene transfection [40]. The highest transfection activity and lowest cytotoxicity were achieved by the linear structures in comparison to branched ones [40]. The particle size, the colloidal stability, the cellular uptake, and the resistance to nuclease degradation were also studied by the same researchers [41]. Zheng et al. demonstrated that poly(ethylene oxide) grafted with short polyethyleneimine gives DNA polyplexes with superior colloidal stability, low cytotoxicity, and potent in vitro gene transfection under serum conditions [42].

Petersen et al. studied the influence of polyethyleneimine-graft-poly(ethylene glycol) copolymer block structure on DNA complexation as a gene delivery system. The blood compatibility, cytotoxicity, and transfection activity were also evaluated [43]. The molecular weight and the degree of PEG grafting were found to be crucial for the biological activities of the resulted polyplexes [43]. Other studies also revealed that the copolymer block structure significantly influenced not only the physicochemical properties of complexes but also their cytotoxicity and transfection efficiency [44–46].

Except for the colloidal stability, the particle size/size distribution and the surface charge of polyplexes are also crucial for their in vitro and in vivo transfection [47, 48]. These parameters also influence the attraction toward serum components [47, 48]. The serum protein binding can lead to the alteration of polyplex structure and properties [47, 48]. For example, poly-L-lysine (PLL) polyplexes are quickly removed from blood circulation because they interact extensively with plasma proteins [49–52]. The steric stabilization of poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes complexed with plasmid DNA showed colloidal stability in vitro, extended circulation times, and tumor targeting in mice [50]. The same polymers

were also used for gene transfer into human ovarian carcinoma cells via active targeting [51]. The importance of lateral and steric stabilization of polyelectrolyte gene delivery vectors for extended systemic circulation has also appeared in the literature several years ago [52]. Oupicky et al. studied polyplexes containing DNA. They used PLL or polyethyleneimine (PEI) systems, surface-modified with PEG, or multivalent copolymers of N-(2-hydroxypropyl)methacrylamide (PHPMA) via intravenous administration [52]. According to the findings, the molecular characteristics and the dose of the coating were the most important parameters for the prolonged circulation of the complexes in the plasma [52]. Furthermore, the ideal ratio of cationic and hydrophobic content of PEGylated siRNA polyplexes can ameliorate the colloidal stability, endosome escape, blood circulation half-life, and bioactivity/effectiveness of the polyplexes in vivo [53]. A polyplex library was designed for the investigation of combinatorial optimization of PEG architecture and hydrophobic content [54]. These design parameters can improve ternary siRNA polyplex stability, the pharmacokinetics of the cargo, and effectiveness in vivo [54]. Sarett et al. demonstrated that hydrophobic interactions between polyplex vector and palmitic acid-conjugated siRNA improve PEGylated complexes' colloidal stability and enhance in vivo pharmacokinetics, as well as the tumor gene silencing [55]. In the same line, Jackson et al. declared that charge ratio optimization maximizes the safety and avoids the rapid clearance of polyplexes from circulation [56]. Recently, thermo-responsive polymers were utilized as vectors for the transfection of nucleic acids [57]. The combination of polyoxazoline moieties, possessing great biocompatibility, with DNA-binding PEI into a single copolymer chain was found as a good candidate for improved transfection efficiency [57]. It should be pointed out that in the majority of the published data, PEGylation is a successful strategy to improve gene delivery via polyplexes and enhance the prolonged circulation times [49–58].

4 Lipoplexes for Targeted Gene Therapy

In the following section, we are going to discuss several examples from the recent literature regarding the targeted gene therapy of lipoplexes. An interesting approach to evaluate the efficiency of the non-covalent association of folate to lipoplexes (FA-associated lipoplexes) was demonstrated by Duarte and coworkers. They studied the ability of this novel gene delivery lipoplex system in two different cancer cell lines (SCC-VII and TSA cells). They found that the addition of 40 μg of FA to lipoplexes was positive for transfection and permitted to get over the prohibitive action induced under physiological conditions. Also, they compared the transfection efficacy between the FA-associated lipoplexes and FA-conjugated lipoplexes. The data presented that the electrostatic association of FA to the lipoplex results in significantly higher levels of biological action than that involving the covalent coupling of FA. Furthermore, the FA-associated lipoplexes provide better DNA protection than FA-conjugated lipoplexes. In conclusion, the FA-associated lipoplexes

seem to have better efficacy in gene delivery than FA-conjugated lipoplexes, and this fact made them potential candidates for in vivo gene delivery [59]. Buñuales et al. designed a rapid, simple, and reliable technique based on lipoplexes, which was utilized in gene delivery [60]. For this reason, they prepared liposomes with a common cationic lipid, 1,2 diodeoyl-3-trimethylammonium propane (DOTAP), and cholesterol (Chol) as the neutral helper lipid. Mixing of cationic lipid and DNA at variable ratios leads to the spontaneous formation of lipoplexes by electrostatic interactions. This work aimed to determine the ability of targeted lipoplexes to improve transgene expression in EGF receptors (EGFR, overexpressed in tumor cells) utilizing lipoplexes. The EGF-lipoplexes presented sizes at the nanoscale and were able to transfect different cancer cell lines effectively in comparison with non-targeted systems. Also, these EGF-lipoplexes showed an augmentation transfection action and were noncytotoxic and extremely capable of protecting DNA from DNase I attack. These observations indicate that these vectors could be a sufficient alternative to viral vectors for gene delivery [60].

The impact of the different structural orientations of amide linkers in modulating in vitro gene transport efficacy of cationic amphiphiles is reported by Srujan et al. They found that the reversible structural orientation of amide linkers strongly affected the serum compatibility and lung transfection efficacy of these amphiphiles. Significantly, cationic lipoplexes of the amide linker-based amphiphiles presented a more effective mouse lung eclectic gene transport ability than a commercial lipid (DOTAP) utilized in liposomal lung transfection [61]. Duarte and coworkers developed a novel formulation of lipoplexes via electrostatic non-covalent interactions of folate (FA) to 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (EPOPC)/cholesterol liposomes composed of various lipid/DNA ratios. They investigated the potential of the biological action in different cell lines, and they found that the FA-lipoplexes had highly improved transfection efficiency in both cell lines and decreased the tumor growth in an animal model of oral cancer [62]. Furthermore, Cardarelli et al. prepared two different lipoplex formulations to explore the efficiency of intracellular delivery of DNA. The first lipoplex formulation was formed by DOTAP and schizophrenic helper lipid dioleoylphosphocholine (DOPC), while the second one was prepared by the cationic lipid 3β-[N-(N,N-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) and the schizophrenic lipid dioleoylphosphatidylethanolamine (DOPE). They utilized a combination of pharmacological and imaging approaches, and they concluded that both DOTAP-DOPC/DNA and DC-Chol-DOPE/DNA lipoplexes are taken up in Chinese hamster ovary living cells, mainly via fluid-phase micropinocytosis. The obtained results demonstrate that lipoplex micropinocytosis is a cholesterol-responsive uptake process. Also, the exploration of the intracellular fate of these lipoplexes was accomplished, and it was found that low efficacy DOTAP-DOPC/DNA lipoplexes are highly degraded in the lysosomes, in contrast to DC-Chol-DOPE/DNA lipoplexes that successfully escape the endosome pathway [63]. In another important example, a group of formulation scientists utilized O-(2R-1,2-di-O-(1′Z-octadecenyl)-glycerol)-3-N-(bis-2-aminoethyl)-carbamate (BCAT) to construct targeted cationic lipoplexes with mannose-poly(ethylene glycol, MW3000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (Mannose-PEG3000-DSPE) and

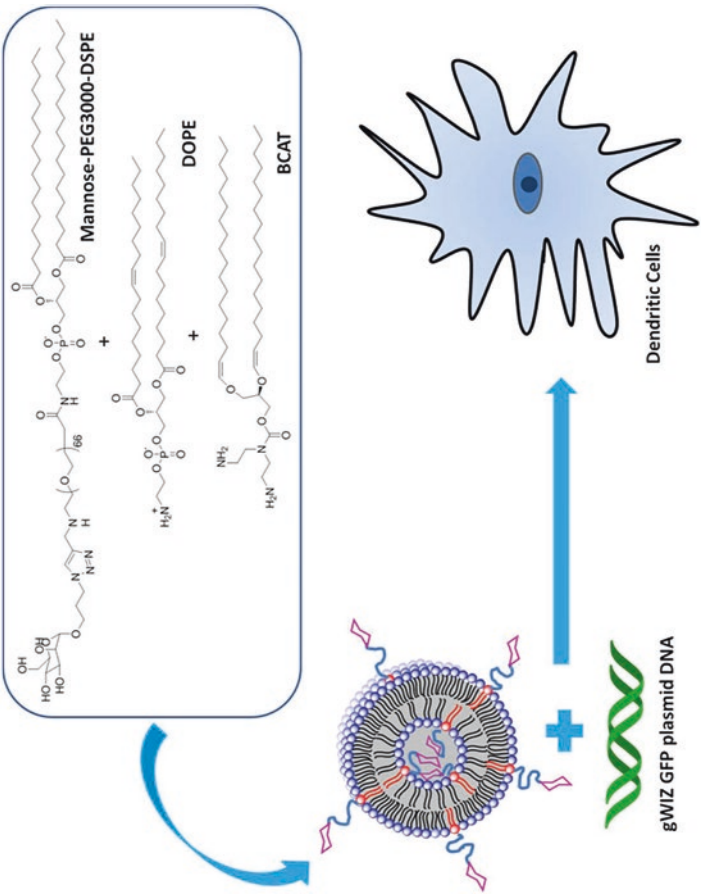

Fig. 1 Conceptual diagram of gWIZ GFP pDNA delivery to DCs via lipoplex composed of Mannose-PEG3000-DSPE, BCAT, and DOPE. (Adapted from Ref. [64])

DOPE for effective delivery of gWIZ GFP plasmid DNA into dendritic cells (DCs) [64]. The structure of the system is visualized in Fig. 1. The transfection performance presented by Mannose-PEG_{3000}-DSPE (10%), BCAT (60%), and DOPE (30%) demonstrates that the most productive delivery into DCs takes place by the synergistic interaction between mannose targeting and acid-labile fusogenic BCAT/DOPE formulations. These outstanding results suggest that these cationic lipoplexes could be potential candidates for gene delivery vectors to DCs [64].

Xu et al. reported the preparation of lipoplexes containing the ideal lipid composition with different conjugates of the folate ligand included in or kept out from the cholesterol component. Using a xenograft tumor model, it was able to evaluate the influence of locating the ligand within the cholesterol segment. The acquired results of the lipoplexes containing the ligand within the cholesterol component revealed a considerably higher luciferase expression and plasmid accumulation in tumors, in comparison to lipoplexes in which the locating ligand was kept out of the cholesterol component. These important data indicate that the environment of the ligand can influence gene delivery to tumors [65]. Wojcicki et al. designed a lipoplex system containing hyaluronic acid (HA) and the lipid DOPE. Subsequently, HA-DOPE has complexed with DNA at different lipid/DNA ratios, where the most efficient formulation of them was the 10%w/w HA-DOPE including lipid/DNA ratio of 2. The transfection efficacy was defined on the CD44-expressing A549 cells via flow cytometry. The desired HA-DOPE rate leads to the creation of the most effective formulation for transfection and increases significantly the GFP expression. Also, a slow transfection of these lipoplexes was presented when a higher level of GFP was achieved after 6 h of incubation. The acquired data indicate the strong ability of DNA targeting via the CD44 receptor utilizing HA as a ligand [66]. Hattori and coworkers reported a promising approach to treating blood lipoplex aggregation in the context of systematic gene delivery work. Specifically, they focused on the coating of liposomes based on DOTAP and Chol, with a series of anionic polymers such as HA, chondroitin sulfate (CS), and poly-L-glutamic acid (PLE), which obstruct accumulation in the lung and enhanced DNA expression in tumor via inhibition of the lipoplex interactions with erythrocytes. The dimensions of HA, CS, and PLE-coated lipoplexes were ca. 200 nm. CS and PLE-coated lipoplexes did not exhibit aggregation after mixing with erythrocytes, while the HA-coated lipoplexes revealed aggregation. Furthermore, CS and PLE-coated lipoplexes did not reveal high gene expression levels in the lung and mainly accumulated in the liver. Their results for CS and PLE-coated lipoplexes determined that these anionic polymers-coated lipoplexes are exceptional tools for effective and secure gene delivery [67].

An interesting work was reported by He and coworkers. They utilized a folate receptor α (FRα)-targeted lipoplex which then was complexed with short hairpin RNA (shRNA) targeting claudin3 (CLDN3) gene forming a folate-modified lipoplex (F-P-LP/CLDN3) to investigate the pharmaceutical properties of the complex. Afterward, the antitumor study of F-P-LP/CLDN3 was carried out in an in vivo model of ovarian cancer. In vivo antitumor experiments presented benign differentiation of tumor and managed to achieve about 90% tumor growth inhibition.

Considering these lipoplexes, it is observed that they are an ideal formulation for ovarian cancer therapy [68].

Additionally, Puras et al. developed a novel niosome system based on the 2,3-di(tetradecyloxy)propan-1-amine cationic lipid in combination with squalene and a nonionic surfactant, namely, polysorbate 80, to investigate the transfection efficiency in rat retinas. These new niosomes, acquired by the solvent emulsification-evaporation procedure, were mixed with pCMSEFGP plasmid to form lipoplexes. In vitro experiments were carried out to determine transfection efficacy and viability in HEK-293 and ARPE-19 cells. The results displayed the successful transfection of HEK-293 and especially of ARPE-19 cells, without affecting the viability. Furthermore, the lipoplexes entered mainly retina cells by clathrin-mediated endocytosis, whereas HEK-293 cells exhibited an important caveolae-dependent entry. This novel niosome formulation leads to a promising process to deliver genetic material into the retina for the treatment of inherited retinal illness [69]. Naicker et al. explored the effect of PEGylation degree in terms of physicochemical properties, cytotoxicity, and transfection efficiency of lipoplexes including cationic liposomes. These lipoplexes contained the cytofectin 3β-[N-(N′-, N′-dimethylaminopropane)-carbamoyl] cholesterol (Chol-T), helper lipid (DOPE), the asialoglycoprotein receptor (ASGP-R) targeting cholesteryl-β-D-galactopyranoside (Chol-β-Gal) ligand, and plasmid DNA in ASGP-R-negative (HEK293) and receptor-positive (HepG2) human cell lines. The results presented important advantages of PEGylated lipoplexes in comparison to non-PEGylated lipoplexes such as well-defined and colloidally stable nanoparticles. Moreover, the transgene efficacy raised by 63% and 77% when HepG2 was encountered by the 2 and 5mole% PEGylated lipoplexes, respectively, in comparison to non-PEGylated lipoplexes. In the case of Chol-T Chol-β-Gal 5% PEG, lipoplexes recorded an achievement of 164% augmentation in transfection activity in the ASGP-R-positive cell line (HepG2) in contrast to HEK293(ASGP-R negative) [70]. Huang's team developed a series of cyclen-based cationic lipids with histidine-containing ester groups or amide bonds in the backbone for gene delivery. The preparation of cationic liposomes was done by mixing the lipids and the neutral lipid DOPE in a proper molar ratio. The synthesized liposomes presented excellent stability and formed lipoplex-encapsulated pDNA into nanoparticles. Cell viability studies based on CCK-8 cells revealed low-level cytotoxicity of these lipoplexes in comparison to the commercial Lipofectamine® 2000. Green fluorescent protein and luciferase experiments were performed to explore the in vitro transfection efficacy of these lipoplexes. The experimental data presented that the structures of the hydrophobic chain and the linking bond influenced notably the transfection efficiency. Also, the imidazole group is indicated to have an important role in the transfection by such a type of lipid. In summarizing, these data recommend that the cyclen-imidazole cationic lipids could be a promising nonviral gene delivery vector [71].

Luo et al. reported an FRα-targeted lipoplex loading plasmid interleukin-12 (PIL12) forming a folate-modified lipoplex (F-PLP/PIL12). In vivo studies were performed, such as an in vivo model of CT26 colon cancer to explore the antitumor effect of these lipoplexes. F-PLP/PIL12 could prevent about 56.6% tumor growth,

and also IL12 expression rise in the F-PLP/PIL12 group, while at the same time, FRα expression was downregulated. Furthermore, toxicity studies of F-PLP/PIL12 indicated no toxicity in the mice. Therefore, the F-PLP/PIL12 could be a promising formulation for clinical colon cancer immunogenic therapy [72]. Zheng's team synthesized two amino acid-based cationic lipids, which included an α-tocopherol moiety and a biocompatible amino acid head group (histidine or lysine) linked by a biodegradable disulfide and carbamate bond. These lipoplexes were evaluated in cell culture experiments as nonviral DNA delivery vehicles. DNA and cationic liposomes formed lipoplexes which present low cytotoxicity and comparable transfection efficacy compared with the commercially available Lipofectamine 2000. Under physiological conditions, especially in the presence of 10% serum, the transfection efficacy of the cationic lipid based in histidine was 4.3 times higher than that of branched polyethylamine. The acquired results indicate that the amino acid-based lipids are capable of a safe and efficient gene delivery [73]. Cardarelli et al. constructed a Lipofectamine/DNA lipoplex to evaluate the intracellular trafficking mechanism of them in live cells. They discovered that Lipofectamine (LFN), in comparison with alternative formulations, could effectively avoid intracellular transport along microtubules and the following encapsulation and degradation of the payload within acidic lysosomal components. This observation is accomplished by following the random Brownian motion of LFN-including vesicles into the cytoplasm (Fig. 2). Also, they indicate that Brownian diffusion is an effective process for LFN/DNA lipoplexes to avoid metabolic degradation, thus resulting in optimal transfection. Based on their results, it would be possible to developed new generations of more optimized nonviral, lipid-based, gene delivery vectors [74].

Another important work was reported by Rak et al. They utilized a set of cationic polyprenyl derivatives (trimethylpolyprenylammonium iodides (PTAI)) as part of effective DNA vectors. Optimization experiments were accomplished for PTAI combined with lipid DOPE on DU145 human prostate cancer cell lines. The acquired data present that the lipofection action of PTAI enhances transfection efficiency of pDNA complexes in negatively charged lipoplexes into cells with no important side effects on cell physiology and incidence of eukaryotic cell proliferation. Considering these results, the PTAI-based lipoplexes could be promising candidates for gene delivery to eukaryotic cells [75].

Another interesting study was the construction of the DNA-liposome complex (lipoplex) by Rasoulianboroujeni et al. This work refers to cationic liposomes and their modified preparation process utilizing the dry lipid film method, including lyophilization, for DNA delivery applications. Using a particle size instrument, it was possible to determine the dimensions of liposomes before and after lyophilization and of course the mean particle size of DNA-lipoplexes. Transfection assays were accomplished by using human embryonic kidney 293 (HEK-293) cell lines. Overall, the acquired data indicated that the DNA expression of these lipoplexes is almost equal to the Lipofectamine® 2000. Furthermore, the acquired cellular protein of the developed lipoplexes was at higher levels than in Lipofectamine® 2000 based on studies [76]. Mohammed-Saeid et al. developed a novel cyclic arginyl-glycyl-aspartic acid (cRGD)-modified gemini surfactant-based lipoplexes for use and

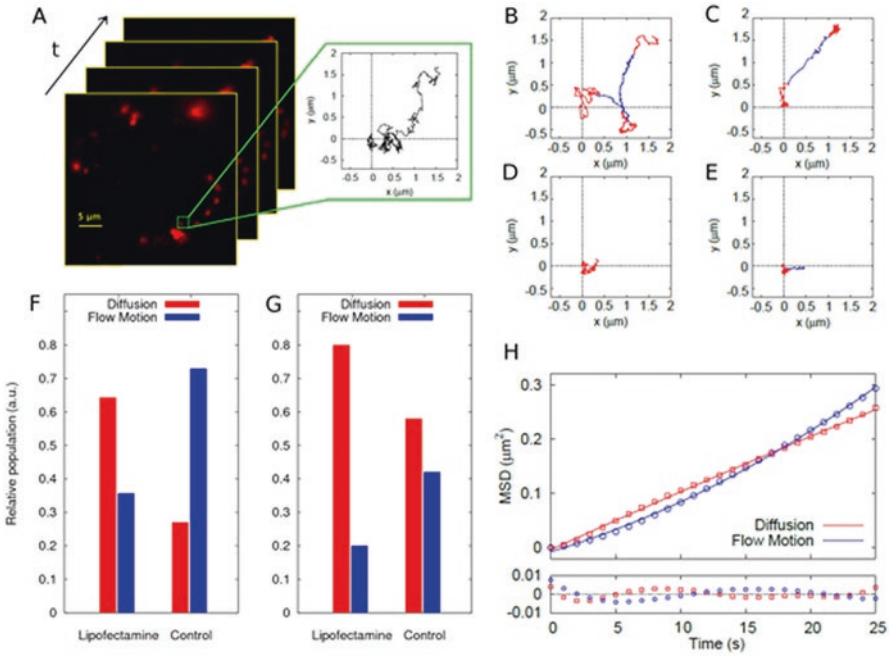

Fig. 2 (**a**) Schematic evaluation of a single particle track from a set of confocal images acquired within 300 s, with a time lapse $\Delta t = 1$ s. Representative trajectories of complexes in not treated Chinese hamster ovarian (CHO) cells: (**b**) Lipofectamine/DNA; (**c**) DOTAP/DOPC/DNA (DD/DNA). Representative trajectories of complexes in nocodazole (NCZ)-treated CHO cells: (**d**) Lipofectamine/DNA and (**e**) DD/DNA. Diffusion (red) and flow motion (blue) segments are shown. Relative populations of the acquired tracks for Lipofectamine and DD in not treated (**f**) and NCZ-treated (**g**) CHO cells. (**h**) Mean square displacement (MSD) analysis of two representative tracks. MSD calculation was used for the measurement of the dynamic parameters, i.e., diffusion coefficients and flow speed. (Adapted from Ref. [74])

evaluation in an in vitro human melanoma model (A375) cell line as a different option to conventional chemotherapy. These peptide-modified lipoplexes exhibited an important enhancement in gene transfection action in A375 human melanoma cell lines in contrast to the standard non-targeted formulations, specifically when RGD was chemically coupled to the gemini surfactant (RGD-G). The IFN-γ expression in A375 cells at 48-h posttreatment with lipoplexes is presented in Fig. 3. These results demonstrate the useful action of RGD-modified gemini surfactant-based lipoplexes in melanoma therapy [77].

A colloidal stable lipoplex formulation of single unilamellar vesicles (SUV) containing a PEGylated stearyl amine (pegSA), which maintains the SUV properties after complexation with DNA, was designed for targeted purposes [78]. The pegSA lipoplexes presented a lower N/P ratio (1.5) for BMP-9 gene complexation, suitable for intravenous infusion for delivery to bone marrow mesenchymal stem cells via sinusoidal vessels in the bone marrow. Furthermore, these lipoplexes exhibited low

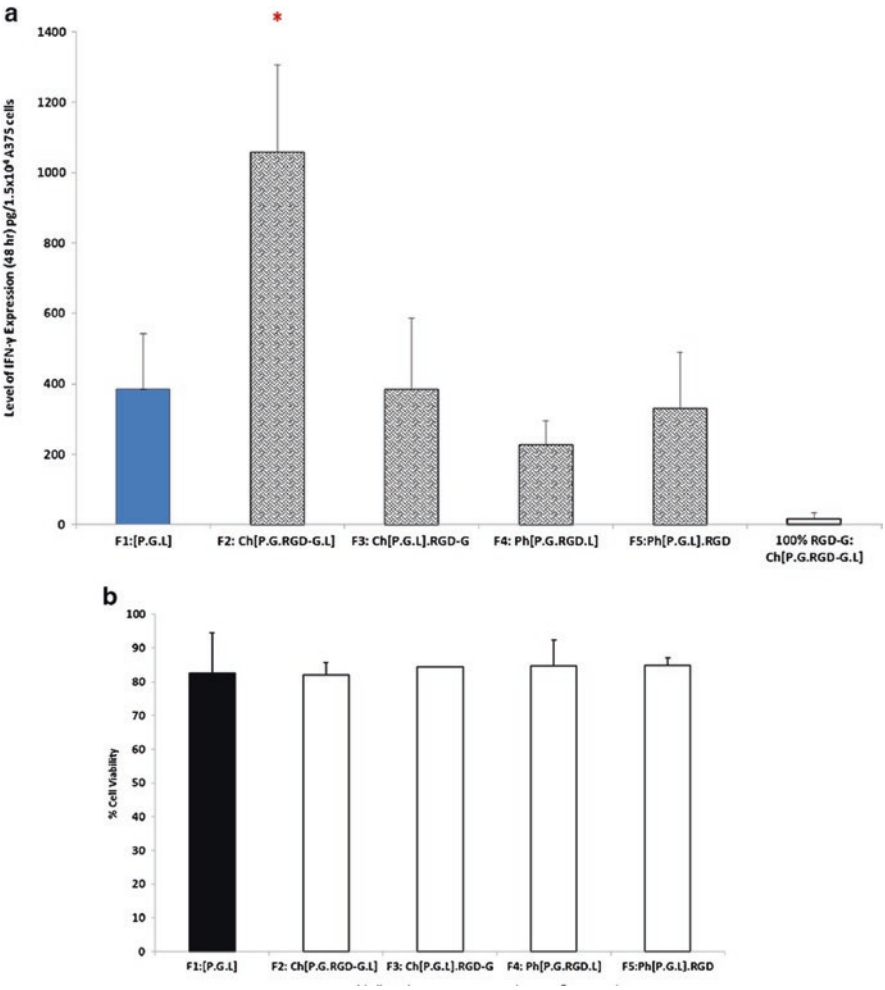

Fig. 3 (**a**) IFN-γ expression in A375 cells at 48-h posttreatment with lipoplexes constructed at 1:10 −/+ charge ratio. P, pDNA; G, 12-7 N(GK)-12; L, DOPE; RGD-G, 12-7 N(RGD)-12; RGD, RGD peptide. (Ch) indicates that the lipoplexes were built using chemically conjugated RGD-gemini, and (Ph) indicates a physical co-formulation of free RGD with the non-targeted lipoplexes. IFN-γ level was determined by ELISA. Significant increase (*$p < 0.01$, one-way ANOVA) in IFN-γ expression was observed when cell treated with RGD chemically conjugated lipoplexes (F2: Ch[P.G.RGD-G.L]) compared to non-modified lipoplexes (F1:[P.G.L]). (**b**) Cell viability in A375 cells after a 48-h treatment with RGD-modified lipoplex formulations as determined by MTT assay. Cell viability was calculated as % relative to non-transfected cells. Four wells of each formulation were loaded in three different experiments. The results are expressed as mean of the three experiments ($n = 3$). Bars represent standard deviation. * Indicates significance at $p < 0.01$ in comparison to standard formulation [P.G.L] (F1). (Adapted from Ref. [77])

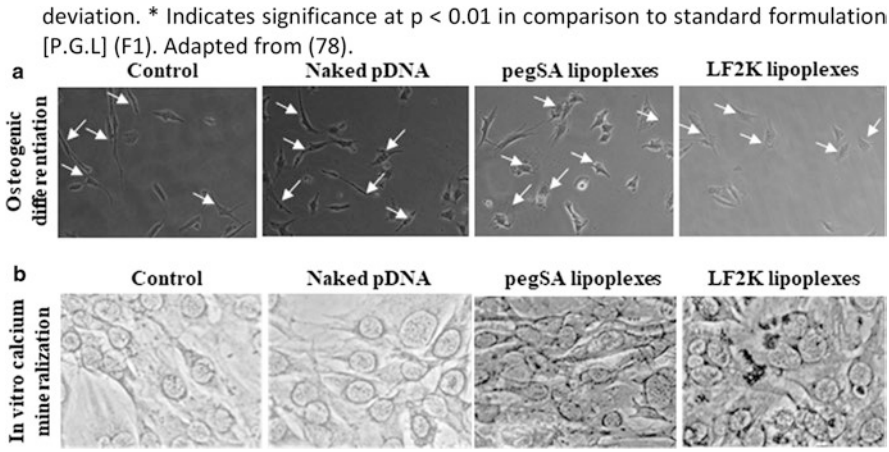

Fig. 4 In vitro BMP-9 transfection in C2C12 cells by pegSA lipoplexes. (**a**) Osteogenic differentiation of C2C12 cells by BMP-9 lipoplexes (n = 3), (**b**) in vitro calcium mineralization after transfection by BMP-9 lipoplexes (n = 3) (All images were captured on a Nikon-2000 microscope, Nikon, Japan). (Adapted from Ref. [78])

toxicity to the C2C12 and NIH3T3 cells and erythrocytes. Also, transfection experiments presented an effective gene delivery to C2C12 cells inducing osteogenic differentiation via BMP-9 expression (Fig. 4). Complementary in vivo studies further proved the safety of the constructed lipoplexes [78].

Lipoplexes based on pH-responsive cationic liposomes were also developed by varying molar mass ratios with respect to pDNA utilized [79]. The authors evaluated the lipoplex abilities for in vivo tumor-targeted gene transfection compared to the conventional reagent Lipofectamine® 2000. In vitro hemocompatibility estimation of pDNA lipoplexes presented <8.5% of hemolysis in contrast to the hemolysis by Lipofectamine® 2000 which was 15.9%. Cell viability studies exhibited >80% values along with 4.42, 5.18, and 5.00 higher transfection efficacy than Lipofectamine® 2000 in MCF-7, HeLa, and HEK-293 cells, respectively. Also, pDNA lipoplexes revealed higher tumor transfection compared with Lipofectamine® 2000, demonstrating outstanding abilities for in vivo gene delivery [79]. Nie et al. by utilizing a flash nanocomplexation controlled procedure mixed the 1,2-dimyristoyl-rac-glycero-3-methoxy poly(ethylene glycol)-2000(DMG-PEG)/DOTAP liposomes with plasmid DNA and formed lipoplex nanostructures with small sizes (60 nm). DMG-PEG acted as a hydrophilic and neutral layer-coated cationic lipid in the DNA-containing nanoparticles to decrease the barrier of penetration via the mucus. Lipoplexes with a PEG surface showed improvement of transportation in the mucus layer of the GI tract. A repetitive transgene expression was verified, and the expressed insulin was found to retain the blood glucose level for 24 h, presenting repetitive therapeutic properties by multiple doses. The results indicate the fast translation effects of DMG-PEG/DOTAP-DNA nanostructures in type I diabetes through oral delivery [80].

Buck et al. developed a combinatorial process for the synthesis of short-chain aminolipids with various headgroups, containing aliphatic and heterocyclic groups [81]. These lipids in combination with the cationic lipid DOTAP can be used to increase the delivery of DNA. This combination leads to the development of novel lipoplex systems able to complex minicircle DNA and explore the transfection efficiency in human liver-derived cell lines (HuH7). Cytotoxicity studies revealed that the combination of these lipoplexes remarkably mitigated the cytotoxicity and increased the transfection ability in HuH7 cells in vitro, in contrast to common DOTAP/chol lipoplexes. These new lipoplex systems would be a potential candidate to promote effective DNA delivery [81].

Recently, Harrys and coworkers reported an interesting work aimed to investigate the utility of LFN as a lipid-based alternative to positively charged polymers and lentiviral transduction for T-cell gene delivery. The cationic lipid LFN facilitates the formation of a lipoplex containing negatively charged DNA and positively charged liposomes capable of transfecting Jurkat cells. Transfection of Jurkat cells was accompanied with high efficiency by transfecting cells with LFN in X-VIVO15 media. On the other hand, a much lower transfection efficacy was observed in T cells. This observation, made by confocal microscopy, revealed that the lipoplexes did not enter the primary T cells. Pyrin and HIN (PYHIN) DNA sensors which could prompt apoptosis after complexation with cytoplasmic DNA were also recorded at high concentrations in primary T cells. Consequently, transfection of primary T cells seems to be restricted in the process of cellular uptake [82].

5 Polyplexes for Targeted Gene Therapy

As mentioned above, polyplexes are complexes formed by cationic polyelectrolytes and nucleic acids via the development of electrostatic interactions. Polyplexes comprise an alternative, safer, and propitious approach, toward addressing or averting acquired and hereditary diseases since the cationic polymer carriers facilitate the nonviral delivery/distribution of DNA or RNA in diseased tissues [83–86]. Since the breakthrough investigation by Tang and Szoka, who introduced cationic polymers as nonviral carriers for nucleic acids directly to target cells [37, 87], fundamental advancements involving design approaches, preparation, and exploration of polyplexes by numerous scientific groups have been added to the constantly increasing field of targeted gene therapy. New-generation vectors based on synthetically and physicochemically evolved cationic polymer classes have overcome several barriers concerning polyplex detection by the host's immune system, while their dismissal from the organism has been averted [88–90]. The most essential qualities that polyplexes should possess are the decreased cytotoxicity to block unwanted adversary actions in patients and to maintain the functionality of the transfected cells to employ therapeutic efficacy, the obstruction of the nuclease-intervened decomposition prior to approaching target cells, and the evasion of host eviction procedures, such as filtration in the spleen and absorption by collector cells [91].

The fact that DNA undergoes extensive folding and is finally condensed when connected with cationic polymers has been confirmed by numerous investigations [92]. Hitherto, polyplexes of globular, rodlike, and toroid structures have been reported as the outcome of cationic polyelectrolyte complexation with DNA molecules [93–95], poly(amidoamine) (PAA) [96], poly(dimethylamino ethyl methacrylate) (PDMAEMA) [97], and poly{N-[N-(2-aminoethyl)-2-aminoethyl]aspartamide} [PAsp(DET)] [98] being some typical examples of positively charged polyelectrolytes. Nevertheless, polyplexes tend to aggregate when the complexation process occurs at charge stoichiometric conditions [88]. Incorporation of a neutral hydrophilic block such as poly(ethylene glycol) (PEG) to a cationic block toward the preparation of block copolymers can inhibit further aggregation and permits the compaction of a DNA molecule inside a polyplex nanostructure protected by a PEG corona (Fig. 5) [94].

A detailed study regarding the effect of PEG functionalization of polymeric carriers on the efficient distribution of gene material to target cells was presented by Clima and her collaborators [99]. They created a collection of vectors consisting of the hydrophobic component PEGylated squalene SQ-PEG-NH_2, namely, poly-(ethyleneglycol)-bis(3-aminopropyl)) (NH_2-PEG-NH_2) of different molecular weight (Mn 1500, 2000, and 3000 Da), and branched polyethyleneimine (bPEI) of low molecular weight (Mn 800 Da). The concept behind the creation of the nonviral vector collection was to alter the composition of the vectors by slowly increasing both the H_2N-PEG-NH_2 ratio and the molecular weight of H_2N-PEG-NH_2 (from 1500 Da to 3000 Da). TEM and DLS techniques showed that an increase in molecular weight of H_2N-PEG-NH_2 induced the formation of structures of smaller dimensions due to steric interactions among PEG clocks and the scaffolding components (Fig. 6). Additionally, it was determined that increasing the molecular weight of H_2N-PEG-NH_2 to 3000 Da in the carrier composition pDNA-binding capability was becoming more fragile, resulting in the formation of larger polyplexes, ascribed to the shaping of a protective veil over the bPEI800 units triggered by PEG units. The group also performed biological evaluation studies on HeLa cell culture to determine transfection efficacy and cytotoxicity parameters of the aforementioned nonviral vectors. All examined samples exhibited transfection efficacy higher at an N/P ratio equal to 100 than at an N/P ratio equal to 50. The chain length of H_2N-PEG-NH_2 exhibited an important effect on both the cytotoxicity and transfection efficacy. Finally, the group concluded that the presence of H_2N-PEG-NH_2 component with comparatively high molecular weight as part of the polymeric vector contributed at the enhanced biocompatibility of the latter, yet reduced transfection efficiency of the cells.

Haladjova et al. studied the physicochemical properties of DNA carriers based on novel poly(vinyl benzyl trimethylammonium chloride) (PVBTMAC) homopolymers and block copolymers [100]. The investigations involved the utilization of two types of linear DNA in terms of chain length. The outcome of the complexation process between the homopolymers and block copolymers with the DNAs was determined by factors such as the length of a cationic block, total polymer composition and architecture, N/P ratio, and salt concentration, which were expected to

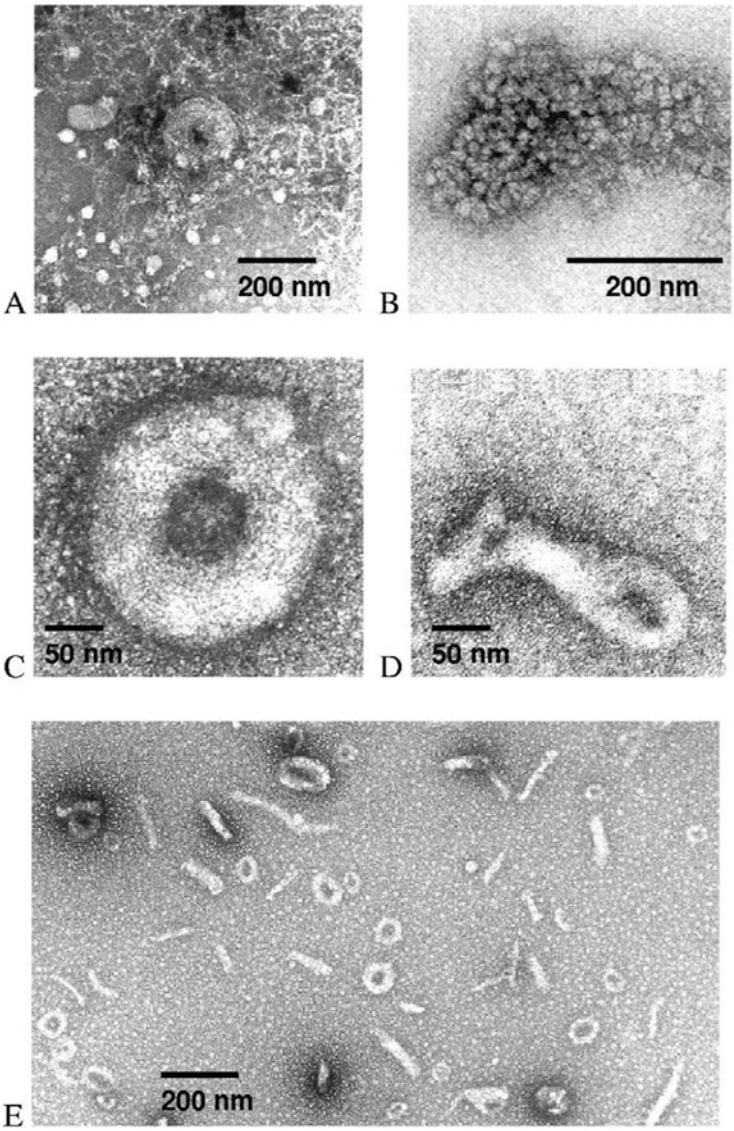

Fig. 5 Transmission electron microscopy pictures of poly-L-lysine/pDNA complexes at 1/2 charge ratio 4:1. Toroid "in a net," size of 200 nm formed by unpegylated third-generation dendrimer (**a**). Aggregate of unpegylated linear PLL 20 kDa, about 500 nm in size (**b**). A perfect complex of unpegylated third-generation dendrimer and plasmid DNA (**c**). The size is about 200 nm. Twisted complex formed by pegylated fifth-generation dendrimer (**d**) ⌊250 nm size. General picture of usual forms of rods and toroids with grafted PLL (**d**); linear PLL showed similar forms. The size of rods is about 150–250 nm and toroids about 100 nm. (Adapted from Ref. [94])

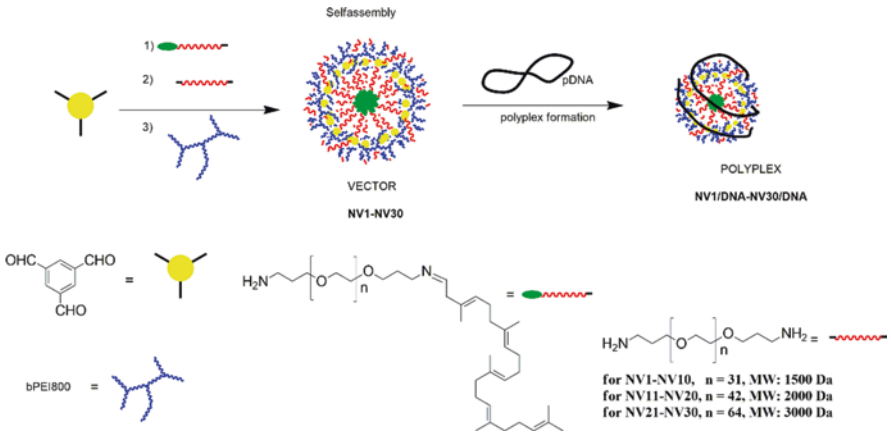

Fig. 6 Schematic representation for the formation of vectors NV1–NV30 and polyplexes. (Adapted from Ref. [99])

affect the structure, stability, and efficacy of the formed polyplexes. The group ascertained that the presence of POEGMA groups provided stabilizing and shielding functionality that eventually prevented aggregation and precipitation and assured colloidal stability to the polyplexes. In fact, precipitation phenomena were monitored only in the case of complexation of the shorter DNA with the shorter PVBTMA homopolymer (20 K), as the absence of POEGMA block allowed the polyplexes to aggregate by forming structures of high dimensions at a specific solution ionic strength. Moreover, they established PVBTMAC as a very promising gene vector as they discovered that the high content of charged segments and the average hydrophobicity contributed to compressing DNA dimensions. Specifically, they declared that as long as the molecular weight of the polyelectrolyte block increased, it acted more efficiently in shrinking DNA regardless of the molar mass and secondary structure of the nucleic acid.

Another important contribution to the field was reported by Tan and coworkers [101]. The group compared the efficacy of the poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) homopolymer and poly(ethylene glycol)-block-poly(2-(dimethylamino) ethyl methacrylate) (PEG-b-PDMAEMA) double hydrophilic diblock copolymer pair along with poly(2-(dimethylamino)ethyl methacrylate)-block-poly(n-butyl methacrylate) (PDMAEMA-b-PnBMA) amphiphilic diblock copolymer and poly(ethylene glycol)-block-poly(2-(dimethylamino)ethyl methacrylate)-block-poly(n-butyl methacrylate) amphiphilic triblock terpolymer pair, as plasmid DNA (pDNA) carriers. Upon complexation with pDNA, PDMAEMA and PEG-b-PDMAEMA formed polyplexes, while PDMAEMA-b-PnBMA and PEG-b-PDMAEMA-b-PnBMA micelleplexes. The polyplexes were aggregates of high mass and size in the case of PDMAEMA and of globular morphology in the case of PEG-b-PDMAEMA. In both cases, pDNA was compressed in the core of the nanostructure. On the contrary, micelleplexes formed beads-on-a-string morphologies with pDNA chains surrounding and connecting with

multitudinous micelles. The results of the study demonstrated that even though both kinds of complexes exhibited similar cytotoxicity, micelleplexes outperformed polyplexes regarding transfection efficacy. Indeed, the micelleplexes presented four times greater transfection efficiency than the polyplexes. The fact is correlated to multiple aspects of the nanosystems. First, the micelleplexes seem to be incorporated more efficiently. Moreover, the introduction of a greater number of amine segments to each micelleplex might help endosomal getaway. More significantly, the "beads on a string" morphology of the micelleplexes imitate the way the cells enclose DNA all over histones in chromatin and preserve the endogenous geometry of helix B of DNA chains. The preservation of DNA geometry is essential since it prompts more beneficial protein expression relative to that where DNA is located within polyplexes, in complexed and compacted conformation, and with its B-form considerably modified.

Equally interesting is the case of the double DNA delivery ensemble, nanoparticle-in-microsphere (NIM) toward DNA vaccine formulations, proposed by Lu and his group [102]. They designed and developed a hybrid system (NIM) that would combine the features of nano-dimensional polyplexes along with the continuous release microsphere concept for DNA vaccine preparations. Specifically, they synthesized polyethylene glycol-graft-polyethyleneimine (PEG-g-PEI) copolymers that acted as DNA vectors. In the next step, lyophilization of DNA along with PEG-g-PEI occurred to condense DNA into a nanostructure for DNA micronization. The resulted polyplexes were formulated into NIMs by following the solid-in-oil-in-water (S/O/W) emulsion protocol to encapsulate DNA polyplexes. In this case, PEG contributed to the colloidal stability of the hybrid system and to maintain the solubility of the polyplexes in a nonaqueous environment. The authors stated that by implementing this strategy, the DNA vaccine could be shielded by increased homogenization and by avoiding the water-oil interface that would trigger DNA denaturation. NIMs comprise a novel, beneficial technology for gene distribution to phagocytic cells that would speed up the intracellular release of DNA vaccine. Further investigations proved that this particular DNA vaccine ensemble presents the ability to provoke immune responses even at low DNA doses in big animals. Therefore, this type of vaccine technology offers great potential for application in humans to treat life-threatening diseases.

Similarly, Soares et al. proposed a novel approach to developing vaccine formulations [103]. They introduced nonviral vectors consisting of poly(β-amino ester) (PβAE) and poly[2-(dimethylamino)ethylmethacrylate] (PDMAEMA) polymers to address low transfection efficiency and immune endurance, parameters that create obstacles to the effectiveness of DNA vaccine technology. The group via a series of methods, such as size measurements, gel retardation studies, cell viability assays, transfection experiments, blood compatibility investigations, etc., established that pDMA/PDMAEMA/PβAE polyplexes can be adjusted to acquire a small size by increasing the pDNA ratios and are eligible for utilization in mice vaccination investigations. Subsequently, they accomplished to include two types of β-glucan in vaccine formulations to finally collect polyplexes of ameliorated transfection efficiency in RAW 267.4 macrophages. The conducted experiments revealed that the

cytotoxicity and the hemocompatibility are strongly influenced by the dose rate. Thus, a secure operating set was arranged. Mice vaccination investigations through the subcutaneous route were not successful as originally anticipated producing a 40% HBsAg seroconversion, unaffected by the β-glucan presence.

Other works involving the formation of polyplexes for nonviral gene delivery include that of Faria and coworkers [104]. Hitherto, conventional delivery vehicles that present serious limitations such as restricted lifetime in the patient body and undesirable side effects are the ones mostly used in combating most cancer cases. The group conducted studies that combine drug and gene delivery strategies. Specifically, they incorporated the anticancer drug methotrexate (MTX) into the polyplexes that resulted from the complexation of polyethyleneimine (PEI) with p35 encoding pDNA. The polymeric vehicles, apart from being capable of encapsulating MTX, acquire a plethora of advantages such as size, structure, surface charge, and cargo complexation for intracellular transportation. The MTX cancer cell-targeting quality tested over folate receptors has been established and produces proof for receptor-intervened endocytosis. Confocal microscopy studies established the integration of vectors into cells and pDNA access in the nucleus, along with the regulation of the cell transfection efficacy with the aim of ameliorating protein expression rates.

Another group that has dealt among others with the formation of DNA polyplexes is that of Valente. In one particular case, they studied the complexation ability of chitosan (CH) or polyethyleneimine (PEI) with different lengths of pDNA [105]. ζ-Potential and encapsulation efficacy experiments confirmed the hypothesis of synthesizing cationic polymeric vehicles, able to load DNA in dependence on the ratio of both components. Cell viability assays were conducted in cases of CH/pDNA at N/P = 0.75 and PEI/pDNA at N/P = 100, where the encapsulation efficiency, zeta potential, and size values were evaluated as the most suitable. Cytotoxicity evaluation demonstrated that CH/pDNA polyplexes were biocompatible, but PEI/p53-pDNA polyplexes presented exiguous cytotoxicity in healthy cells which could prevent their future employment as therapeutic agents. The results obtained by transfection assays showed that all the studied polyplexes can transfect the cell lines utilized (Fig. 7). However, higher transfection rates were monitored in the case of complexation with the smaller DNA. In the next step, the group investigated the possibility of P53 protein expression via employing the Hela cancer cell line. P53 rates increased up to 54.2% and 32% when chitosan and PEI polymers, respectively, acted as vectors. A direct comparison between the two types of polymers based on experimental conditions and the obtained results defined chitosan/pDNA polyplexes at N/P = 7.5 as less cytotoxic, more productive regarding cell transfection, and more effective triggering agents toward protein expression. To sum up, chitosan/pDNA polyplexes are more eligible to pDNA delivery approaches relative to PEI/pDNA polyplexes.

Sentoukas et al. described the complexation process of (2-[dimethylamino]ethyl methacrylate)-b-poly(hydroxypropyl methacrylate) (PDMAEMA-b-PHPMA) dual-responsive block copolymers, in terms of pH and temperature, and their quaternized derivatives QPDMAEMA-b-PHPMA with short, 113-base DNA [106].

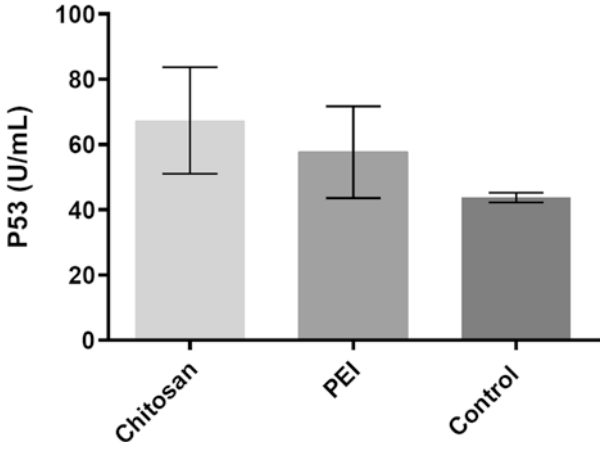

Fig. 7 Evaluation of p53 protein expression following administration of p53-pDNA polyplexes of CH and PEI in HeLa cervix carcinoma. Data are represented as mean ± S.D., $n = 2$. (Adapted from [105])

Studies have shown that complexation with a block copolymer of smaller length resulted in well-defined polyplexes only at N/P = 1, while the one with the longer chain length formed well-structured polyplexes at all N/P ratios. However, salt addition induced the formation of aggregates of large dimensions due to the occurrence of charge screening effects that eventually triggered a decrease in the electrostatic interactions between the positive charges of the block copolymer and DNA. The occurrence of stronger interactions between DNA molecules and the PDMAEMA segment compared with the quaternized one was determined by optical absorption studies. Fluorescence quenching studies demonstrated that the block copolymer interacts efficiently with DNA, suggesting a distinctive inner association of the preorganized copolymer nanoparticles, regardless of the low ionization degree of the PDMAEMA block. Surface charge measurements indicated that the most suitable N/P ratio is equal to 1, producing polyplexes under the highest complexation with DNA. Finally, the authors suggested that for all cases of polymers used, the most possible scenario about the location of the DNA is on the surface of the resulted complexes, because the cationic segments, interacting with DNA, should also be sited on the surface of the preorganized polymer aggregates.

Along the same lines, Chroni et al. proposed an innovative multirole nanosystem that entailed the cationic poly[oligo(ethylene glycol) methacrylate]-b-poly[(vinyl benzyl trimethylammonium chloride)] (POEGMA-b-PVBTMAC) diblock copolymer, combined with hydrophilic negatively charged magnetic nanoparticles (MNPs), and its subsequent complexation with linear DNA, comprised of 113 base pairs [107]. The preassembled nanostructures were spherical. The design and investigation of the novel triple-functional DNA delivery system were based on the development and subsequent monitoring of the electrostatic interactions between the positively charged PVBTMAC segments and the negatively charged magnetic nanoparticles and DNA phosphate groups. Parameters such as solution

concentration, solvent, and ionic strength strongly affected both the self-assembly behavior of the diblock copolymer and the co-assembly of DNA and cationic block copolymer with the magnetic nanoparticles. DLS measurements showed the formation of magnetopolyplexes resulting from the complexation of the hybrid MNPs/copolymer aggregates along with the short DNA molecule. The obtained magnetopolyplexes presented a hydrodynamic radius equal to 283 nm at N/P = 4. Physicochemical investigations revealed that surface charge, size, mass, and complexation ability between the hybrid MNPs/copolymer aggregates and DNA were regulated by the N/P ratio. The group observed the formation of magnetopolyplexes of lower mass as the salt concentration increased, while the size was relatively constant. Analysis of the images obtained by cryo-TEM disclosed an important inclination toward aggregation phenomena of the NMPs/copolymer structures upon interaction with the DNA molecules. The observed behavior is assigned to the accretion of the DNA molecules on the surface of the hybrid NMPs/copolymer complexes. The magnetic properties of the MNPs were preserved after the complexation process with DNA, prompting the multifunctional MNP/polyelectrolyte copolymer/DNA system eligible for therapeutics and bioimaging applications.

Another noteworthy study was reported by Rumschöttel et al. [108]. The group explored polyplexes formed between DNA extracted from salmon tastes along with either hyperbranched poly(ethyleneimines) (PEIs) of molecular weights equal to 5000 g/mol and 25,000 g/mol or modified PEI (5000 g/mol) with maltose segments (PEI-Mal), according to molar N/P ratio adaptation by implementing DLS, surface charge, DSC, STEM, and cryo-SEM techniques. Particularly, polyplexes derived from hyperbranched PEI and DNA exhibit dissimilar tendencies, depending on the N/P ratio. Polyplexes of small dimensions (ca. 80 nm) were observed when DNA content was higher relative to that of the polymer. Those polyplexes were formed by relaxed stacked DNA network regions, linked with DNA strands. However, when cationic PEI content was in excess, every DNA molecule was located inside the cationic polyplex. At the specific N/P ratio equal to 8, the majority of DNA molecules are placed in the core of spherical configurated polyplexes, encircled by PEI chains. At an extremely high excess of PEI (ca. N/P = 40), polyplexes of onion-like configuration and 200 nm dimension are detected. Therefore, the morphology of the resulted polyplexes is tremendously affected by the N/P ratio. Moreover, the authors discovered a deviation at the melting point of DNA from 88 °C to 86 °C attributed to the strong electrostatic interactions developed between DNA and PEI. In the case of interaction of the less toxic PEI-MaI with DNA, polyplexes of positive charges were detected at lower N/P ratios. Moreover, the participation of PEI-Mal instead of unmodified PEI forges larger aggregates a tendency ascribed to the occurrence of more hydrogen bonds. According to DSC studies, solely on the occasion of a high quantity of PEI-Mal at N/P ratio above 40, dense polyplexes characterized by two melting points are detected. Relatively to polyplexes obtained as the result of the interaction between the unmodified PEI and DNA, the ones derived by PEI-Mal and DNA are of spherical morphology and dimensions of 100–250 nm. The latter exhibited enhanced stability, attributable to the combination of electrostatic and H-bonding interactions.

6 Conclusions

In this chapter, we presented the current technology and the applications of lipoplexes and polyplexes in the field of targeted gene delivery. These nanosystems are very useful for the delivery of nucleic acids for the treatment of several diseases like cancer. We also gave special attention to the mechanisms by which lipoplexes and polyplexes are used for the delivery and the release of the complexed nucleic acids. The physicochemical characteristics of nucleic acid complexes were discussed, in some detail. Several examples from the recent literature were analyzed. Current research which has already utilized several types of lipo- or polyplexes to target a plethora of molecular and cellular mechanisms has been presented. The research outcomes show that both of these cationic carriers open new horizons for the fast clinical translation of nanomedicines with added value for the treatment of several diseases.

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