Nanoparticles as Nonviral Transfection Agents

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Abstract

A series of studies have been carried out for delivery and controlled release of genes, miRNAs, peptide structures, siRNAs, and pharmacological agents to the target tissues through different nanoparticles. Agents to be delivered are either attached on or entrapped in nanoparticle structure. In the delivery process, the nanocarriers face many different delivery tasks and different physiological microenvironments. Considering the changes in the environment, nanocarriers

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are designed and synthesized in such a manner that enables these structures to overcome the challenges faced during delivery. In this chapter nanoparticle structures as cationic lipids, polycationic polymers, and dendrimers used in drug and gene delivery are reviewed.

Keywords
Nanocarriers • Controlled release • Gene therapy

Introduction

For medicine and healthcare, the ability to design and synthesize efficient drug delivery systems is very important. Progressions in drug delivery systems have been achieved by innovations in material chemistry, which produces biodegradable, environment-responsive, biocompatible, and targeted delivery systems, and in nanotechnology, which allows one to control the size, multi-functionality, and shape of particulate drug delivery systems [1]. Simple drug-containing capsule systems are not an effective way of drug delivery. An ideal drug delivery system has to release the drug at a steady and uniform rate. It is very hard to achieve required specifications for controlled drug delivery by adopting conventional formulations. Controlled drug delivery is the release of pharmaceutical compound from a material in accordance with the quantities required for the therapeutic effect. Various polymeric systems have been developed for the sustained release of the therapeutic agent in a controlled manner. Polymeric material used to prepare a drug carrier could be natural or synthetic. Various controlled drug delivery systems such as nanoparticles, microspheres, liposome-based systems, and drug–polymer conjugates have thus been developed.

Nanoparticles are particles of less than 1 μm in diameter that are prepared from natural or synthetic polymers. Nanoparticles have ability to deliver a wide range of drugs to different regions of the body for sustained periods of time. A successful nanodelivery system should have a high drug-loading capacity, thereby reducing the quantity of matrix materials for administration. Drug solubility in the excipient matrix material, which is solid polymer or liquid dispersion agent, determines drug-loading and entrapment efficiency. This depends on the matrix composition, drug–polymer interactions, molecular weights, and the presence of end functional groups such as ester or carboxyl in either the matrix or the drug [2–6].

When developing a nanoparticulate delivery system, drug release rate and polymer biodegradation are important components. In general, the determinants of drug release rate are as follows:

(i) The solubility of the drug
(ii) The desorption of the adsorbed drug
(iii) The diffusion of the drug through the nanoparticle matrix
(iv) The erosion or the degradation of the nanoparticle matrix
(v) The combination of erosion and diffusion processes

The five main factors above determine the release process of the drug from matrix. Drug release from nanosphere occurs by diffusion from the matrix or erosion of the matrix. The drug has to be uniformly distributed in the matrix. The drug release from the polymeric membrane is controlled by diffusion in the cases where the nanoparticle is coated by a polymer. Membrane coating acts as a drug release barrier; therefore, drug solubility and diffusion in or across the polymer membrane becomes a determining factor in drug release. The mechanism of release is largely controlled by a diffusion process where the diffusion of the drug is faster than matrix erosion. The rapid, initial release, or "burst," is mainly attributed a state where the drug is weekly bound to the carrier. It is clear that the method of incorporation has an effect on the release profile. Additionally, the release rate can be affected by ionic interactions between the drug and auxiliary ingredients [6].

The current focus of research on nanoparticle drug delivery system is on selecting and combining carrier materials to achieve the optimum drug release speed, on modifying the surface of nanoparticles in order to improve their targeting capability, on preparing nanoparticles in ways that will increase their drug delivery ability in clinical applications, and on investigating in vivo processes to shed light on how nanoparticles interact with blood, targeting tissues and organs, and so on.

Polymeric materials used for preparing nanoparticles for drug delivery must be biocompatible at its best and nontoxic at the very least [7]. The purity of natural polymers varies and they often need cross-linking, which can denature the embedded drug. Consequently, synthetic polymers have been used significantly more in this field. The polymers most widely used for nanoparticles are poly (lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers, poly (lactide-co-glycolide) (PLGA), chitosan, solid lipids, liposomes, block copolymers, poly(ethylene glycol), polycaprolactone, polycyanoacrylate, dextran, poly-L-lysine, silica, gelatin, etc.

Nanoparticles are solid colloidal particles. Based on the preparation process, two types of nanoparticles exist: nanospheres that have a monolithic-type structure in which drugs are dispersed or adsorbed onto their surfaces and nanocapsules that have a membrane-wall structure, which entraps the drugs in the core or adsorbs them onto their exterior. Usually the nanocapsules contain an outer surfactant adsorption layer. Polyalkylcyanoacrylates and polylactides are some of the polymers used for the outer coating. The term "nanoparticles" is adopted because it is often very difficult to unambiguously establish whether these particles are of a matrix or a membrane type [8]. The most important goal in the controlled drug delivery is to increase therapeutic effect and minimize side effects. Ongoing researches are based on the tissue or cell-selective targeting of drugs which can be achieved by delivering the drug to the target area of the body. Over the past few decades, there has been considerable interest in developing polymeric nanoparticles for targeted delivery of pharmaceutical compounds, gene peptide structures, siRNAs, etc. Naturally, researches to improve already the existing drug delivery systems or to invent novel and more effective controlled drug delivery ones are still going on.

Gene therapy is the transfer of genetic material into specific cells of a patient in order to correct or supplement defective genes responsible for the development of the disease. Transferring genetic material into the target cell is maintained by using two major vectors: viral and nonviral. Viral vectors are also called biological nanoparticles. However, disadvantages of viral vectors such as immunogenic/inflammatory responses, low loading capacity, large-scale manufacturing, and quality control have limited their application in gene delivery. In contrast to viral vectors, nonviral vectors have several advantages, such as ease of synthesis, cell/tissue targeting, low immune response, and unrestricted plasmid size [9, 10]. In general, transfection efficiency and gene expression levels of nonviral vectors are low compared to viral vectors. In recent years, newly developed nonviral methods adopted in vector technology have yielded nonviral vectors whose transfection efficiencies are similar to those of viral vectors. Consequently, the advantages stated above resulted in the use of nonviral vectors such as liposomes (lipoplexes), polycationic polymers (polyplexes), and organic or inorganic nanoparticles (nanoplexes) in the ongoing researches on the matter.

The Importance of Polycationic Vectors in Gene Therapy

Cationic phospholipids and cationic polymers are the two major types of nonviral gene delivery vectors currently being investigated. Polycationic vectors are indispensible for delivering therapeutic agents/genetic materials to target tissue. In gene therapy, plasmid DNA is introduced to the target cell and expresses the therapeutic proteins. Dissimilarly, in antisense therapy, oligonucleotides are used to suppress the expression of a disease-causing gene. In the event of in-cell transfection, the naked plasmid can only exceed a trace amount of cell membrane. Cationic polymers have become increasingly popular among nonviral vectors due to their ability to easily form polyelectrolyte complexes between plasmid DNA and cationic polymers. Moreover, they protect DNA from enzymatic degradation, facilitate transfection by condensing DNA into nanoparticles, and ease cell uptake and endolysosomal escape [11]. An ideal carrier system should be biocompatible and non-immunogenic. Nowadays, studies focus on how to reduce the toxicity while increasing the transfection efficiency of polycationic carriers. For an effective transfection of a genetic material, nanoparticle has to compact genetic material and the complex has to migrate through the blood circulation which is expected to arrive at the target tissue without any harm.

Importance of Particle Size and Surface Charge During Its Travel

Through the travel, vector has to prevent genetic material from getting degradated and prevent interactions by the reticuloendothelial system (RES) components, enter the cell via endocytosis, and deliver genetic material to the nucleus (Fig. 1).

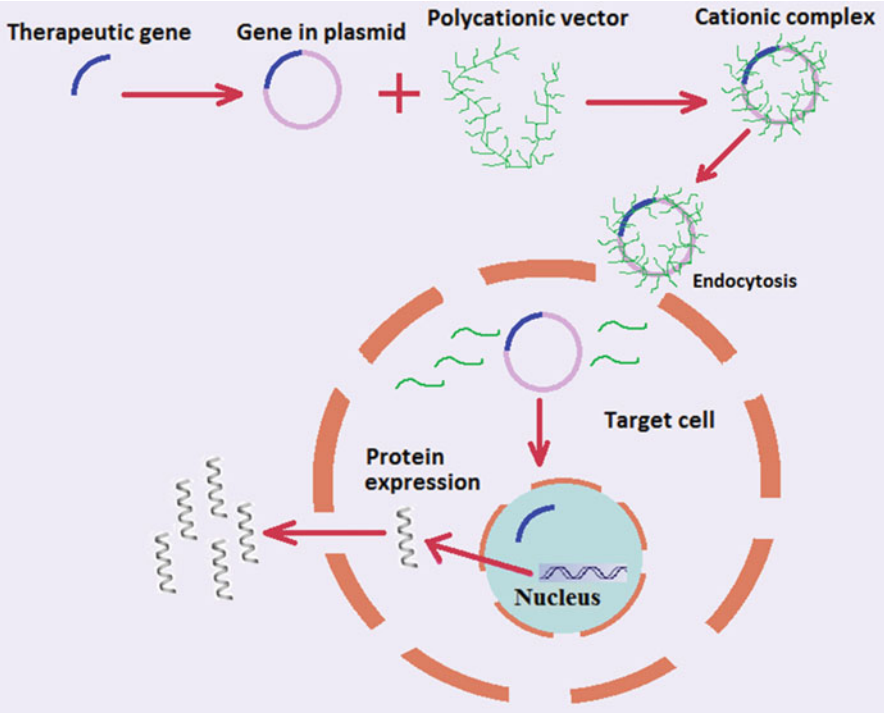

Fig. 1 Schematic representation of gene delivery by polycationic nanoparticles

Escape from RES depends on three main factors: particle size, particle charge, and surface hydrophobicity.

In drug delivery applications, size of the particulate is important for treatment efficacy. Macro size has important drawbacks compared to nanosize in biomedical applications. Conventional micron-sized drug delivery techniques in cancer therapy carry the following disadvantages: delivery inefficiency, toxic effects on health, and impaired transport to tumor sites. Yet, delivery vehicles that are micron sized (μm) are not able to passively traverse through cells and cell pores, including tumor cells with pore sizes as large as 380–780 nm. Consequently, nanodelivery would be the ideal system for biological applications [12, 13].

The submicron size of nanoparticles offers a number of distinct advantages over microparticles. Cell uptake efficiency of nanoparticles is relatively higher in comparison with microparticles. The cell uptake efficiency of 100 nm sized particles has been 15–250-fold higher than larger-sized microparticles [14].

The approaches to modify surface charge and hydrophilicity are initially based on the adsorption of hydrophilic surfactants, such as block copolymers of the poloxamer and poloxamine series. Size, surface charge, and chemistry of nanoparticles affect their clearance by the RES. In general, nature and concentration of the surfactant play an important role in determining the particle size, as well as the surface charge.

PEGylation of a carrier system (usage of PEG for surface modification) makes system invisible against RES. PEG contains the terminal primary hydroxyl groups suitable for derivatization. Polyethylene glycol (PEG) is nontoxic, non-immunogenic, nonantigenic, and highly soluble in water, thanks to these favorable properties that have been approved by the FDA for human use. PEG is a highly preferred polymer in drug delivery systems due to its ease of preparation, relatively low cost, and controllable molecular weight. Looking at in vitro particle internalization by transmission electron microscopy reveals that unmodified polyplexes enter the cells as large aggregates, whereas PEGylated particles stay small and discrete both within and outside the cells. Unmodified and PEGylated particles enter cells via the endocytic pathway, and they assemble in a perinuclear region. Immunolabeling shows unpackaged exogenous DNA in the nuclei and the cytoplasm. All particle types seem to travel toward the nucleus in vesicles and undergo degradation in vesicles and/or the cytoplasm. Then eventually some exogenous DNA enters the nucleus, where it is transcribed. Polyplexes and their PEGylated variants are significantly different in their cellular uptake, particle morphology, and resultant expression [15].

PLA, PLG, or poly(caprolactone) nanospheres coated with PEG are suitable to use for intravenous drug delivery. PEG and PEO are essentially identical polymers used for the same purpose. Their only difference is that PEO's methoxy groups may replace PEG's terminal hydroxyls. The PEG coating of the nanospheres protects against interaction with blood components and removes foreign particles from the blood.

Due to the safety and stability of the hydrophilic coat, the use of diblock copolymers made of poly(lactic acid) (PLA) and poly(ethylene oxide) (PEO) is widely accepted. To achieve this purpose, the copolymer is dissolved in an organic solvent and is then emulsified in an external aqueous phase to orient the PEO toward the aqueous surrounding medium. In a different method, the PLA–PEO copolymer is adsorbed onto preformed PLGA nanoparticles. This was found to effectively prolong the nanosphere circulation time after following intravenous administration. In a study, the poly(lactide-co-glycolide) nanoparticles coated with a 5–10 nm thick layer of polypropylene (PPO)–PEO block copolymer or with tetrafunctional $(PEO–PPO)_2$–N-CH_2-CH_2-N–$(PPO–PEO)_2$ have been prepared by nanoprecipitation technique. The result is that PEO chains have formed a steric barrier which hinders the adsorption of certain plasma proteins onto the surface, and the PEO-coated nanospheres have not been recognized by macrophages as foreign bodies and are not attacked by them.

Recently, by adopting recombinant DNA techniques, many proteins were produced in large quantities, and these have become important new drugs. Protein drugs have certain disadvantages such as susceptibility to degradation by proteases, low solubility, short circulating half-life in vivo, rapid kidney clearance, and propensity to generate neutralizing antibodies, which may limit their usefulness. Thus, extensive research has been conducted in recent years to overcome these inherent problems of protein drugs. Scientists investigated different strategies to enhance the clinical properties of proteins, which include changing amino acid

sequencing via protein engineering techniques in order to decrease proteolytic degradation and antigenic side effects, producing chimeric protein drugs fused to albumin in order to improve half-life or incorporation into appropriate drug delivery vehicles. Among these strategies, nowadays surface modification of protein drugs via covalent attachment of poly(ethylene glycol) (PEG) is viewed as a very important technique that makes protein drugs more water soluble, non-aggregating, non-immunogenic, and more stable to proteolytic digestion [16].

Biodegradability of a Vector Is an Important Feature

Biodegradable polymers have enjoyed significant interest in the past few decades especially for applications in drug delivery. Biodegradability of a polymeric vesicle is an important feature in order to release drugs or bioactive agents in a controllable manner. A variety of biodegradable polymers have been used to deliver drugs, macromolecules, cells, and enzymes. The important feature of these polymers is the manipulation of biodegradability. Poly(lactic acid) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) have been the most extensively investigated polymeric structures for drug and biomolecule delivery.

Poly(D,L-lactic-co-glycolic acid) is the most commonly used structure thanks to its biodegradable feature and metabolizable decay products, which make it far more preferable in comparison with other structures. PLGA is approved by FDA for therapeutic use in humans. Release of drugs from such structures occurs by controlled biological degradation of polymeric structure or diffusion-controlled mechanism. One can adjust the size of PLGA particles by modifying the chemical composition as well as the method of fabrication. The rate of drug release from PLGA NPs can be controlled by changing the molecular weight of PLA, which determines the rate by which the vesicle degrades [17].

PLGA nanoparticles are generally formulated by using emulsion solvent evaporation or solvent displacement techniques [18]. For example, pDNA (alkaline phosphatase, AP, a reporter gene) has been encapsulated in submicron-sized poly (D,L-lactide-co-glycolide) particles. Transfection efficiency of pDNA–NP has resulted in significantly higher in comparison to naked pDNA, in vitro. Additionally, a sustained release of pDNA has been observed for a month [19]. In those years, Labhasetwar et al. have prepared nanoparticles containing bovine serum albumin (BSA) as a model protein and 6-coumarin as a fluorescent marker by a double-emulsion/solvent evaporation technique and observed the endocytosis, exocytosis, and intracellular retention of poly(D,L-lactide-co-glycolide) nanoparticles in vitro. They have observed the model protein carried along with nanoparticles inside the cells [20]. In subsequent studies, the rapid endolysosomal escape of the PLGA nanoparticle carrier has been demonstrated. It has also been suggested that endolysosomal escape of these NPs occurs thanks to their selective surface charge reversal in the acidic endolysosomes. Additionally, NPs deliver their cargo in the cytoplasm at a slow rate, leading to a sustained therapeutic effect [21]. Biodegradable PLGA–PEG–PLGA triblock copolymer systems have been developed by

researchers for nonviral gene transfection in vitro and in vivo [22, 23]. Another study to control blood glucose has been performed by encapsulation of the incretin hormone glucagon-like peptide (GLP-1) into biodegradable triblock copolymer of PLGA–PEG–PLGA (Choi et al. 2004). Besides, many pharmaceutical agents such as 9-nitrocamptothecin, paclitaxel, cisplatin, dexamethasone, triptorelin, and insulin have been entrapped into PLGA nanoparticles for several therapeutic purposes [24–27].

The PLA–PEG and PLGA–PEG are especially useful for encapsulation of hydrophobic drugs. They have also been investigated for the intravenous and mucosal delivery of proteins, oligonucleotides, and genes. All results have proved to be encouraging [28].

For the inhibition of restenosis and to decrease intimal hyperplasia, anti-MCP-1 plasmid-encapsulated PLGA NPs synthesized by double-emulsion/solvent evaporation technology were used, and it has been observed that NPs have a steady in vitro release of 95 % of the total enclosed DNA within 30 days and a significant decrease in intimal hyperplasia [29]. In recent years, a new modified nanoprecipitation method was suggested to fabricate DNA-loaded PLGA nanoparticles instead of the conventional double-emulsion/solvent evaporation method [30].

Semete et al. have conducted an in vitro cytotoxicity study to assess the cell viability following exposure to PLGA nanoparticles. Greater than 75 % cell viability has been observed for PLGA nanoparticles. The extent of tissue distribution and retention following oral administration of PLGA particles have shown that the particles remain detectable in the brain, heart, kidney, liver, lungs, and spleen after 7 days of the issue. 40.04 % of the particles have been localized in the liver, 25.97 % in the kidney, and 12.86 % in the brain. The lowest percentage of PLGA nanoparticles has been observed in the spleen, and they have suggested that toxic effects observed with various industrial nanoparticles will not be observed with PLGA nanoparticles [31]. Some of the examples for PLGA-based polycationic nanoparticles for drug and biomolecule delivery are given in Table 1.

Targeted Drug Delivery

There are different modes of drug administration such as oral, nasal, transdermal, intra venal, etc., in drug delivery applications. Oral and nasal delivery result in high drug levels in the blood and have poor release profiles. Aerosol design is complex and problematic about loading issues. Transdermal delivery does not have targeting and damages healthy cells as well. These shortcomings resulted in the development of targeted drug delivery as a means of overcoming the delivery problems [32].

In targeted delivery approach, targeting molecules are attached to the surface of vectors so that the vectors travel to the target tissue selectively. Targeting and reduced clearance of nanocarrier lowers the therapeutic agent amount required for the treatment of disease. Targeted delivery occurs by either passive or active targeting. Passive targeting results from extravasation of nanoparticles at the disease site with leaky microvasculature. Tumors and inflamed tissues are examples

Table 1 Poly(DL-lactide-co-glycolide) (PLGA)-based biodegradable polycationic nanoparticles for drug and biomolecule delivery

Polymeric materials	Agent	Usage	Target cells
PLGA	pDNA	Model	NIH 3T3 cells
PLGA	Dexamethasone	Antiproliferative	Human arterial smooth muscle cells (HASMCs)
PLGA	pDNA	Model	(Prostate cancer) PC3 cells
Biodegradable triblock copolymer (PEG–PLGA–PEG)	pDNA	Model	HEK 293 cells
(PLGA) nanosphere	Pigment epithelium-derived factor (PEDF)	Antiproliferative	Ocular transport
Biodegradable triblock copolymer (PEG–PLGA–PEG)	pDNA	Model	Skin wound, in vivo
Biodegradable triblock copolymer (PLGA–PEG–PLGA)	Glucagon-like peptide (GLP-1)	To control blood glucose level	Zucker diabetic fatty rats In vitro, In vivo
PLGA nanosphere	Pigment epithelium-derived factor (PEDF)	Antitumor agent (antiproliferative)	Ocular transport
Poly(ethylene glycol)-modified PLGA (PLGA–PEG) NPs	9-nitrocamptothecin	Anticancer drug	In vitro drug release
PLGA nanoparticles	Paclitaxel	Antitumoral activity	NCI-H69 cell line
PLGA nanoparticles	Dexamethasone	Anticancer drug	In vitro
PLGA nanospheres	Triptorelin Peptide delivery	Anticancer agent	Drug encapsulation
PLGA–mPEG nanoparticles	Cisplatin	Anticancer agents	In vivo BALB/c mice
PLGA nanoparticles	Insulin	To reduce blood glucose level	In vivo
(PLGA–mPEG) nanoparticles	Cisplatin	Antitumoral	LNCaP prostate cancer cells
(PLGA–mPEG) nanoparticles	Cisplatin	Antitumoral	Adenocarcinoma HT29 cells
PLGA NPs	Anti-MCP-1 (antisense)	Inhibition of restenosis	Smooth muscle cell (SMC)
PLGA NPs	Gene delivery	For the treatment of atherosclerotic cardiovascular disease	In vivo
PLGA NPs	VEGF	Myocardial infarction	Rabbit

of diseases where passive targeting of nanocarriers can be achieved. In order for the passive targeting to succeed, the nanocarriers should circulate in the blood for an extended time for the nanocarriers to have multiple possibilities to pass through the

target site. Due to the body's natural defense mechanisms that work to eliminate nanoparticles after opsonization by the mononuclear phagocytic system, nanoparticles usually have short circulation half-lives. Localized diseases such as inflammation or cancer have leaky vasculature and overexpress some epitopes or receptors that can be used as targets. Thus, nanomedicines may also be actively targeted to these sites. Ligands specifically binding to surface epitopes or receptors that are preferentially overexpressed at target sites have been coupled to the surface of long-circulating nanocarriers (Koo et al. 2005).

Targeting of a drug may be provided through two different approaches: direct targeting method and pretargeting multistep method. In direct targeting approach, the targeting ligand is attached onto the nanoparticles. In the pretargeting approach, the ligand, intended to be concentrated and localized in the target tissue, is administered before the administration of drug-loaded carrier.

Popular targeting molecules are monoclonal antibodies (mAb) and their fragments, folate, transferrin, avidin–biotin, RGD(Arg-Gly-Asp) peptide, IKVAV, cell adhesion molecules (E-selectin, ICAM-1, VCAM-1,and P-selectin), etc. In the avidin–biotin targeting system, targeting of drug/biomolecule is maintained at three steps. Firstly, biotinylated targeting ligands are sent to target tissue, after which avidin administration is performed. Finally, biotinylated drug-loaded nanoparticles are administrated (Breitz et al. 1999; Cremonesi et al. 1999; Knox et al. 2000).

MMP-2 is one of the enzymes in MMP family and is essential for angiogenesis. MT1–MMP is linked to metastasis and angiogenesis and is observed to be expressed on endothelial cells and certain types of tumor cells, which include malignancies of lung, gastric, colon, breast, and cervical carcinomas, gliomas, and melanomas. One of the main targets of MMP is the membrane type-1 matrix metalloproteinase (MT1–MMP), which is an activator of MMP-2 [33].

For example, anti-HER2 (trastuzumab, Herceptin) and anti-CD20 (rituximab, Mabthera) have been conjugated to poly(lactic acid) nanoparticles. Cell uptake efficiency of nanoparticles with targeting molecules has increased sixfold compared to nanoparticles without targeting molecules (Nobs et al. 2006). In another study, Chung et al. have encapsulated tissue-plasminogen activator (t-PA) into CS-GRGD-coated PLGA of nanoparticles to accelerate thrombolysis (Chung et al. 2008).

In cancer treatments, anticancer drug carriers have to deliver the drug to the target tissue at prolonged times and required rates in a controlled manner. Combination of controlled-release systems with targeted drug delivery systems provides more efficient delivery of the nanocarriers in cancer therapy. Conventional chemotherapeutic agents get nonspecifically distributed in the body, where they influence cancerous and normal cells. This limits the dose that can be achieved within a tumor and results in a suboptimal treatment because of excessive toxicities. In order to overcome the conventional chemotherapeutic agents' lack of specificity, one approach that has emerged is molecularly targeted therapy [34]. In recent years, chemotherapeutic agent loaded in nanoparticles is targeted to improve their therapeutic efficiency and functionality in cancer treatments.

The targeting scheme for the αvβ3 integrin focused on the three amino acid sequence arginine–glycine–aspartic acid (RGD). The αvβ3 integrin is an

endothelial cell receptor for extracellular matrix (ECM) proteins that harbor the RGD sequence, which contains von Willebrand factor, fibrinogen (fibrin), vitronectin, thrombospondin, osteopontin, and fibronectin [35]. Signals from receptors for growth factors and ECM molecules regulate angiogenesis. For instance, integrin αvβ3 inhibition during bFGF stimulation suppresses the sustained phase of extracellular signal-related kinase (ERK) signaling, which leads to endothelial apoptosis and inhibition of angiogenesis. Despite the fact that anti-αvβ3 blocks bFGF-mediated angiogenesis, anti-αvβ5 disrupts VEGF-induced angiogenesis, showing that distinct signaling pathways regulate angiogenesis. Hood et al. have emphasized αvβ3 targeting by an RGD non-peptide mimetic coupled to a nanoparticle for anti-angiogenesis therapies [36].

Another example of targeted drug delivery in cancer treatment is the folate-conjugated PEG-co-poly(lactic-co-glycolic acid) (PEG–PLGA) micelles loaded with the anticancer drug doxorubicin, which express folate on the micelle surface. Increased cytotoxicity and decreased tumor growth for folate-conjugated micelles have been reported compared to nontargeted micelles and free DOX [37].

A different example for active targeting of a nanoparticle is RNA A10 aptamers specific for the prostrate-specific membrane antigen. Compared to nontargeting NPs, these have been successfully conjugated onto PLA-block-PEG polymers and showed increased drug delivery to prostate tumor cells [38].

Blood Brain Barrier

The blood–brain barrier (BBB) is one of the hard-to-pass barriers for drugs such as anticancer agents, antibiotics, peptides, oligo-molecules, and macromolecules. This is due to the presence of "tight junctions between the endothelial cell linings in the brain blood vessels." Nanoparticles seem to be an attractive solution to overcome the BBB. The size and surface modification/functionalization enable the transport of nanodelivery vehicles across the BBB. Yet, there is a widely speculated possibility of unintended intrusion into the brain via the BBB, and thus high selectivity is critical for any BBB uptake in order to avoid unwelcome particles.

One successful drug delivery system to the brain uses nanoparticles coated with polysorbate 80. Nanoparticles of drug vehicles coated with polysorbate 80 result in better uptake across the BBB. Notably, during the delivery of doxorubicin, the drug delivery was more efficient with the polysorbate-coated nanoparticles compared with the non-coated nanoparticles [39].

Smart Polymers Respond Microenvironmental Changes

Smart polymers are known to be stimulus-responsive, intelligent polymers or environmentally sensitive polymers. They have become an important class of polymers and have a significantly increasing application [40]. In the body, some environmental variables, such as pH, temperature, ionic strength, etc., are found.

The characteristic special property that actually makes them "intelligent" is their ability to respond to the changes in the surrounding environment.

The lower critical solution temperature (LCST) is the critical temperature that the polymers are soluble in a solvent (water) at temperatures below LCST but which become insoluble as the temperature rises above the LCST. The LCST behavior of a copolymeric structure depends on the monomer ratios, polymer degree of polymerization, composition, and branching of the polymer. Poly(*N*-alkyl-substituted acrylamides) and poly(*N*-vinylalkylamides) are the common thermosensitive polymers with LCST of 32 °C and 32–35 °C, respectively. As an example:

> Chung et al. have designed thermoresponsive polymeric micelles comprising AB block copolymers of PIPAAm (poly(N-isopropylacrylamide)) blocks and PBMA (poly(butyl methacrylate)) or PSt (polystyrene) blocks that are able to encapsulate adriamycin, which is a hydrophobic drug. PIPAAm-PBMA micelles were observed to release the drug only above the reversible thermoresponsive phase transition of PIPAAm. [41]

pH-responsive polymers respond to the pH changes in the microenvironment by changing their dimensions. Depending on the pH of the environment, pH-responsive polymers become soluble or collapse. This is due to the existence of certain functional groups in the polymer chain. Protonation/deprotonation takes place depending on the presence of ionizable functional groups (–COOH, –NH) in certain pH.

pH-sensitive polymer's pH-induced phase transition usually switches within 0.2–0.3 unit of pH and tends to be very sharp. Copolymers of methyl methacrylate and methacrylic acid go through a sharp conformational transition and collapse at low pH, around 5. Copolymers of methyl methacrylate with dimethylaminoethyl methacrylate are soluble at low pH, but they collapse and aggregate in slightly alkaline conditions [40]. For example, exendin-4 (an insulinotropic agent) incorporated pH-sensitive nanoparticle vehicles that have been developed to administrate this agent in the small intestine. The pH-sensitive nanoparticle vehicles have been designed to stay intact in the stomach and then dissolve in the small intestine. The system has exhibited a prolonged glucose-lowering effect [42].

Tumor Microenvironment

It is common for cancer cells to display increased aerobic glycolysis. Biological adaptation to metabolic changes due to mitochondrial dysfunction, hypoxia, and oncogenic signals makes the malignant cells addicted to glycolysis and dependent on this ATP generation pathway. These changes in the energy metabolism and the following increased glycolytic enzyme expression and other pro-survival molecules give the cancer cells an advantage in surviving.

Moreover, lactate accumulation due to increased glycolysis results in an acidic tumor microenvironment, which provides a tissue environment that selects the cancer cells that have high survival capacity and malignant behaviors.

These biological modifications cause important problems in cancer treatment, evidenced by the cancer cells in hypoxic environment becoming resistant to chemotherapeutic agents and radiation therapy. Yet, the growing dependency of cancer cells on glycolysis to generate energy also presents a biological mechanism to preferentially kill the malignant cells through inhibiting glycolysis. According to strong evidence from recent studies, cancer cells with mitochondrial defects or that are under hypoxia are highly sensitive to glycolysis inhibition. It has been found that several glycolytic inhibitors have promising anticancer activity in vitro and in vivo, and some of them have begun to be tested in clinical trials [43].

There are some improved systems that combine two stimulus-responsive mechanisms into one polymer system such as temperature-sensitive polymers which also responds to pH changes. For example, Zhang et al. have prepared a thermo- and pH dual-responsive nanoparticle, which encapsulates an anticancer drug (paclitaxel) that was assembled from a diblock copolymer comprised of a hydrophilic poly(*N*-isopropylacrylamide-co-acrylic acid) block and a hydrophobic polycaprolactone block. Nanoparticles aggregated in a pH of 6.9 at body temperature. It has been found that faster drug release was associated with higher temperature and lower pH. Both of these conditions are advantageous for tumor-targeted anticancer drug delivery [44].

Another approach is the development of multidrug-loaded nanoparticles against drug-resistant cancers. Advances in nanoparticle-based combination strategies against clinical cancer drug resistance were reached through the co-encapsulation of drugs with differing physicochemical characteristics, organizing ratiometric control over drug loading and temporal sequencing of drug release. These new strategies lead the way for better-tailored combinatorial solutions for clinical cancer treatment [45].

The following studies are some of the examples for stimulus-responsive drug delivery:

Brown et al. have prepared doxorubicin-loaded nanoparticles, formulated by nanoprecipitation of acid-ended poly(lactic-co-glycolic acid) and have achieved the controlled release of doxorubicin in a pH-dependent manner to breast cancer cells (Betancourt et al. 2007). Also, via the copolymerization of NIPAAm and DMAEMA, with Ce4+ ions and tris(hydroxymethyl)methylamine as a redox initiator system, an amphiphilic star block consisting of a hydrophobic PMMA block and a hydrophilic tri-arm poly(NIPAAm-co-DMAEMA) was synthesized. The star copolymer goes through self-assembly to the micellar nanoparticles with a core–shell structure and the thermo-/pH dual response, resulting from the thermosensitivity of PNIPAAm and the pH sensitivity of PDMAEMA [46]. Poly (ethylene oxide)-modified poly(β-amino ester) nanoparticles for tumor-targeted delivery of hydrophobic drugs have been developed as a pH-sensitive system [47]. Acid-sensitive dexamethasone-loaded polyketal nanoparticles in diameter between 200 and 600 nm have been designed as a delivery system to tumors, inflammatory tissues, and phagosomes. Nanoparticles were produced from poly (1,4-phenyleneacetone dimethylene ketal) (PPADK), which is a new hydrophobic polymer containing ketal linkages in its backbone. The polyketal nanoparticles go

through acid-catalyzed hydrolysis to become low-molecular-weight hydrophilic compounds, thus releasing the therapeutics encapsulated in them at a faster rate in acidic environments [48].

Lin et al. have developed pH-responsive liposomes containing synthetic glutamic acid-based zwitterionic lipids and evaluated their properties both in vitro and in vivo. L1 (1,5-dihexadecyl *N*-glutamyl-L-glutamate) and L2 (1,5-dihexadecyl *N*,*N*-diglutamyl-lysyl-L-glutamate) are the glutamic acid-based lipids which are used in liposomal drug delivery systems as the pH-responsive pieces of the vehicle that should give a response to endosomal PH.

Application of pH-responsive liposomes has indicated efficient intracellular drug delivery by the L1- and L2-containing liposomes and higher DOX toxicity toward HeLa cells in comparison with conventional DPPC liposomes [49].

Liposome or Lipid-Based Nanoparticles in Drug/Gene Delivery

The drug delivery system has seen much progress from the design and synthesis of different biocompatible materials. For clinical application, liposome has been the most successful candidate. Most DDS that are approved by the FDA are lipid based or liposome. Liposomes are shown to be useful for the delivery of pharmaceutical agents. "Contact-facilitated drug delivery" that is used by these systems involves binding or interaction with the targeted cell membrane. Such nanosystems can serve as drug depots exhibiting prolonged release kinetics and long persistence at the target site [6].

Liposomes are small, artificial, spherical vesicles that self-associate into bilayers in order to encapsulate genes, drugs, and other biomolecules on aqueous interior. They are composed of nontoxic phospholipids and cholesterol. Liposomes vary in size 25 nm to 10 μm, depending on the method of their preparation. Currently, certain therapeutic agent-loaded liposomes are in the process of being tested comprehensively for targeted delivery against cancers. Liposomes that have certain sizes, typical instance being less than 400 nm, can quickly infiltrate tumor sites from the blood. Yet, they are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. In order to have effective therapeutic concentrations at the tumor site, liposomes are perforated through nanovasculature. These are able to restrict and/or decrease certain common side effects such as headache, nausea, vomiting, and hair loss. Several types of nanoscale liposomes have been widely used in treatments for cancer (Tangri).

Size is an important factor in determining the efficiency of targeting and the associated therapeutic effects of liposomes. It has been shown that size determines the efficacy of therapy, liposomal accumulation in tumor site, cross-vessel permeation, level of toxicity, and overall transport in the body. Moreover, the smaller the size, the better the extent of targeting and therapy efficacy. This can be associated with the drug amount that reaches the site of the tumor. Liposomes that are 100 nm in size and below have shown better targeting and accumulation in the tumor site [50].

Due to the aggregations of liposomes in the presence of plasma proteins and the rapid clearance of liposomes from the bloodstream via the reticuloendothelial system (RES), the in vivo application of liposomes through intravascular injection is limited. In order to avoid detection of the RES, "stealth" or long-circulating liposomes have been designed. This kind of lipid-based drug carriers for in vivo delivery is prepared by using cholesterol, amphiphilic stabilizers, or phosphatidylinositol. Grafting polyethylene glycol (PEG) chains on the liposome surface to make a hydrophilic surface is another approach. These liposomes that are sterically stabilized serve as long-circulating drug reservoirs, and they allow drug targeting to non-RES target sites. In order to achieve specific tissue targeting, ligands such as antibodies can be conjugated to the PEG chains that are on PEG-stabilized liposomes [35].

Some examples for the current liposomal formulations are PEGylated liposomal doxorubicin (Doxil R Ortho Biotech, Caelyx(R) Schering-Plough), non-PEGylated doxorubicin (Myocet R Elan Pharma), and liposomal daunorubicin (DaunoXome R, Gilead Sciences). Not only these approved agents but also many liposomal chemotherapeutics are presently in the process of evaluation in clinical trials. The upcoming liposomal drug generation could be immunoliposomes that may selectively transport the drug to the desired locations.

Wang et al. have developed the folate–PEG-coated polymeric liposome that combines both advantages of polymer nanoparticles and liposomes [36]. Surface-functionalized target liposomes are presently being investigated, and it is hoped that the targeted systems could further ameliorate these drug delivery systems' efficacy and safety qualities. Using liposomal drug delivery systems in combination with the polymeric systems will result in prolonged and more selective drug delivery [51].

Currently, several kinds of cancer drugs have been applied to these lipid-based systems using a variety of preparation methods. Below are some examples for the subject; Chen et al. have developed a FGFR-mediated drug delivery system in order to target the FGFR-overexpressed tumor cells with chemotherapeutic agents. Using electrostatic force, they linked a shortened truncated human basic fibroblast growth factor (tbFGF) peptide to the surface of cationic liposomal doxorubicin (LPs–DOX) and paclitaxel (LPs–PTX) [33].

Similarly, Banerjee et al. have designed anisamide-targeted doxorubicin-loaded stealth liposomes for targeted drug delivery to human prostate cancer cells. It is shown that some human malignancies, such as prostate cancer, overexpress sigma receptor. Sigma receptor is a membrane-bound protein, which binds haloperidol and various other neuroleptics with high affinity. When a polyethylene glycol phospholipid was derivatized with an anisamide ligand and was put into the DOX-loaded liposome, this resulting anisamide-conjugated liposomal DOX had significantly higher toxicity for DU-145 cells than the nontargeted liposomal DOX [52].

Cationic lipids are also used for gene therapy. DNA/lipid complexes formed by the interaction of positively charged lipids at the physiological pH with the negatively charged DNA through electrostatic attractions. Cationic lipids used for gene therapy are composed of three basic domains: a positive-charged headgroup, a

hydrophobic chain, and a linker which joins the polar and nonpolar regions. The polar and hydrophobic domains of cationic lipids may have dramatic effects on both transfection and toxicity levels. The stability and particle size of these delivery vesicles partly determine their transfection efficiency in vitro. Yet, these liposomes or DNA/lipid complexes frequently show decreased efficiency of transfection in vivo. In general, overcharging is toxic to a variety of cell types, different reagents have varying toxicity degrees for cells, and toxicity is cell specific [53]. There are also liposome-based systems developed for the delivery of nucleic acid-based therapeutics such as antisense, aptamers, and RNAi molecules.

Polysaccharide-Based Nanoparticles as Drug Delivery Systems

Polysaccharides, the polymers of monosaccharides, have various resources of algal origin (e.g., alginate), plant origin (e.g., pectin, guar gum), microbial origin (e.g., dextran, xanthan gum), and animal origin (chitosan, chondroitin) in nature. Polysaccharides possess a broad range of molecular weight (MW), a great number of reactive groups, and differing chemical compositions, and these factors contribute to their diverse structures and properties. Polysaccharides are easy to chemically and biochemically modify because of their various derivable groups on molecular chains, and this leads to multiple types of polysaccharide derivatives. Polysaccharides have the following properties: they are highly stable, hydrophilic, biodegradable, nontoxic, and abundant in nature and have low process cost. Specifically, most natural polysaccharides possess hydrophilic groups such as hydroxyl, carboxyl, and amino groups that can form non-covalent bonds with biological tissues, mostly epithelia and mucous membranes, and create bioadhesion. To give an example, chitosan, starch, and alginate are good bioadhesive materials. This is advantageous because nanoparticle carriers that are created using bioadhesive polysaccharides may prolong the resistance time and thereby increase the absorbance of loaded drugs. In recent years, a large number of studies have been conducted on polysaccharides and their derivatives for their potential application as nanoparticle drug delivery systems [54].

Currently, natural polysaccharides are widely preferred when developing solid dosage forms for drug delivery to the colon. The reason for this lies in the following fact: due to the colon being inhabited by a large variety and number of bacteria that secrete many enzymes such as β-D-glucosidase, β-D-galactosidase, amylase, pectinase, xylanase, β-D-xylosidase, and dextranase, there exists large amounts of polysaccharidases in the human colon. Fermentable coating of the drug core, embedding the drug in the biodegradable matrix, and formulating drug–saccharide conjugate are some of the major approaches that use polysaccharides for colon-specific delivery. Many polysaccharides, such as chitosan, pectin, chondroitin sulfate, cyclodextrin, dextrans, guar gum, inulin, amylose, and locust bean gum, have already been investigated as colon-specific drug carrier systems [55].

Dextran, a polysaccharide made up of glucose units that are coupled into long branched chains mainly through a 1–6 and some 1–3 glycosidic linkages, is a

colloidal, hydrophilic, water-soluble substance, which is inert in biological systems and does not influence cell viability. Dextrans have been explored for the delivery of various pharmaceuticals.

Alginate, an anionic polysaccharide, is widely dispersed in brown algae cell walls, and in those cell walls, it forms a viscous gum by binding with water. It absorbs water quickly in its extracted form, and it has the ability to absorb 200–300 times its own weight in water. Alginates are one of the most versatile biopolymers, and they are employed in a wide variety of applications. Alginate's thickening, gel forming, and stabilizing properties underlie its use as an excipient in drug products. In order to achieve prolonged and better control of drug administration, the demand for tailor-made polymers has increased. Hydrocolloids such as alginate may have an important part in the design of a controlled-release product [56].

Aynie et al. have designed a new antisense oligonucleotide (ON) carrier system that was based on alginate nanoparticles. They investigated whether it would be able to protect ON from degradation when the serum was introduced. They have reported that this new alginate-based system was able to protect [33P]-radiolabeled ON from degradation in bovine serum medium [57].

Chitosan (CS) is a polysaccharide consisting linear β(1–4)-linked monosaccharides which is similar to cellulose in structure. The important difference between cellulose and CS is the 2-amino-2-deoxy-h-D-glucan units combined with glycosidic linkages. Chitosan is derived from the deacetylation of chitin, naturally available in marine crustaceans. Chitosan is used extensively in drug delivery applications due to its favorable properties such as positive-charge, biocompatibility, and mucoadhesive character. Chitosan, a cationic polysaccharide in neutral or basic pH conditions, has free amino groups and is thus insoluble in water. Since in acidic pH, amino groups can undergo protonation, which makes it soluble in water, CS solubility is dependent on the distribution of free amino and *N*-acetyl groups. Due to its characteristics of not causing allergic reactions and rejection, chitosan is biocompatible with living tissues. Chitosan slowly breaks down into harmless products, which the human body can completely absorb. Chitosan derivatives are nontoxic and can be easily removed from the organism without resulting in any side reactions [58].

Chitosan-based nanoparticles are attractive gene delivery devices. Huang et al. have evaluated the effects of the molecular weight and the deacetylation degree of chitosan on cellular uptake and gene transfection efficiency. They have reported that an N/P ratio of 6 was optimal for producing the chitosan–DNA NP. Abovementioned N/P ratio is optimal to prepare chitosan nanoparticles with mean size of 150–300 nm which is suitable for gene delivery. They also reported chitosan vectors with lower Mw or DD to be less-efficient retainers of DNA upon dilution. Thus, they were observed to be less able to protect the condensed DNA from DNase and serum component degradation. Decreasing the Mw or DD of the chitosan vector significantly reduced the cellular uptake of the NP [59].

The transfection efficiency of chitosan/pDNA complex greatly depends on the microenvironment's pH, since the protonated amines of chitosan help it bind to negatively charged DNA. Zhao et al. investigated pH's effect on transfection

efficiency, and they obtained the highest expression efficiency at pH 6.8 and 7.0. When pH of transfection medium was increased to 7.4, the transfection efficiency was observed to dramatically decrease. They have defined the decrease in transfection efficiency by the dissociation of free plasmid from the complex at the higher pH [60]. In another study, chitosan-g-poly(ethylene glycol)-folate nanoparticles for gene delivery have been prepared. PEGylation increased its solubility, and folate conjugation improved the efficiency of gene transfection resulting from the promoted uptake of folate receptor-bearing tumor cells. Because of its targeting ability, low cytotoxicity, solubility in physiological pH, and efficiency in condensing DNA, modified chitosan is a favorable gene carrier [34].

In the following years, chitosan nanoparticles functionalized by attachment of different targeting molecules to achieve side-specific/targeted drug delivery have been prepared. As an example, Mohan et al. developed a folic acid (FA)-conjugated carboxymethyl chitosan coordinated to manganese-doped zinc sulfide quantum dot (FA–CMC–ZnS:Mn) nanoparticles. This system has been utilized for controlled drug delivery, targeting, and cancer cell imaging [38]. In another study, an amphiphilic copolymer has been designed. Firstly, *N*-octyl-*N*-phthalyl-3,6-*O*-(2-hydroxypropyl) chitosan (OPHPC) is synthesized and then conjugated with folic acid (FA–OPHPC) in order to create a targeted drug carrier for tumor-specific drug delivery. Paclitaxel is loaded into OPHPC micelles with a loading efficiency of 50.5–82.8 %. The paclitaxel-loaded OPHPC has shown a significantly higher cellular uptake efficiency in human breast adenocarcinoma cell line compared to Taxol®. Moreover, the cellular uptake of the drug in drug-loaded FA–OPHPC micelles (paclitaxel-FA–OPHPC) is 3.2-fold more effective than that of paclitaxel-loaded OPHPC [49]. Recently, chitosan nanoparticles have been studied for the delivery of tamoxifen. Tamoxifen-loaded chitosan nanoparticles have been constructed as pH-responsive drug carries for effective antitumor activity. Because cancer cells have an acidic extracellular tumor environment, this mechanism is especially appealing for cancer therapy. It has been observed that tamoxifen-loaded chitosan nanoparticles augmented the tamoxifen accumulation in tumor cells, caused caspase-dependent apoptosis, and increased anticancer activity.

Peptide and Protein Delivery

The peptides, proteins, and other compounds that are acquired via biotechnological processes have a complex nature, and this causes various challenges when one aims to understand their therapeutic and physicochemical behaviors [61]. The number of amino acid residues generally determines the classification of peptides. Proteins are molecules with more than 50 amino acids, and molecules with 10–50 amino acids are called peptides. A peptide is a chemical compound that has two or more amino acids coupled by a peptide bond. This bond is the linkage of the nitrogen atom of one amino acid with the carboxyl carbon atom of another amino acid. Polypeptide refers to molecules that have molecular weights that range from several thousand to several million daltons. It can be observed that the terms protein and polypeptide

are used interchangeably. In 1901, Emil Fischer in collaboration with Ernest Fourneau discovered the first synthetic peptide glycyl–glycine. In 1953, Vincent du Vigneaud synthesized the first polypeptide (oxytocin – nine amino acid sequence).

Specific primary, secondary, tertiary, and quaternary structures of a protein play key roles in defining the integrity and biological activity of biomacromolecules. The primary structure of a protein is the amino acid linear sequence of the polypeptide chain. The secondary protein structure is the specific geometric shape caused by intramolecular and intermolecular hydrogen bonding of amide groups. Alpha helix and beta sheets are two main types of secondary structure. These secondary structures are defined by patterns of hydrogen bonds between the main-chain peptide groups. The third type of structure found in proteins is called tertiary protein structure. Tertiary structure refers to three-dimensional structure of a single-protein molecule. The tertiary structure is the final specific geometric shape of a protein. Quaternary structure is the three-dimensional structure of a multi-subunit protein. An important point is that whereas a small peptide's function only depends on the functional groups of different amino acids, a protein's function depends on the maintenance of a precise 3D structure. Thus, it is understandable that protein delivery technologies undergo a more hard task than it is in the case of peptide delivery. The nature of 3D structure must not be spoiled while loading into the vector so that the protein functions properly [61].

Oral administration is the most convenient route for drug delivery. Yet, due to the instability of peptide and protein drugs in the gastrointestinal tract and their low permeability across the intestinal mucosa, their bioavailability following oral administration happens to be very low. Nowadays, numerous types of bioactive peptides are available. Several approaches have been investigated to improve their oral bioavailability such as chemical modification of peptide drugs, the use of an absorption enhancer to promote drug absorption, and the use of protease inhibitor to protect drugs against degradation. Encapsulating or incorporating peptides in polymeric nanoparticles seems to be a promising approach. The use of nanoparticles should at least protect peptide drugs against degradation and, in some cases, also enhance their absorption. Below are some examples of nanoparticle-based peptide delivery.

PLGA nanoparticles have also been used to deliver peptides. A model synthetic long peptide has been encapsulated in PLGA nanoparticles. Silva et al. developed an encapsulation method where they used an apparent inner phase pH above the pI of the encapsulated SLP. This can lead to future advances in encapsulating peptides that have amphiphilic and/or hydrophilic qualities. They have also observed encapsulation and release characteristics to depend strongly on the first emulsion's pH [62].

In another study, exendin-4 a glucagon-like peptide-1 mimetic for type 2 diabetes treatment was conjugated to low-molecular-weight chitosan (LMWC). The LMWC–exendin-4 conjugate formed a nanoparticle structure via complexation between the positively charged LMWC backbone and the negatively charged exendin-4, a structure that had a mean particle size of 101 $\pm$ 41 nm; absorbed

exendin-4 showed a significantly increased hypoglycemic effect, suggesting that it may be employed as a possible oral antidiabetic agent for type 2 diabetes treatment [63].

siRNA-/Nanoparticle-Based Therapy

An important part of gene regulation in gene expression is the control of translation and mRNA degradation. Small RNA molecules, which are common and effective modulators of gene expression in many eukaryotic cells, can be either endogenous or exogenous microRNAs (miRNAs) and short-interfering RNAs (siRNAs). There are a lot of studies carried out for the treatment of diseases through the delivery of RNA molecules. Small interfering RNA (siRNA) is short, double-stranded RNA consisting of 20–25 nucleotides. It causes the degradation of target mRNA and blocks the production of the associated protein, thus resulting in RNA silencing. The possibility of silencing genes, involved in the formation of the disease using siRNA, has led to a rapidly evolving area in drug discovery. In case of direct injection of naked siRNA, high doses are required due to RNA instability, besides the nonspecific cellular uptake is a disadvantage.

The most important issue in the use of siRNA-based therapies for gene silencing by systemic administration is to help siRNA to reach the cytoplasm of the target cell without spoiling its structure. Stealth property and surface charge of the siRNA-encapsulated polycationic-based vector are important parameters for siRNA's stability and for the system to escape from RES components as it is the case in polycation-based drug/gene delivery systems. For effective siRNA delivery, positive surface charge of vector/siRNA complex is an advantage because it facilitates binding to negatively charged cell membranes and it induces cell uptake. On the other hand, for in vivo applications, an excessive positive surface charge is rather a handicap because of interactions with negatively charged serum proteins which makes them detectable by the macrophages. Various cationic polymer/siRNA conjugates have been developed for targeted siRNA delivery. Most nonviral vector systems, developed for plasmid DNA delivery, have been adopted for siRNA delivery.

PLGA nanoparticles are widely used in controlled release of oligonucleotides such as siRNAs, thanks to their solid structure. Solid structure of PLGA nanoparticles makes them stable and prevents the degradation of nucleic acids when circulating in the blood stream. Its solid phase is favorable for long-term storage and convenient for clinical use. PLGA is a biodegradable polymer and during its degradation through hydrolysis, there is a slow release of siRNA that results in the sustained knockdown of the target gene. The ability to precisely conjugate up to three different ligands to the nanoparticle surface leads to flexibility during their modification for different potential applications. These multifunctional nanoparticles have multiple benefits over other siRNA delivery technologies [60]. Some of the examples for polymeric nanoparticles used for therapeutic agent delivery are given in Table 2.

Table 2 Polymeric nanoparticles used for therapeutic agent delivery

Polymeric materials	Agent	Usage	Target cells
Liposome-based nanoparticle	KLF5-siRNA	Antitumor	PC3 cell tumors
Cyclodextrin (CDP)–AD–PEG nanoparticles	siRNA	Anticancer therapeutics	PC3 tumors
Chitosan/siRNA NP encapsulated in PLGA nanofibers	siRNA	Gene silencing	H1299 cells
Transferrin (Tf) grafted–poly(lactide-co-glycolide) (PLGA) nanoparticles	Nevirapine (NVP)	To enhance the transport of nevirapine (NVP)	Human brain microvascular endothelial cells (HBMECs)
Penetratin-modified PLGA nanoparticles	miR-155	miR-155 replacement	pre-B cell tumors
Nonaarginine-modified PLGA nanoparticles	Antisense oligonucleotide	miR-155 inhibition	Inhibition of proto-oncogene, MCL1
CDP–AD–PEG–Tf nanoparticles	pDNA	Anticancer therapeutics	PC3 tumors
PLGA nanoparticles	Synthetic peptides	Immunotherapy of cancer	CD8+ T cells
Low-molecular-weight chitosan (LMWC)	Glucagon-like peptide-1 (GLP-1) (Exendin-4)	Treatment of type 2 diabetes	INS-1 cell line
PLGA nanoparticles PLGA–PEI nanoparticles	siRNA	Target gene silencing	MDA-kb2 cells
Lactosylated gramicidin-based lipid nanoparticles (Lac-GLN)	Anti-miR155	microRNA-155 inhibition	Hepatocellular carcinoma (HCC) cells
PLA–PEG NPs PLGA NPs	Procaine hydrochloride	Drug delivery	In vitro drug release
PLGA–PVA nanoparticles	Dexamethasone	Antiproliferative	Human vascular smooth muscle cells (VSMCs)
Polyalkylcyanoacrylate nanoparticle	CyA (as a chemosentizing drug) with doxorubicin (Dox)	Anticancer drug	P388 resistant cells
PLGA nanoparticles	Vincristine and verapamil	For the treatment of drug-resistant cancers	MCF-7/ADR, a human breast carcinoma cell line
Liposomal NPs	pDNA	Gene delivery	Mouse bone marrow-derived dendritic cells (BMDC)
PLGA nanoparticles/CPP penetrated PLGA	Plasmids encoding for luciferase	Gene delivery	A549 human lung epithelial cells

Another method for the efficient protection and delivery of siRNAs in vitro and in vivo relies on polyethylenimine complexation. Urban-Klein et al. have reported that upon PEI complexation, siRNAs are efficiently protected from RNase and nuclease degradation. Additionally, it has been stated that no siRNAs were found neither nor in nontargeted organs. The results have indicated that the detected signals were derived from siRNA molecules actually internalized by the cells of the respective target organ [64].

Below are some of the examples about the topic. Angiogenesis is essential for tumor proliferation. The suppression of gene expression of VEGF is an important approach for the prevention of tumor growth. Vascular endothelial growth factor (VEGF) is a critical mitogen that induces angiogenesis. Huang et al. have developed amine-modified PVA–PLGA/siRNA nanoparticles for pulmonary siRNA delivery. This polymer is considered to be promising siRNA carrier for pulmonary gene therapy because of its following qualities: nanoparticle stability during nebulization, high specific knockdown, and fast degradation in conjunction with low cytotoxicity [59].

Yagi et al. have developed a systemically injectable siRNA vehicle, which contains siRNA and a cationic lipofection complex in a core that is fully enveloped by a neutral lipid bilayer and hydrophilic polymers. Nanoparticle system has provided the protection of siRNA from enzymatic digestion for 24 h. The result is that the complex has leaked from blood vessels within tumors into the tumor tissue and transfected into the tumor cells [65]. Davis et al. developed a cyclodextrin- and PEG-containing polymer with a human transferrin molecule on its surface, which is a targeting ligand to directly transferrin receptors that are typically found on cancer cells [66]. Dohmen et al. have prepared siRNA-encapsulated nanoparticles on which there is a ligand targeting folic acid receptor-expressing cells. Targeted nanoparticles have been shown to be specifically internalized into folic acid receptor-expressing cells, and efficient receptor-specific gene silencing is achieved [67].

Inhibition of miRNAs

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression at the posttranscriptional level. miRNAs take part in various cellular mechanisms such as proliferation, apoptosis, etc. In recent years, the role of miRNAs in human cancers has been discovered and used for cancer treatment quite popularly. Current RNA-based therapeutics are based on the association of synthetic nucleic acids with cellular RNA targets. The gene therapy method antisense oligonucleotide bound to mature microRNA inhibits microRNA-mediated gene regulation, and the method of splicing junctions on pre-mRNA induces alternative splicing [52].

Several studies have demonstrated the utility of inhibiting miRNAs by using complementary anti-miR molecules, chemically modified antagomirs, and peptide nucleic acids (PNAs) which show particular promise in vivo. The backbone of peptide nucleic acid (PNA), which is an artificially synthesized polymer, consists of

repeating *N*-(2-aminoethyl)-glycine units that are linked via peptide bonds. The various purine and pyrimidine bases are connected to the backbone. The backbone of PNA contains no charged phosphate groups. Thus, the absence of electrostatic repulsion in the binding between PNA/DNA strands makes it stronger than the one between DNA/DNA strands. PNA oligomers have great specificity when binding to complementary DNAs, and this same specificity and strength are present in PNA/RNA duplexes as well. PNAs are stable over a wide pH range, and nucleases and proteases cannot easily recognize them. Because of their stability and excellent binding affinity, PNAs are ideal anti-miRs. A naked PNA cannot be across cell membrane, easily.

Cheng et al. have stated that PNA encapsulation into nanoparticles is uniquely independent from electrostatic interactions between cargo and delivery vehicle due to their charge-neutral backbone unlike most nucleic acids. They have also reported that with standard preparation methods, less than 5 % of the naked siRNA can be encapsulated into PLGA nanoparticles. However, precomplexing siRNA with a polycation can improve loading efficiency into nanoparticles to approximately 50 %. They have used the same method for encapsulation of PNAs and have reported similar encapsulation efficiency (approximately 50 %). Chang et al. state that even though polycations are commonly used for nucleic acid delivery, they may contribute to adverse side effects in cellular and physiological environments due to their charge density. For a nucleic acid delivery system that is potentially more benign and more effective than other strategies, charge-neutral PNAs can be loaded into nanoparticles. Chang et al. attached a cell-penetrating peptide, penetratin, to the nanoparticle (100–2,000 nm in diameter) surface in order to increase the capability of PLGA nanoparticles to deliver cargo intracellularly. To achieve this, they utilized an effective surface attachment strategy, which permits conformational flexibility and a high density of ligand deposition. Chang et al.'s experiments show that pre-B cells uniquely favored the uptake of nanoparticles that were coated penetratin (ANTP–NP) and not the uptake of other cell-penetrating peptides such as TAT and polyarginine [68].

miRNA-Restoration-Based Therapy

The genomic loss or downregulation of miRNAs can be restored using miRNA mimetics or mimics, which are synthetic double-stranded RNA where the guide strand is identical to the endogenous mature miRNA needing to be restored and the passenger strand is completely complementary to the guide strand. miRNA mimics need chemical modification via the strategies explained above for the anti-miRs so that their stability can be increased and nuclease degradation can be avoided. Yet, ensuring the proper loading of the guide strand into the RISC complex and the degradation of the passenger strand requires that the chemical modifications of both strands be different. miRNA mimics are usually conjugated or encapsulated into different carriers, whereas anti-miRs can successfully be delivered in vivo naked.

Similar to the miRNA transfection methods developed in vitro, the field has also seen the development of lipid-based strategies for in vivo delivery. Liposomes are vesicles that have a lipidic bilayer, which can be loaded with various molecules including miRNAs. The use of cationic lipids compensates for the negative charge of nucleic acids, leading the particles to have a net positive charge, and this facilitates the cellular uptake. Neutral lipid liposomes were developed following the observation of the adverse immune response effects resulting from cationic liposome administration. Neutral lipid liposomes are utilized in delivering miRNAs locally and systemically without apparent toxicity [69].

Some Other Polycations in Drug/Gene Delivery

Poly-L-lysine (PLL), one of the first polymers used in nonviral gene delivery, is biodegradable but its high toxicity prevents its use in vivo. Transfection efficiency of PLL−nucleic acid complexes remain lower when compared to other transfection agents. It is believed that inefficient transfection is due to the lack of amino groups with a pKa ∼5−7 which offers endosomolysis and nucleic acid release. Many hydrophilic polymers have been linked covalently to poly-L-lysine and copolymers such as poly-L-lysine–poly(ethylene glycol) block. Reports are also available on synthesis of lipid-bearing poly-L-lysines, where lipid units are attached to terminal lysine amino units. The covalent attachment of both hydrophilic and hydrophobic units to poly-L-lysine and further formation of nanoparticles are available as well. As an example, Kim et al. have synthesized a cationic diblock copolymer, poly(L-lysine)−poly(ethylene glycol)−folate (PLL−PEG−FOL), to improve their site-specific intracellular delivery against cancer cells that overexpress folate receptor. PLL−PEG−FOL-coated PLGA nanoparticles have demonstrated enhanced cellular uptake into KB cells even in the presence of serum proteins [70].

Whereas most proteins have negative charges at the physiological pH, the amine side chains of poly(L-lysine) are positively charged. Because of multiple electrostatic interactions, the polymer can encapsulate protein molecules in aqueous solutions. Block copolymers composed of the cationic segment and hydrophilic segments are expected spontaneously to associate with polyanionic DNA to form block copolymer micelles. Wei et al. constructed a hydrophilic star block copolymer PEI–PLL–b-PEG with a poly(L-lysine) inner shell as a potential nanocarrier that may easily encapsulate proteins such as insulin. At the physiological pH, the loaded insulin in the star block copolymer displayed a sustained release; it showed a significantly accelerated release upon charge switching of the protein molecules [51].

Poly(alkylcyanoacrylate)-based nanosystems include various types of nanoparticles suitable to use in vivo drug delivery in a well-controlled manner. Thanks to its favorable properties such as biocompatibility and biodegradability, simple preparation process for the entrapment of bioactives, especially proteins and peptides and poly(alkylcyanoacrylate) nanoparticles, has sparked extensive interest as drug delivery systems.

DeVerdière et al. have loaded polyalkylcyanoacrylate (PACA) nanoparticles with doxorubicin. Previously they found that tumor cells do not digest PACA nanoparticles, and here they report the crucial necessity of a direct interaction between nanoparticles and cells to overcome resistance. They showed that the degradation products of PACA, mainly polycyanoacrylic acid, have the ability to increase both accumulation and cytotoxicity in the presence of doxorubicin. They interpret this finding to suggest that a doxorubicin–polycyanoacrylic acid ion pair has formed. They conclude that both the increased doxorubicin diffusion by the accumulation of an ion pair at the plasma membrane and the adsorption of nanoparticles to the cell surface work together to overcome resistance.

Hillaireau et al. have encapsulated cidofovir (CDV) and azidothymidine-triphosphate (AZT-TP) in poly(iso-butylcyanoacrylate) (PIBCA) aqueous-core nanocapsules. However encapsulation efficiency is low and additionally the rapid leakage of the small and hydrophilic molecules through the thin polymer wall of the nanocapsules is observed. Then, various water-soluble polymers as increasing Mw adjuvants are used for the entrapment of mononucleotides (CDV, AZT-TP) has been done in the study conducted with oligonucleotides into these PIBCA aqueous-core nanocapsules. It has been reported that in the presence of cationic polymers (i.e., poly(ethyleneimine) (PEI) or chitosan), encapsulation of AZT-TP and ODN has been successful. Nanocapsule of poly(iso-butylcyanoacrylate) finds a chance of applications in drug delivery as well [71].

Polyethylenimines (PEIs) are synthetic linear or branched polymers. Their molecular weights vary in the range of <1 to $>1{,}000$ kDa. PEIs have a protonable amino group in every third position and a high cationic charge density. These properties allow PEIs to make non-covalent interpolyelectrolyte complexes with DNA. Cells can efficiently take up these small colloidal particles, which intracellularly buffer the low endosomal pH. The proton-sponge effect results in an increased proton and water influx, leading to the eventual burst of endosomes and the release of complexes into the cytoplasm. Making use of this mechanism enabled the introduction of certain PEIs as transfection agents to a variety of cell lines and to animals for DNA delivery.

In general, higher molecular weights associated with augmented cytotoxicity and low-molecular-weight PEIs (<25 kDa) with a branched rather than linear structure are superior for gene transfer. The DNA/PEI ratio, which is defined as the nitrogen/phosphorus (N/P) ratio, is another important determinant of the PEI's lack of toxicity and transfection efficiency alongside with the molecular weight and degree of branching [72].

PEIs are able to form non-covalent complexes with DNA, siRNA, and antisense oligodeoxynucleotide due to their high cationic charge density at physiological pH. However, this polymer is rather toxic among some of other polycations, and its non-degradability is a major drawback for its in vivo use. Following studies are some examples to PEI usage in gene delivery:

Poly(lactide-co-glycolide) (PLGA)-based nanoparticles for drug delivery have been studied commonly due to their excellent biocompatibility and low toxicity. However, their low DNA encapsulation capacity, instability of genetic material,

and the difficulty of regulating the release ratio are the drawbacks in gene therapy applications. Since PLGA nanoparticles have a negative surface charge, negatively charged pDNA does not adsorb on PLGA particles. Therefore, developing copolymers of PLGA cationic polymers for gene delivery could enhance the amount of DNA adsorbed on nanoparticle.

Benita et al. have prepared DNA-loaded poly(D,L-lactide-co-glycolide) nanoparticles bearing polyethyleneimine on their surface. Zeta potential has observed to be strongly positive (above 30 mV) for all the PEI–DNA ratios while the loading efficiency has exceeded 99 % [73]. Later on, Shau et al. have prepared positively charged PLGA nanoparticles in a one-step process by the addition of PEI to an aqueous PVA solution in which a PLGA solution in ethyl acetate has emulsified. PLGA/PEI nanoparticles have been examined for pDNA transfection efficiency. Their results show that the nanoparticles prepared via the one-step procedure have a small size distribution and a spherical morphology. Compared to particles prepared in a two-step process, these particles were found to have a much better DNA binding capacity. What is most important is that they were demonstrated to have a relatively low cytotoxicity and a considerably better transfection activity of PEI polyplexes even in the presence of serum [53].

A new type of poly(D,L-lactide-co-glycolide) (PLGA)-based nanoparticles for gene delivery has been developed that is able to overcome the polyethylenimine (PEI)- or cationic liposome-based gene carrier's disadvantages, some of which include the cytotoxicity resulting from excess positive charge and aggregation on cell surface. The size of 60 nm PLGA/PEI nanoparticles used for miRNA transfection in HepG2 cells has been successful [74].

The PEI-based core−shell nanoparticles have been prepared as carriers for gene delivery by Zhu et al. The poly(methyl methacrylate) (PMMA)–PEI core−shell nanoparticles in diameters that range between 130 and 170 nm displayed zeta potentials approximately +40 mV. Then the nanoparticles were incubated with plasmid DNA. After conjugation, nanoparticles squeezed pDNA and they formed complexes of approximately 120 nm in diameter. Cytotoxicity studies suggest that the PMMA–PEI core−shell nanoparticles are three times less toxic than the branched PEI (25 kDa) and that their transfection efficiencies are significantly higher [75]. In another study, PEI–PMMA nanoparticles have been complexed with pGL3 plasmid and delivered to HeLa cells [76].

Sethuraman et al. have developed a pH-sensitive sulfonamide/PEI polymer for tumor-specific gene delivery and complexed polyethyleneimine nanoparticles with a pH-sensitive diblock copolymer, poly(methacryloyl sulfadimethoxine) (PSD)-block-PEG (PSD-b-PEG), for utilization in the delivery of pDNA [77]. Laçin et al. have prepared poly(St/PEG-EEM/DMAPM), PEGylated nanoparticles for the gene transfer of tissue inhibitor of matrix metalloproteinase-2 to prevent restenosis via inhibition of smooth muscle cell proliferation [78].

Although PLGA polymers have been widely utilized for drug delivery, PLA polymers have not been broadly used because of their slow degradation rate. Nowadays, PLA is being used for the surface modification of organic microsphere poly(hydroxyethyl methacrylate) (PHEMA). It has been shown that PLA-modified

microspheres have an increased loading capacity and a better antitumor effect than the unmodified microspheres [79].

Another approach that enhances the systemic circulation lifetime of the drugs and decreases their exposure to normal tissues is covalently attaching therapeutic agents to water-soluble polymers. Polymer conjugation resulted in improved pharmacokinetic profiles and clinical efficacy for multiple low-molecular-weight anticancer drugs, such as paclitaxel, doxorubicin (DOX), and camptothecin. Some polymers, such as poly(L-glutamic acid) and *N*-(2-hydroxypropyl) methacrylamide (HPMA), have been accepted at clinical practices. The advantages of multivalent functional groups on these polymers have led to an increase in the amount of research focusing on them. Because drugs need to detach from the polymer conjugates to take effect, drug release kinetics can be modified with enzyme-sensitive linkers according to the environment in which the delivery process will take place [45].

Conclusion

Presently, there have been a lot of studies conducted in the areas of designing novel polymeric nanoparticulate systems as well as the ones on the existing controlled delivery vehicles. The results of the studies have taken their place in relative publications giving us the chance to follow up the improvements and innovations in the area. Thanks to their high stability and tunable properties, such polymer nanoparticles have a great potential in controlled delivery of genes, miRNAs, peptide structures, siRNAs, and pharmacological agents. An ideal drug/biomolecule delivery system has to be nontoxic, non-immunogenic, and nonantigenic. Additionally, nanoparticle system has to deliver the cargo to the target tissue at prolonged times and at required rates in a controllable manner. Targeted drug or biomolecule delivery systems are very effective thanks to their specificity and selectivity. Targeted delivery of nanoparticles enables increased specific localization, decreased toxic side effects, and reduced dose. Additionally, the nanosize of vectors also allows access into the cell and various cellular compartments including the nucleus. Although significant advances have been made in this area, many theoretical and technical problems remain to be solved. Currently, the researchers working on nanoparticle drug delivery system focus on the optimization of the preparation of nanoparticles, increasing their drug delivery capability, applications in clinic, and the possibility of industrial production. Nowadays, the possibilities of developing multifunctional nanoparticulate are attracting more interest. To date, there are a lot of clinically approved nanoparticle-based therapeutics and many more are under clinical investigation.

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