Chapter 5
Potential of Chitosan-Based Nanocomposites for Biomedical Application in Gene Therapy

Manoj Trivedi and Sanjay Kumar

Abstract The application of gene therapy in the field of molecular medicine is an extremely promising approach to curing distinct varieties of illnesses and disorders of the human race. Currently, challenges of the gene therapy are to find secure and effective vectors which might be capable of delivering genes to the specific cells and getting them to express inside the cells. Because of safety concerns, artificial delivery systems are desired in comparison to viral vectors for gene delivery so numerous attention has been centered on the development of the effective vectors. However, Researchers are confronted with numerous problems consisting of low gene transfer efficiency, cytotoxicity, and lack of cell-targeting capability for the usage of these synthetic vectors. Chitosan, which is the biodegradable and non-toxic cationic polysaccharide, is generally preferred to the other cationic polymers as a non-viral vector mainly due to its properties of chemical versatility, excellence in transcellular transport, effectiveness as a DNA-condensing agent, and efficient and permanent transfection. The objective of this chapter is to indicate the importance and give an overview of the applications of chitosan and its derivatives as novel non-viral vectors for gene delivery.

Keywords Chitosan · Polymer · Carrier · Cationic · Gene · Transfection · Ligand

Abbreviations

DNA	Deoxyribonucleic acid
mPEG	Methoxy Polyethylene glycol
mV	Millivolts
PDMAEA	Poly[2-(dimethylamino) ethyl acrylate]
PDMAEMA	Poly[2-(dimethylamino) ethyl methacrylate]
PEC	Polyelectrolyte complex

M. Trivedi (✉) · S. Kumar
Department of Chemistry, Sri Venkateswara College, University of Delhi, New Delhi 110021, India
e-mail: mtrivedi@svc.ac.in

S. Gulati (ed.), *Chitosan-Based Nanocomposite Materials*,
https://doi.org/10.1007/978-981-19-5338-5_5

PEG	Polyethylene glycol
PEI	Polyethylenimine
PEI	Poly(L-lysine)
pHPMA	Poly[N-(2-hydroxypropyl methacrylamide]
p-NIPAM	Poly(N-isopropylacrylamide)
RNA	Ribonucleic acid

1 Introduction

Since the discovery of DNA's structure, its functions, and gene transfer applications have gotten a lot of interest, from cell transfection to the manufacture of transgenic animals by getting transgenic embryos to gene therapy. Several studies were conducted, as follows:

- Changing the gene code and looking into the roles of genes.
- Transfer of DNA to the organism or its cells (transgenesis).
- Gene therapy is used to treat gene mutations or deficiencies.
- Therapeutics are produced using transgenic prokaryotes and eukaryotes, particularly pharmaceutical animals such as goats and cattle (bio-pharming of therapeutics).
- Model laboratory animals are created to study genetic illnesses caused by genetic damage or mutations.
- Farm animals, particularly pigs, are being studied for use as a tissue bank for human transplants (xenotransplantation).

In these investigations, many successful outcomes have been obtained, as well as innovative methodologies. Apart from in vitro experiments, in vivo applications have also been carried out, but due to some unsolved problems and restrictions or difficulties, such as targeting of gene carrier particles, undesirable acute or late side effects of genes, and their carrier systems, obstacles relating to human applications have yet to be overcome. Transfection (gene transfer) is a process in which a gene is transferred to the nucleus of another cell and implanted in its DNA. Many approaches and protocols for transgenesis applications have been created by researchers. The gene is transferred to the tissue/cells using a variety of ways.

- Electroporation.
- Direct injection of genes into the nucleus or pronucleus.
- Using viral vectors to transfer genes.
- Non-viral vectors are used to deliver genes.

Because the electroporation technique is only utilized in cell suspension, it has limited use. Furthermore, microinjection of the gene directly into the nucleus/pronucleus necessitates specialized and costly equipment, and this technique can be utilized in cell culture systems and after egg fertilization. The reliability of

viral vectors in vivo experiments is still an open question, and the results of these studies are generally poor. As a result, researchers created a novel targeted gene carrier system, and non-viral gene carrier systems have become more commonly used in transgenesis and gene therapy applications. However, these methods have issues such as difficulty in targeting the gene carrier system, early cytoplasmic enzymatic activity degradation of the particles and the gene in the cells, and non-viral agent cytotoxicity. Currently, research is focused on resolving these issues. By delivering genetic material to the patient, gene therapy has been utilized to prevent genetic abnormalities. With this technique, the patient's therapy, which is the regeneration of damaged biological activities or the restoration of homeostasis, is carried out at the molecular level. The basic goal is to overcome biological barriers that prevent therapeutic genes from reaching the desired location. Preclinical and clinical gene therapy research has advanced considerably in the last 15 years. Gene therapy has recently gained popularity as a promising treatment option for genetic illnesses, cancer, cardiovascular disease, and viral infection. Gene therapy not only tries to treat diseases but also to transfer recombinant genetic material to the nucleus, where gene expression, which activates or deactivates protein synthesis, occurs. It is clear that well-targeted, non-toxic gene carrier mechanisms are required to transport the gene to the nucleus. Chitosan is a non-viral gene carrier that is commonly used in gene therapy.

2 Chitosan as a Gene Carrier

The carrier system that delivers a gene for gene expression is called a vector. Vectors are mainly divided into two groups: non-viral vectors and viral vectors. Effective transfection and gene expression of viral vector therapy genes are of great clinical importance for gene therapy. Due to its structure, the virus performs gene transfection very effectively. Due to this property of viral vectors, the required carrier system is preserved and improved. Recently, viral vectors with different genomic characteristics such as retrovirus, adenovirus, adeno-associated virus, herpesvirus, and poxvirus have been commonly used for the efficient provision of gene transport capacity and gene expression. Retroviruses are the most popular of these viral vectors because of their highest gene transfection efficiency and highest expression of therapeutic genes. While these functions emphasize the importance of safe RNA and DNA viral carrier systems, the same report reveals the difficulty of using clinical viral vectors [1, 2]. For example, adenovirus provides the highest gene expression and can infect dividing and non-dividing cells, but it elicits an immune response through viral protein and transient gene expression [3–5]. Similarly, retroviruses facilitate the manipulation of the viral genome. Although easy to combine with DNA, their advantages are difficult to target, complex combinations within the genome, and instability [5]. The first clinical study deals with viral vector compliance and reliability. There are numerous viral vector systems that have been tested in ex vivo and in vivo studies. In recent years, studies have focused on virus targeting, cell type expression, and time of expression

to enhance existing effects [5]. Researchers prefer viral vectors for their effectiveness in transfection studies, but the use of these vectors has characteristics such as cytotoxicity, immunosensitivity to viral antigens, and possible viral combinations. Is limited because it is low. Because of these negative features, researchers have been drawn to non-viral vectors. Today, gene therapy is clinically effective in treating many illnesses. Since 1989, when gene therapy was first introduced, this approach has been used in more than 3000 patients with approximately 600 interventions, but no gene therapy product raises toxicity concerns. In addition, there are significant usage differences between viral and non-viral vectors. Viral vectors are used in about 75% of clinical treatment cases, while non-viral vectors are used in less than 25% [6, 7]. Some highlights of the gene transfer application are shown in chronological order in Fig. 5.1 [8–15]. Due to these instabilities, researchers not only compared viral vectors with non-viral vectors but also sought to understand the potential and limitations of non-viral systems. In many of these studies, the researchers of gene therapy and pharmaceutic technologies recognize that viral vectors are merged with the genome and the cell is mutated, so cancer occurs in vivo. Besides that, the immune response is activated, and that's why the treatment becomes more difficult [7, 16]. Especially, although the numerous non-viral vectors are synthesized and their features designed, these systems are not effective enough for gene transfer, so there are not any commercial products. The non-viral vectors are divided into two groups lipophilic vectors and polymeric vectors.

Non-viral vectors containing cationic liposomes are commonly used before and during clinical trials, but an important part of non-viral lipophilic vectors has the same toxicity as viral vectors. And although there are clinical problems, the main advantage of these polymers is that they can create various modified cations in the structure of the polymer to enhance their potency. As a result, plasmid DNA is released in a controlled manner, increasing stability to enzymes in the blood such as nucleases, reducing non-specific uptake, regulating interactions with cells and plasma molecules, and simultaneously eliminating the immunizing of the system. These modifications are applied to improve the release properties of the polymer, allowing the genetic material to be released in a controlled manner at the target site [17]. On the other hand, non-viral gene transfer systems with different polymer structures are

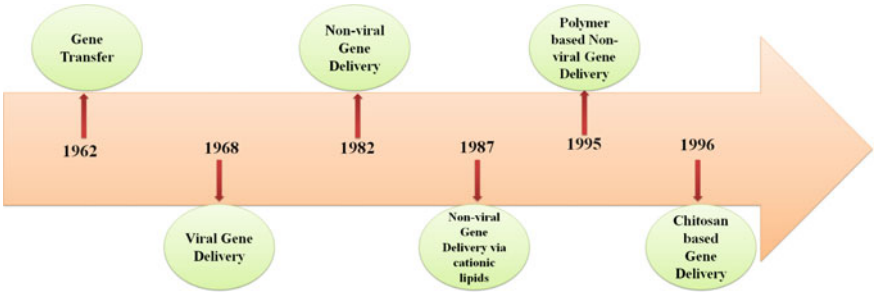

Fig. 5.1 The Continuous growth of gene transfer applications

safe and economical gene transfer systems with improved various synthetic vectors, some of which are commercially available. In addition, non-viral vectors are not as efficient as viral vectors and generally solve as some of the cationic carrier systems used with anionic genes to generate ion complexes capable of exhibiting cytotoxic effects [18, 19]. The common goal of these studies is the synthesis of polymer carrier systems that are as effective and less toxic as viral vectors.

3 Application of Polymers in Gene Delivery

3.1 Artificial Polymers

There are several non-viral gene delivery systems for transferring genetic material to the cell nucleus. The non-viral gene delivery system consists of polymers and lipids or liposomes. Cationic polymers have many advantages for use in gene delivery such as low toxicity and immune response, being easy to handle, and being stable [20, 21]. However, some issues need to be resolved, including toxicity, reduced transfection efficiency, and lack of biodegradability. These properties of the polymer need to be modified in different ways. These biodegradable non-viral polymer gene delivery systems are called transgenic polymers. Transgenic polymers can be divided into two groups: natural transgenic polymers and synthetic transgenic polymers. Synthetic transgenic polymers are generally preferred for gene delivery because of their ease of modification. According to Amiji [6], synthetic transgenic polymers include non-biodegradable transgenic synthetic polymers [polyethylenimine (PEI), polyethylene glycol (PEG) conjugates, etc.], biodegradable transgenic synthetic polymers (poly-β-aminoesters, polyamido amines, poly-imidazoles, etc.), polyethylene oxide/polypropylene oxide copolymers and polymeric polyethylene oxide, polyalkylcyanoacrylate nanomicrospheres. However, the main drawbacks of these polymers are their high toxicity. The main reasons for toxicity are the polymer skeleton and the density and distribution of positive charges along with the molecular weight. PEI's with a molecular weight of 22–25 kDa are used for gene therapy because of their reduced cytotoxicity and high transfection efficiency [22, 23]. Other non-biodegradable transgenic polymers are used to deliver genetic material such as DNA, oligonucleotides, and small interfering RNAs. Some of these polymers are poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA), poly[2-(dimethylamino) ethyl acrylate] (PDMAEA), and *N*-vinyl pyrrolidone. They show high transfection efficiency like PEI, but due to their negative properties that reduce stability in blood, high interaction with serum components, etc., these polymers are PEG and poly[*N*-(2-hydroxypropyl methacrylamide)] (pHPMA) is bound [24]. PEG and pHPMA materials mask the instability of nanoparticles in serum due to the presence of nanoparticles in the hydrophilic layer on the polyplex. However, the PEG and pHPMA groups interfere with complex: DNA complex formation, thereby reducing

the efficiency of polyplex transfection. Researchers are working on improving various ways to solve these problems [24–26].

3.2 Natural Polymers

Cationic polymers (polycations) are one of the most commonly used carrier systems in molecular gene transfer systems. The polyelectrolyte complex (PEC) obtained by the interaction of DNA and polycations protects DNA from enzymes such as DNAse. In addition, transgenic polycations interact with serum DNA due to their cationic properties at physiological pH.PEC systems are easier to manufacture and have a lower immune response than viral vectors, but researchers are pursuing further research due to negative properties such as biodegradability problems and reduced transfection efficiency. The main transgenic polycations are PEI, poly(L-lysine), dendrimers, gelatin, and chitosan, a natural cationic polysaccharide. As mentioned above, one of these polycations, chitosan, is natural, biodegradable, biocompatible, non-toxic, and does not contain negative charges in the PEC system [6], so it is used in transfection studies. It is attracting the attention of researchers. Natural polymers commonly used in gene delivery are poly (amino acids) such as poly-L-lysine (PLL), polyornithine, polyarginine, chitosan, dextran, collagen, gelatin, and their modified derivatives. PLLs and other polys (amino acids) are important polymers for use in gene delivery systems due to their biodegradability, but these polymers are highly toxic [22]. Other polycations, namely, dextran [27], collagen [28], gelatin [29, 30], and their modified derivatives are used in gene delivery systems, but researchers have observed sufficient transfection efficiency so did not do it. Therefore, chitosan has been widely used in many studies due to its characteristic properties.

4 General Characteristics of Chitosan

Chitosan is a linear polysaccharide composed of glucosamine and N-acetyl-glucosamine units bound by β (1–4) glycosidic bonds and a partially deacetylated product of the natural polysaccharide chitin. Chitin, a biopolymer, is the most abundant organic compound in nature and is an important component of the exoskeleton of animals, mainly found in the shells of crustaceans such as crabs, shrimp, and krill. Since chitosan is an N-deacetylated derivative of chitin, the degree of acetylation determines whether the biopolymer is chitin or chitosan. When the degree of deacetylation of chitin, which is the content of glucosamine, exceeds about 50%, it dissolves in acidic aqueous solutions such as acetic acid, lactic acid, hydrochloric acid, and aspartic acid, and is called chitosan [31, 32]. Chitosan does not dissolve at basic pH values. Chitosan exhibits varying degrees of solubility in dilute aqueous media, depending on the free amine content of the chain. The molar ratio of acetylated amine groups to deacetylated amine groups in chitosan also determines the

sensitivity or biodegradability of the enzyme. Chitosan is an inexpensive, biocompatible, animal/human biodegradable, non-toxic cationic polymer. In addition to these properties, it exhibits other excellent biological properties such as immunological, antibacterial, and wound healing activity. Chitosan degradation products are also non-toxic, non-immunogenic, and non-carcinogenic. The chemical modification of chitosan that results in a variety of derivatives is easy to apply. A variety of possible modification reactions can be applied, including nitration, phosphorylation, sulfation, thiolation, acylation, hydroxyalkylation, graft polymerization, amination, and combinations of chitosan derivatives with cyclodextrin. In particular, the physical, mechanical, chemical, bioactive properties, and commercial availability of chitosan make chitosan a very attractive biomaterial in biomedicine. Therefore, since the nineteenth century [33–35], chitosan derivatives have found widespread use in many areas, including biotechnology (especially biomedicine) and environmental applications. For example, a future and important use of chitosan in biomedicine for gene delivery. Chitosan has a high positive charge density due to the D-glucosamine unit in the structure. It exhibits polycationic properties at acidic and neutral pH. The amine group of chitosan is protonated and chitosan forms a PEC with negatively charged DNA [6, 36, 37]. Currently, positively charged groups (amino groups) and negatively charged ones, nucleic acids that form stable complexes or biological membranes and in vivo targets (e.g., PEI or polyamide amine dendrimers) due to their low toxicity and immunogenicity [33, 34]. Therefore, chitosan and its derivatives have recently been recognized as safe and efficient cationic carriers for gene delivery [6, 33, 34, 38–41].

5 Factors Affecting Gene Transfer in Chitosan Delivery System

Many delivery systems have been developed to maximize transfection efficiency and minimize side effects. For this purpose, Kasyua and Karudahave been used in vivo, versatile payload acceptability, low toxicity or non-toxicity, low immunogenicity, stealth, active targeting, proper size, and proper surface charge. It provided information on the optimized properties of these carrier systems for efficient cellular penetration: activity, intracellular targeting mechanism and high productivity [42]. Bhavsar and Amiji pointed out other properties like reactivity, biocompatibility, non-heat resistance, impurities, availability in medicinal grade, load capacity, permeability, swelling, viscoelasticity, and local environment sensitivity [43]. As defined above, we have looked at these two classifications for rectification of the non-viral vectors compared to viral vectors. Size and charge density are very playing a crucial role in vitro and in vivo overall performance of polymeric gene delivery systems. The polymeric gene delivery systems have a positive charge which is complex with the DNA having a negative charge. These cationic densities boom the encapsulation performance and enhance the uptake of DNA into the cells through the interplay of the negatively charged cell membrane [18, 44–46].

5.1 Surface Charge and Zeta Potential

The polymeric gene delivery systems that are complexed with DNA are called polyplex. The positive charge density of the polymer increases the DNA encapsulation efficiency and stability as well as immunity. Therefore, in various studies, PEG or the same other molecule forms a complex with the polymer carrier to provide charge balance, and these modifications make these carriers safe [47, 48]. Charges are also important for cell penetration. Cationic nanoparticles enter cells via endocytosis. Positively charged particles react with the negatively charged sugar coating on the outside of the cell membrane (on the extracellular polymer material on the surface of the cell membrane) and are taken up by cells by various intracellular mechanisms. When particles (which did not invade cells by passive and active transport) are taken up by endocytosis, multiple acidic groups cause endosome destabilization at low pKa values, resulting in a proton sponge effect. The polymer is then protected by moving protons to the endosome and increasing the ion charge density until the endosome becomes unstable [10]. From the formation of polymer support-DNA complexes to cell invasion and gene transfer to the nucleus, the required surface charge is called the zeta potential [49, 50]. Expressed as colloidal stability of particles distributed in a liquid (usually water) and when an electric field is applied to the liquid, it moves to the negative or positive electrode according to the surface charge ratio. As is well-known, in the case of colloidal systems, when a net surface charge is formed, a reverse charge begins to be generated in the outer layer, thereby forming an electric double layer. The innermost layer that surrounds the opposite layer for each surface charge of a particle is called the star layer. Each particle acts as a single entity consisting of this bilayer whose positive charge is equal to its negative charge. The potential difference between this field and the surface charge of the particle is called the zeta potential (ζ). The unit is millivolts (mV) and is measured with a zeta meter [51]. Particles are stable below -30 mV and above $+30$ mV. For stable nanoparticles used in gene delivery applications, the average zeta potential is up to $+30$ mV. The ionization of the terminal groups of the non-viral polymer carrier depends on the degree of surface ionization proportional to the pH of the dispersion. For zwitterionic particles, the surface charge is positive at low pH and the surface charge is negative at high pH. These charges equilibrate at a zero point called the isoelectric point [51].

5.2 Particle Size

In 1860, nanoparticle technology was born from nanoscale or nanometer (nm) scale materials. Currently, the nanoscale concept is described as a material with a particle size of 1–1000 nm. The scope of nanotechnology lies in the manufacture of nanoscale materials with various properties and the study of these properties. While chemistry, physics, molecular biology, and materials science are related to nanotechnology,

many areas of science are beginning to associate nanotechnology with the development of technological methods [52, 53]. The evolution of nanotechnology is not limited to the position of atoms in the structure of materials as they are transported from one location to another. Moreover, nanomaterials, which have a high surface area due to their nanosize, are synthesized with exclusive properties throughout their controlled size. Therefore, nanomaterials are widely used in many fields such as electronic equipment, automobiles, military, medical, manufacturing of conductive and semi-conductive materials, ceramics, surface coating materials, ink manufacturing, and so on. This technological revolution is seen by scientists as the starting point for more development over the next 10–15 years. The concept of nanotechnology in gene therapy began in 1930 with the discovery of the intracellular nanoscale structure. Today, nanotechnology approaches in gene therapy are known for developing nanoparticle systems below 200 nm. The size of the polyplex is important for functionality. The diameter limit of the polyplex is about 10 nm at the first uptake in the liver. In addition, the upper limit size of the polyplex should be less than 200 nm. The size of the polyplex is changed by changing the DNA: polymer ratio [54, 55]. In particular, factors that influence the transfection efficiency of the chitosan-DNA complex are the degree of deacetylation and the molecular weight of chitosan, pH, serum, chitosan charge ratio to DNA, and viscosity, and cell type [6, 38, 56, 57]. Transfection efficiency and DNA loading capacity increase with the degree of deacetylation, and extracellular DNA protection and intracellular DNA release increase with molecular weight. The optimum pH for transfection media is 6.8–7.0 [56].

5.3 Chitosan Modification Reactions

Chitosan is insoluble in physiological pH and has low transfection efficiency, so modification studies are needed. For this purpose, several modification reactions are performed on chitosan such as modified with PEG or glycol [58], synthesized quaternized chitosan [59], low molecular weight chitosan [60], and reducing or thiolated chitosan (Fig. 5.2) [61]. Generally, PEG, glycol, or pHPMA is used to mask the instability of nanoparticles in serum and the formation of the hydrophilic layer onto the polyplex. The introduction of grafted PEG units onto the galactosylated chitosan was investigated by Parket al. which increases stability in water and cellpermeability [62]. Mao et al. have been studied grafted methoxyPEG (mPEG) units of different molecular weights onto the trimethylchitosan [63] to produce modified chitosan such as PEG-aldehyde [64, 65], PEGcarboxylicacid [66, 67], PEG-carbonate [68], PEG-iodide [69], PEG-epoxide [70], PEG-diacrylate [71], PEG-NHS ester [72], and PEG-sulfonate [73–75]. The introduction of colloidal stabilities of polyplexes with a pHPMAlinker was studied by Luten et al. [76] which enhanced the stability in serum for in vitro transfection with low cytotoxicity. Later on, a lot of studies based on this concept have been carried out to date [72, 77–84].

This modification is preferred over the others because of improvement in transfection efficiency and solubility in water. Numerous quaternized chitosans such as *N*-(4-pyridinylmethyl)chitosans [38], *N*-trimethylated chitosan oligomers [85], methylated *N*-(4-*N*,*N*-dimethylaminobenzyl) chitosan [86], octadecyl quaternized carboxymethyl chitosans [87], PEG*graft*-quaternized chitosan [88], and other low molecular weight chitosan have been studied in non-viral gene delivery. Richardson et al. studied the effect of molecular weight of chitosan on the cytotoxicity, complexation with DNA, and relation to the protection of DNA from nuclease degradation. He found that the low molecular weight of chitosan is more effective than poly(L-lysine) for complexation with DNA, and there is no cytotoxic effect for use in gene delivery [89]. Various research articles related to the modification reactions of the low molecular weight chitosans appeared in the literature [90–102]. Reducing or thiolated chitosan is commonly used to dissociate DNA and vectors. In addition, modifications containing ester bonds [103–105] or biological macromolecules [106, 107] such as heparin and proteoglycans are used but not generally preferred by the researchers. The disulfide bonds are delivered to the gene switch through diverse strategies. One of those strategies is the cationic ligands, which can be coiled at the polymer segments with the disulfide bonds, then DNA is bonded through the electrostatic interaction, and so DNA is added into the cytosol via the dissociation of disulfide bonds from the polyplex. Alternatively, other methods are the formation of cross-linking points via disulfide bonds and the reaction with disulfide bonds via polymer segments. These bonds present in the polymer segment not only release DNA but also reduce cytotoxicity through the degradation of small molecule components [24, 25]. Lee et al. stated the thiol modification of chitosan for sustained gene transport. In this study, the thiolated chitosan/DNA nanocomplexes exhibited significantly stepped forward gene transport in vitro and in vivo via way of means of the oxidation of thiol groups to crosslink the thiolated chitosan [108]. Numerous reducible chitosan research had been stated so far [109–113].

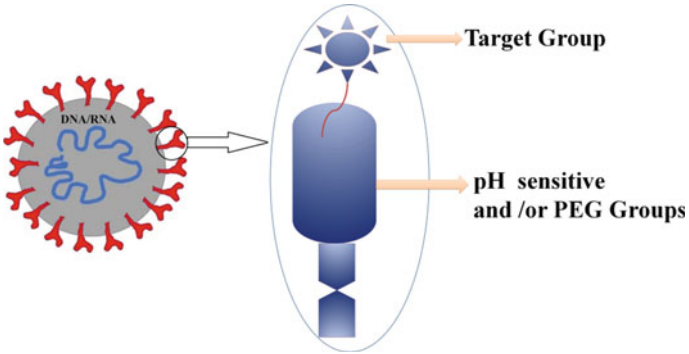

Fig. 5.2 Scheme for representation of chitosan-modified nanoparticles

6 Application of Chitosan-Based Nanocomposites in Gene Therapy

Chitosan is the most studied natural macromolecule for gene delivery. It has a positive charge, binds to negatively charged cell membranes with high affinity, and forms a complex with DNA via electrostatic interaction [22, 114]. Mumper et al. [15, 115] have introduced firstly the role of chitosan in gene therapy. Afterward, in 1996–1997, Murata et al. synthesized the chitosan gene carrier systems having galactose residues for the transportation of the DNA molecule [116, 117]. Although gene transport mechanisms of cationic nanoparticles had now no longer been absolutely understood in those years, numerous reports related to the chitosan gene carrier systems were reported in the literature. Polymeric gene transport applications are quite tough to classify. In well-known, classification is performed further to the polymer type as natural and artificial polymeric gene transport structures. However, researchers understood that this type does not explain all the gene delivery applications so, in place of making the classifications, they realized that the gene shipping mechanisms want to be understood in aggregate with polymer-DNA out of the mobile to the occurrence of the related proteins into the mobile nucleus. Despite the fact that these mechanisms have now not been completely defined yet, the modification reactions are done in step with the diagnosed mechanisms. Wong et al. described seven essential steps for the transport of a gene to the nucleus: (1) healing genes packaging; (2) entry to the cellular; (3) endolysosomal getaway; (4) the impact of DNA/provider system release; (5) progression throughout the cytoplasm and transition into the nucleus; (6) gene expression; and (7) biocompatibility [24]. Gene packaging strategies are very critical for gene delivery structures. For the prevention of the same charge impact on the cell membrane resulting from the phosphate corporations of DNA, the condensation of the cumbersome shape of DNA, and the safety of DNA from the degradation extracellularly or intracellularly, researchers stepped forward with two packaging strategies for chitosan gene transport: (1) electrostatic interplay and a couple of encapsulation [24, 118, 119]. Usually, electrostatic interaction strategies are desired for the chitosan gene transport systems. Erbacher et al. used the electrostatic interplay methods for growing chitosan/DNA complexes and they located that this complicated required some feature modifications for strong and small complexes [107]. Chitosan has amino businesses that are protonated in impartial pH and have interaction with DNA spontaneously. Due to those features of chitosan, Lee et al. studied the hydrophobically modified chitosan complexes with plasmid DNA to put together self-aggregated nanoparticles in aqueous media with adjusted pH [120]. Aside from this examination, there are various articles reported relating to those capabilities of chitosan [121–128]. Especially because the encapsulation strategies are explored, the researchers have not generally favored the electrostatic interaction techniques. The encapsulation methods are used for the protection of genes from enzymatic degradation and offer the controlled launch of DNA via the biodegradable groups of chitosan [24]. Buddy et al. classified the techniques of obtaining the encapsulated chitosan-based totally on the nanoparticle systems;

those are covalently connected nanoparticles, ionically pass-linked nanoparticles, and desolvated nanoparticles [129]. The covalently pass-related method is carried out with a chemical cross-linking agent, including glutaraldehyde, for cross-linking of chitosan [130]. The ionically cross-connected nanoparticles method or ionotropic gelation approach is the most famous technique for the use of polyanions, such as tripolyphosphate, for forming cross-connected chitosan [131–134]. In the desolvated nanoparticles approach, the desolvating agent is used for the precipitation of chitosan to extract water from chitosan polymeric chains. The complex coacervation approach is one of the desolvated strategies [129]. Bozkır and Saka studied the complicated formation of chitosan and plasmid DNA with the use of complicated coacervation and solvent evaporation techniques. In addition, they investigated the crucial parameters which include encapsulation efficiency, molecular weight, and deacetylation degree of chitosan [135]. The restrictions of the encapsulation techniques are that the polymeric provider systems are uncovered the natural solvents and high temperatures, which disrupt the genetic materials, much less encapsulation efficiency, much less DNA biocompatibility because of inadequate launch from the polymeric carrier structures, and the degradation of DNA due to the hydrolysis of ester bonds in low pH [24]. The polymer-DNA complexes as polyplexes come upon the primary barrier on the mobile, and it is far referred to as the plasma membrane. Polyplexes do not undergo passive diffusion because the transition membrane's pores and canals are very constrained dimensionally. Various strategies have been employed to overcome bodily obstacles. Endocytic uptake of the molecules, which are not handed from the cell membrane via the easy diffusion or active delivery, is passed essentially from the mobile membrane through 3 approaches: (1) phagocytosis; (2) pinocytosis; (3) receptor-mediated endocytosis. In general, the particles which are bigger than 250 nm pass via phagocytosis, and the smaller ones skip through endocytosis on the mobile membrane [136]. Polyplexes are uptaken in the mobile by means of receptor-mediated endocytosis. The focus on the agent in the provider structures is used for specific uptake of the gene in a mobile as for reaching endocytosis. The endogenous ligands, inclusive of folate and transferrin, are widely utilized in phrases of growing the biocompabilities and transfection efficiencies. However, the exogenous ligands have very constrained utilization in terms of generated immune reaction because of their overseas structures [24, 78, 114, 137–145]. The endolysosomal getaway of chitosan polyplexes is explained while after the endocytic uptake of polyplexes, they go back to the mobile floor, which is facilitated by means of lysosomes, intracell organelle, etc. This concept is expressed with the pK_a value of polycations, which is stricken by a trade-in buffer capability. For chitosan polyplexes, which have a pK_a value of approximately 6.5, the amino organizations are protonated within the cellular cytoplasm, but this function is applied for the endolysosomal break out of chitosan polyplexes [146]. In line with the effectiveness of molecular weight and degree of deacetylation, Huang et al. studied the transfection efficiency of chitosan. They said that chitosan has 2.5 times higher proton absorption ability than PLL [147]. However, Höggard et al. studied the connection between ultrapure chitosan and PEI and studied their characteristics. Consistent with their experimental consequences, the ultrapure chitosan does now not offer a sponge effect due to its primary amine

groups as compared with PEI. In addition, its buffering capacity is lower than PEI on the acid endosomal pH interval of 4.5–5.5 [148]. due to these disadvantages, researchers have carried out a few modification reactions on chitosan. Moreira et al. studied to improve the transfection performance of chitosan with the aid of promoting the endosomal break out potential and buffer capability of chitosan polyplexes. For this cause, the chitosan backbone is modified with imidazole moieties with a view to escape endolysosomal degradation, much like PEI [149]. In another study, Chang et al. modified chitosan with histidine as buffering potential of histidine might help the escape of DNA inside the endosomal pH variety [150]. Comparable research was finished with the aid of others as well [151–154]. The most important steps for the transport of a gene to the nucleus are DNA/carrier device dissociation, cytosolic carrier, launch into the nucleus, and gene expression. The maximum crucial step for chitosan carrier systems is the DNA/vector dissociation among those primary steps because green gene transfer is completed with a minimum retention time of non-protective DNA in the cytosol. Efforts had been made to enhance the modification of the chitosan backbone, and charge reduction or modification of chitosan is done by modifying it with thermoresponsive groups, ester bonds, or disulfide bonds (reducible polymers). Among them, the most critical and typically desired strategies are modification with thermoresponsive groups and disulfide bonds. The thermoresponsive polymers are transformed to reversible frizz-circular form relying upon the temperature. Thus, the degree of DNA condensation is decided by the change in temperature. The frizz phase has the flexible, hydrophilic, the long-wide chain conformation, whereas the circular form has the collapse, hydrophobic, small stretched conformation. If the carrier system is circular up to the transition temperature, and frizz forms below the transition temperature, this transition temperature is called lower critical solution temperature (LCST) [155]. In fact, the thermoresponsive provider device that has an LCST value underneath the frame temperature is used for the condensation DNA with stretching form into the cell. In this regard, poly(N-isopropylacrylamide) (p-NIPAM), with an LCST value of 32 °C, is extensively utilized in transfection studies. This polymer offers excessive transfection efficiency, endosomal escape, cationic character, and hydrophobicity and NIPAM has been used for chitosan modification in many studies [156–159]. The thiolated polymers are commonly desired in gene transfer systems as a promising tool [160]. The disulfide linkages are modified to shape the gene carrier systems by the usage of various strategies; (1) electrostatic interactions, (2) reversible cross-linking, and (3) direct affiliation of the disulfide linkages at the polymer backbone. As cited above, those linkages preserve the polymer structure solid inside the cytosol, launch DNA into the cytosol, and furthermore the cytotoxicity of the carrier system is decreased with the dissociation of the carrier system to lower molecular weight components [24, 25, 160]. The thiolated chitosan carrier systems that enable gene transfection efficiency were developed by Schmitz et al. [161]. Jia et al. advanced a redox-responsive chitosan carrier system by using the PEG, PEI, and disulfide bonds for greater effective gene transfection in HeLa cells [162]. Targeted gene delivery is a significant step in chitosan carrier systems for obtaining selective and enhanced gene delivery to the

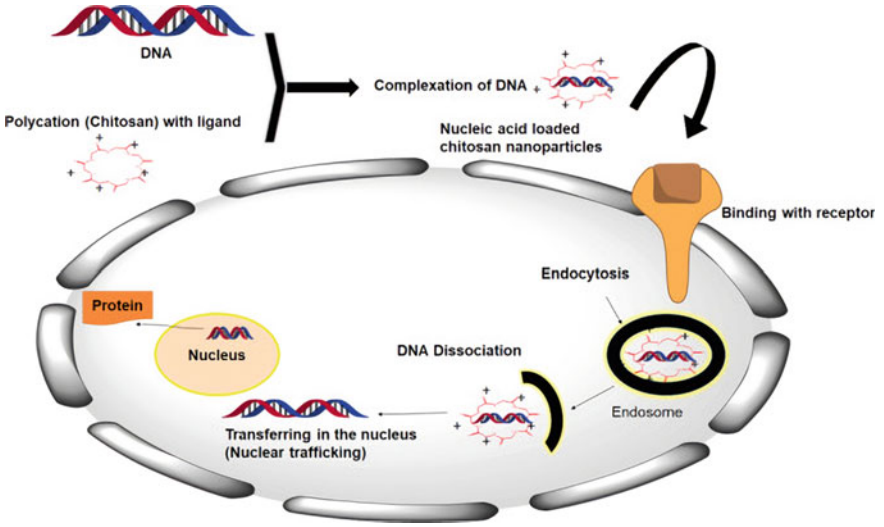

Fig. 5.3 Schematic Pathway for Chitosan nanoparticles in targeted gene delivery. Reproduced from M. Junaid Dar et. al. with permission from Elsevier 2019

target site. By using the in vivo approaches, the chitosan polyplex is administered to the body, it must be targeted to the specific sites as shown in Fig. 5.3.

Numerous reports are reported in the literature relating to targeting these specific sites, such as tumors [163], liver [164, 165], lung [166, 167], and brain [168, 169]. In most of these studies, chitosan is conjugated with the protein, transferrin, peptide, antibody, etc. [170]. A peptide functionalized chitosan-DNA nanoparticles were reported by Talvitie et al. for cellular targeting which is targeted to the required cell receptors in a specific and time-dependent manner [171]. In addition, Wang et al. synthesized the pH-sensitive gene delivery system for cancer cell-targeting which improved gene delivery by the introduction of pDNA nanocomplexes in the core and a pH-sensitive anionic polymer folic acid-modified PEG tethered carboxylated chitosan coating on the surface [172]. Numerous studies focused on targeted chitosan carrier systems have been reported in the literature [62, 173–180]. Currently, a combination of both the viral vectors and chitosan is used for efficient and permanent transfection [181]. Lameiro et al. coupled the adenovirus into the chitosan microparticle for mucosal vaccination. The main reason behind this study was to defend viruses, lower the immune response, and prolonged release. However, there are some boundaries including the difficulty of controlled release, loss of viral activity, and less loading efficiency. More studies are needed to triumph over these shortcomings [181, 182].

7 Conclusion

The idea of gene delivery was introduced in 1963, and viral vectors have been most effectively utilized in the gene therapy area. Because early 2000, these vectors were in the main abandoned because of the adverse side effects of viral-based gene therapies. Researchers have targeted the synthesis and applications of non-viral vectors, which are lipophilic or polymer-primarily based gene delivery systems. Cationic polymers are desired as a non-viral vector inside the field of gene transport. Chitosan and chitosan derivatives are commonly desired by the other cationic polymers due to their superior properties. Chitosan and chitosan derivatives are especially biodegradable and biocompatible polysaccharides. These are chemically versatile for undergoing varieties of reactions having different physicochemical properties which were tuned through modification having lower cytotoxicity, and high transfection properties. They are also called effective DNA-condensing agents and provide protection against DNAase-degradation. In connection with this fact that polymer-based gene delivery systems are yet to gain a massive presence in medical trials. Chitosan and its derivatives have been utilized in gene delivery studies after numerous modifications. A number of in vitro and in vivo studies confirm that chitosan and its derivatives are suitable and promising materials for efficient non-viral gene and DNA vaccine delivery. It is evident that chitosan and its derivatives are strong candidates to be used as the most preferred non-viral vector for gene delivery clinical trials in the future.

References

1. Thomas CE, Ehrhardt A, Kay MA (2003) Progress and problems with the use of viral vectors for gene therapy. Nat Rev Genet 4(5):346–358
2. Verma IM, Somia N (1997) Gene therapy-promises, problems and prospects. Nature 389(6648):239–242
3. Kircheis R, Wightman L, Wagner E (2001) Design and gene delivery activity of modified polyethylenimines. Adv Drug Deliv Rev 53(3):341–358
4. Boulaiz H, Marchal JA, Prados J, Melguizo C, Aranega A (2005) Non-viral and viral vectors for gene therapy. Cell Mol Biol 51(1):3–22
5. Walther W, Stein U (2000) Viral vectors for gene transfer. Drugs 60(2):249–271
6. Amiji MM (2010) Polymeric gene delivery: principles andapplications. CRC Press, Bosa Roca
7. Kwon GS (2005) Polymeric drug delivery systems. Taylor & Francis, Boca Raton
8. Wirth T, Ylä-Herttuala S (2013) History of gene therapy. Gene 525(2):162–169
9. Al-Dosari MS, Gao X (2009) Nonviral gene delivery: principle, limitations, and recent progress. AAPS J 11(4):671–681
10. Szybalska EH, Szybalski W (1962) Genetics of human celllines, IV. DNA-mediated heritable transformation of a biochemical trait. Proc Natl Acad Sci USA 48(12):2026–2034
11. Rogers S, Pfuderer P (1968) Use of viruses as carriers of added genetic information. Nature 219(5155):749–751
12. Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider P (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO J 1(7):841–845

13. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM,Lipofection Danielsen M(1987) A highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci 84(21):7413–7417
14. Boussif O, Lezoualéh F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr J-P (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci 92(16):7297–7301
15. Mumper R,Wang J,Claspell J, Rolland A (1995) Novel polymeric condensing carriers for gene delivery. In: Proceedings of the 22nd international symposium on controlled releasebioactive materials, controlled release society, vol 2, Seattle,Washington, July 30-August 2, pp 178–179
16. Xu F-J, Li H, Li J, Zhang Z, Kang E-T, Neoh K-G (2008) Pentablock copolymers of poly(ethylene glycol), poly((2-dimethyl amino) ethyl methacrylate) and poly(2-hydroxyethyl methacrylate) from consecutive atom transfer radical polymerizations for non-viral gene delivery. Biomaterials 29(20):3023–3033
17. Thomas M, Klibanov A (2003) Non-viral gene therapy: polycation-mediated DNA delivery. Appl Microbol Biotechnol 62(1):27–34
18. Vorhies JS, Nemunaitis JJ (2009) Synthetic vs. natural/biodegradable polymers for delivery of shRNA-based cancer therapies. In: Macromolecular drug delivery; Belting, M.,Ed.; Humana Press, Springer: New York, pp 11–29
19. Luo D, Saltzman WM (2000) Synthetic DNA delivery systems. Nat Biotechnol 18(1):33–37
20. Wu GC, Zhou F, Ge LF, Liu XM, Kong FS (2012) Novelmannan-PEG-PE modified bioadhesive PLGA nanoparticles for targeted gene delivery. J Nanomater 2012:1–9
21. Mandke R, Singh J (2012) Cationic nanomicelles for delivery of plasmids encoding interleukin-4 and interleukin-10 for prevention of autoimmune diabetes in mice. Pharm Res 29(3):883–897
22. Tiera MJ, Winnik FM, Fernandes JC (2006) Synthetic and natural polycations for gene therapy: state of the art and new perspectives. Curr Gene Ther 6(1):59–71
23. Jaeger W, Bohrisch J, Laschewsky A (2010) Syntheticpolymers with quaternary nitrogen atoms-Synthesis and structure of the most used type of cationic polyelectrolytes. Prog Polym Sci 35(5):511–577
24. Wong SY, Pelet JM, Putnam D (2007) Polymer systems for gene delivery-Past, present, and future. Prog Polym Sci 32(8):799–837
25. Osada K, Christie RJ, Kataoka K (2009) Polymeric micelles from poly(ethylene glycol)-poly(amino acid) block copolymer for drug and gene delivery. J R Soc Interface 6(Suppl 3):S325–S339
26. Lin S, Du F, Wang Y, Ji S, Liang D, Yu L, Li Z (2007) Anacid-labile block copolymer of PDMAEMA and PEGas potential carrier for intelligent gene delivery systems. Biomacromolecules 9(1):109–115
27. Hosseinkhani H, Azzam T, Tabata Y, Domb A (2004) Dextran-spermine polycation: an efficient nonviral vector for in vitro and in vivo gene transfection. Gene Ther 11(2):194–203
28. Chevallay B, Herbage D (2000) Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy. Med Biol Eng Comput 38(2):211–218
29. Kaul G, Amiji M (2005) Tumor-targeted gene delivery using poly(ethylene glycol)-modified gelatin nanoparticles: invitro and in vivo studies. Pharm Res 22(6):951–961
30. Kommareddy S, Amiji M (2007) Antiangiogenic gene therapy with systemically administered sFlt-1 plasmid DNA in engineered gelatin-based nanovectors. Cancer Gene Ther 14(5):488–498
31. Bsme WCS (2011) Scaleless dieting: the essential SurvivalKit for the overweight. Obese and Diabetics; Author-House, Bloomington
32. Elkins R, Hennen WJ (1996) Chitosan; Woodland Publishing, TX, USA
33. Park S, Jeong EJ, Lee J, Rhim T, Lee SK, Lee KY (2013) Preparation and characterization of non aarginine modified chitosan nanoparticles for siRNA delivery. Carbohydr Polym 92(1):57–62
34. Jeevitha D, Amarnath K (2013) Chitosan/PLA nanoparticles asa novel carrier for the delivery of anthraquinone: synthesis, characterization and in vitro cytotoxicity evaluation.Colloid Surf B 101:126–134

35. Jagani H, Rao JV, Palanimuthu VR, Hariharapura RC, Gang S (2013) A nanoformulation of siRNA and its role in cancer therapy: In vitro and in vivo evaluation. Cell Mol Biol Lett 18(1):120–136
36. Cho Y-W, Han S-S, Ko S-W (2000) PVA containing chito-oligosaccharideside chain. Polymer 41(6):2033–2039
37. Saranya N, Moorthi A, Saravanan S, Devi MP, Selvamurugan N (2011) Chitosan and its derivatives for gene delivery. Int J Biol Macromol 48(2):234–238
38. Opanasopit P, Sajomsang W, Ruktanonchai U, Mayen V, Rojanarata T, Ngawhirunpat T (2008) Methylated N-(4-pyridinylmethyl)chitosan as a novel effective safe gene carrier. Int J Pharm 364(1):127–134
39. Sajomsang W, Gonil P, Ruktanonchai UR, Petchsangsai M, Opanasopit P, Puttipipatkhachorn S (2013) Effects of molecular weight and pyridinium moiety on water-soluble chitosan derivatives for mediated gene delivery. Carbohydr Polym 91(2):508–517
40. Alves N, Mano J (2008) Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. Int J Biol Macromol 43(5):401–414
41. Zohuriaan-Mehr MJ (2005) Advances in chitin and chitosan modification through graft copolymerization: a comprehensive review. Iran Polym J 14(3):235–265
42. Kasuya T, Kuroda SI (2009) Nanoparticles for human liver specific drug and gene delivery systems: in vitro and in vivo advances. Expert Opin Drug Deliv 6(1):39–52
43. Bhavsar MD, Amiji MM (2007) Polymeric nano-and microparticle technologies for oral gene delivery. ExpertOpin. Drug Deliv. 4(3):197–213
44. Park TG, Jeong JH, Kim SW (2006) Current status of polymeric gene delivery systems. Adv Drug Deliv Rev 58:467–486
45. Schwartz B, Ivanov M, Pitard B, Escriou V, Rangara R, Byk G, Wils P, Crouzet J, Scherman D (1999) SyntheticDNA-compacting peptides derived from human sequence enhance cationic lipid-mediated gene transfer in vitro and in vivo. Gene Ther 6(2):282–292
46. Gaspar V, Sousa F, Queiroz J, Correia I (2011) Formulationof chitosan–TPP–pDNAnanocapsules for gene therapyapplications. Nanotechnology 22(1):1–12
47. .Plank C, Mechtler K, Szoka Jr FC, Wagner E (1996) Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene delivery. Hum Gene Ther 7(12):1437–1446
48. Barron LG, Gagne L, Szoka FC (1999) Lipoplex-mediated gene delivery to the lung occurs within 60 minutes of intravenous administration. Hum Gene Ther 10(10):1683–1694
49. Kunath K, Harpe A, Fischer D, Petersen H, Bickel U, Voigt K, Kissel T (2003) Low-molecular-weight polyethylenimine as a non-viral vector for DNA delivery: comparison of physicochemical properties, transfection efficiency andin vivo distribution with high-molecular-weight polyethylenimine. J Control Release 89(1):113–125
50. Mansouri S, Cuie Y, Winnik F, Shi Q, Lavigne P, Benderdour M, Beaumont E, Fernandes JC (2006) Characterization of folate-chitosan-DNA nanoparticles for gene therapy. Biomaterials 27(9):2060–2065
51. Hunter RJ (1981) Zeta potential in colloid science: principles and applications. Academic Press, London
52. Lundin KE, Simonson OE, Moreno PM, Zaghloul EM, Oprea II, Svahn MG, Smith CE (2009) Nanotechnologyapproaches for gene transfer. Genetica 137(1):47–56
53. Amiji MM (2006) Nanotechnology for cancer therapy. CRCPress. Taylor & Francis Groups, Boca Raton
54. . Venturoli D, Rippe B (2005) Ficoll and dextran vs. globular proteins as probes for testing glomerular perm selectivity: effects of molecular size, shape, charge, and deformability. Am J Physiol Renal Physiol 288(4):F605–F613
55. Schatzlein AG, Zinselmeyer BH, Elouzi A, Dufes C, Chim YTA, Roberts CJ, Davies MC, Munro A, Gray AI, Uchegbu IF (2005) Preferential liver gene expressionwith polypropylenimine dendrimers. J Control Release 101(1):247–258
56. Kim T-H, Jiang H-L, Jere D, Park I-K, Cho M-H, Nah J-W, Choi Y-J, Akaike T, Cho C-S (2007) Chemicalmodification of chitosan as a gene carrier in vitro and invivo. Prog Polym Sci 32(7):726–753

57. Özbas-Turan S, Akbuga J (2011) Plasmid DNA-loaded chitosan/TPP nanoparticles for topical gene delivery. DrugDeliv. 18(3):215–222
58. Vlerken LE, Vyas TK, Amiji MM (2007) Poly(ethyleneglycol)-modified nanocarriers for tumor-targeted and intracellular delivery. Pharm Res 24(8):1405–1414
59. Li T, Shi XW, Du YM, Tang YF (2007) Quaternized chitosan/alginate nanoparticles for protein delivery. J Biomed Mater Res A 83(2):383–390
60. Xu Y, Du Y (2003) Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. Int J Pharm 250(1):215–226
61. Li Z-T, Guo J, Zhang J-S, Zhao Y-P, Lv L, Ding C, Zhang X-Z (2010) Chitosan-graft-polyethylenimine with improved properties as a potential gene vector. Carbohydr Polym 80(1):254–259
62. Park IK, Kim TH, Park YH, Shin BA, Choi ES, Chowdhury EH, Akaike T, Cho CS (2001) Galactosylatedchitosan-graft-poly(ethyleneglycol) as hepatocytetargetingDNA carrier. J Control Release 76(3):349–362
63. Mao SR, Shuai XT, Unger F, Wittmar M, Xie XL, Kissel T (2005) Synthesis, characterization and cytotoxicity ofpoly(ethylene glycol)-graft-trimethyl chitosan block copolymers. Biomaterials 26(32):6343–6356
64. Wu J, Wang XF, Keum JK, Zhou HW, Gelfer M, Avila-Orta CA, Pan H, Chen WL, Chiao SM, Hsiao BS, Chu B (2007) Water soluble complexes of chitosang-MPEG and hyaluronic acid. J Biomed Mater Res A 80A(4):800–812
65. Zhang W, Pan SR, Zhang X, Luo X, Du Z (2008) Preparation of monomethyl poly(ethylene glycol)-g-chitosan copolymers with various degrees of substitution: Their ability to encapsulate and condense plasmid DNA. J Appl Polym Sci 108(5):2958–2967
66. Ghiamkazemi S, Amanzadeh A, Dinarvand R, Rafiee-Tehrani M, Amini M (2010) Synthesis, and characterization, and evaluation of cellular effects of the FOL-PEG-g-PEIGALnanoparticles as a potential non-viral vector for gene delivery. J Nanomater 2010:1–10
67. Fernandez-Megia E, Novoa-Carballal R, Quinoa E, Riguera R (2007) Conjugation of bioactive ligands to PEG-grafted chitosan at the distal end of PEG. Biomacromol 8(3):833–842
68. He H, Li Y, Jia X-R, Du J, Ying X, Lu W-L, Lou J-N, Wei Y (2011) PEGylated Poly(amidoamine) dendrimer based dual-targeting carrier for treating brain tumors. Biomaterials 32(2):478–487
69. Hu Y, Jiang H, Xu C, Wang Y, Zhu K (2005) Preparation and characterization of poly(ethylene glycol)-g-chitosan with water-and organo solubility. Carbohydr Polym 61(4):472–479
70. Casettari L, Vllasaliu D, Mantovani G, Howdle SM, Stolnik S, Illum L (2010) Effect of PEGylation on the toxicity and permeability enhancement of chitosan. Biomacromol 11(11):2854–2865
71. Prabaharan M, Mano J (2004) Chitosan-based particles as controlled drug delivery systems. Drug Deliv 12(1):41–57
72. Chan P, Kurisawa M, Chung JE, Yang Y-Y (2007) Synthesis and characterization of chitosan-g-poly(ethyleneglycol)-folate as a non-viral carrier for tumor-targeted gene delivery. Biomaterials 28(3):540–549
73. Casettari L, Vllasaliu D, Castagnino E, Stolnik S, Howdle S, Illum L (2012) PEGylated chitosan derivatives: synthesis, characterizations and pharmaceutical applications. Prog Polym Sci 37(5):659–685
74. .Deng L, Qi H, Yao C, Feng M, Dong A (2007) Investigation on the properties of methoxy poly(ethylene glycol)/chitosan graft co-polymers. J Biomater Sci Polym Ed 18(12):1575–1589
75. Amiji MM (1997) Synthesis of anionic poly(ethylene glycol)derivative for chitosan surface modification in blood-contacting applications. Carbohydr Polym 32(3):193–199
76. Luten J, Akeroyd N, Funhoff A, Lok MC, Talsma H, Hennink WE (2006) Methacrylamide polymers with hydrolysis-sensitive cationic side groups as degradable gene carriers. Bioconjug Chem 17(4):1077–1084

77. Varkouhi AK, Lammers T, Schiffelers RM, van Steenbergen MJ, Hennink WE, Storm G (2011) Gene silencing activity of siRNA polyplexes based on biodegradable polymers. Eur J Pharm Biopharm 77(3):450–457
78. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT, Leong KW (2001) Chitosan-DNAnanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J Control Release 70(3):399–421
79. Park IK, Kim TH, Kim SI, Park YH, Kim WJ, Akaike T, Cho CS (2003) Visualization of transfection of hepatocytes by galactosylated chitosan-graft-poly(ethyleneglycol)/DNA complexes by confocal laser scanning microscopy. Int J Pharm 257(1):103–110
80. Yun YH, Jiang HL, Chan R, Chen WL (2005) Sustainedrelease of PEG-g-chitosan complexed DNA from poly(lactide-co-glycolide). J Biomater Sci Polym Ed 16(11):1359–1378
81. Jiang X, Dai H, Leong KW, Goh SH, Mao HQ, Yang YY (2006) Chitosan-g-PEG/DNA complexes deliver gene to the rat liver *via* intrabiliary and intraportal infusions. J Gene Med 8(4):477–487
82. Germershaus O, Mao SR, Sitterberg J, Bakowsky U, Kissel T (2008) Gene delivery using chitosan, trimethyl chitosan or polyethylenglycol-graft-trimethyl chitosan block copolymers: establishment of structure-activity relationships in vitro. J Control Release 125(2):145–154
83. Wu YD, Liu CB, Zhao XY, Xiang JN (2008) A new biodegradable polymer: PEGylated chitosan-g-PEI possessing a hydroxyl group at the PEG end. J Polym Res 15(3):181–185
84. Zhang T, Yu YY, Li D, Peng R, Li Y, Jiang Q, Dai P, Gao R (2009) Synthesis and properties of a novel methoxypoly(ethylene glycol)-modified galactosylated chitosan derivative. J Mater Sci Mater Med 20(3):673–680
85. Thanou M, Florea BI, Geldof M, Junginger HE, Borchard G (2002) Quaternized chitosan oligomers as novel gene delivery vectors in epithelial cell lines. Biomaterials 23(1):153–159
86. Rojanarata T, Petchsangsai M, Opanasopit P, Ngawhirunpat T, Ruktanonchai U, Sajomsang W, Tantayanon S (2008) Methylated N-(4-N, N-dimethylaminobenzyl)chitosan for novel effective gene carriers. Eur J Pharm Biopharm 70(1):207–214
87. Liang XF, Tian H, Luo H, Wang HJ, Chang J (2009) Novel quaternized chitosan and polymeric micelles with cross-linked ionic cores for prolonged release of minocycline. J Biomater Sci Polym Ed 20(1):115–131
88. Liang XF, Sun YM, Duan YR, Cheng YS (2012) Synthesis and characterization of PEG-graft-quaternized chitosan and cationic polymeric liposomes for drug delivery. J Appl Polym Sci 125(2):1302–1309
89. Richardson SCW, Kolbe HJV, Duncan R (1999) Potential of low molecular mass chitosan as a DNA delivery system:Biocompatibility, body distribution and ability to complex and protect DNA. Int J Pharm 178(2):231–243
90. Huang Z, Dong L, Chen JJ, Gao FB, Zhang ZP, Chen JN, Zhang JF (2012) Low-molecular weight chitosan/vascular endothelial growth factor short hairpin RNA for the treatment of hepatocellular carcinoma. Life Sci 91(23):1207–1215
91. Yang XR, Yuan XY, Cai DN, Wang SY, Zong L (2009) Low molecular weight chitosan in DNA vaccine delivery *via* mucosa. Int J Pharm 375(1):123–132
92. Heo SH, Jang MJ, Kim DG, Jeong YI, Jang MK, Nah JW (2007) Characterization and preparation of low molecular weight water soluble chitosan nanoparticle modified with cell targeting ligand for efficient gene delivery. Polym Korea 31(5):454–459
93. Ercelen S, Zhang X, Duportail G, Grandfils C, Desbrieres J, Karaeva S, Tikhonov V, Mely Y, Babak V (2006) Physicochemical properties of low molecular weight alkylated chitosans: a new class of potential nonviral vectors for gene delivery. Colloid Surf B 51(2):140–148
94. Mao SR, Shuai XT, Unger F, Simon M, Bi DZ, Kissel T (2004) The depolymerization of chitosan: effects on physicochemical and biological properties. Int J Pharm 281(1):45–54
95. Janes KA, Alonso MJ (2003) Depolymerized chitosan nanoparticles for protein delivery: preparation and characterization. J Appl Polym Sci 88(12):2769–2776
96. Yang F, Cui XQ, Yang XR (2002) Interaction of low-molecular-weight chitosan with mimic membrane studied by electrochemical methods and surface plasmon resonance. Biophys Chem 99(1):99–106

97. Lee M, Nah JW, Kwon Y, Koh JJ, Ko KS, Kim SW (2001) Water-soluble and low molecular weight chitosan-based plasmid DNA delivery. Pharm Res 18(4):427–431
98. Tripathi SK, Goyal R, Kashyap MP, Pant AB, Haq W, Kumar P, Gupta KC (2012) Depolymerized chitosansfunctionalized with bPEI as carriers of nucleic acids andtuftsin-tethered conjugate for macrophage targeting. Biomaterials 33(16):4204–4219
99. Fernandes JC, Qiu XP, Winnik FM, Benderdour M, Zhang XL, Dai KR, Shi Q (2012) Low molecular weight chitosan conjugated with folate for siRNA delivery invitro: optimization studies. Int J Nanomedicine 7:5833–5845
100. Yuan H, Lu LJ, Du YZ, Hu FQ (2011) Stearic acid-g-chitosan polymeric micelle for oral drug delivery: in vitro transport and in vivo absorption. Mol Pharm 8(1):225–238
101. Yoksan R, Akashi M (2009) Low molecular weight chitosan-g-L-phenylalanine: preparation, characterization, and complex formation with DNA. Carbohydr Polym 75(1):95–103
102. Gao SY, Chen JN, Xu XR, Ding Z, Yang YH, Hua ZC, Zhang JF (2003) Galactosylated low molecular weight chitosan as DNA carrier for hepatocyte-targeting. Int J Pharm 255(1):57–68
103. Lee K, Kwon I, Kim Y-H, Jo W, Jeong S (1998) Preparation of chitosan self-aggregates as a gene delivery system. J Control Release 51(2):213–220
104. Mintzer MA, Simanek EE (2008) Nonviral vectors for gene delivery. Chem Rev 109(2):259–302
105. Zhang S, Xu Y, Wang B, Qiao W, Liu D, Li Z (2004) Cationiccompounds used in lipoplexes and polyplexes forgene delivery. J Control Release 100(2):165–180
106. Liu Z, Jiao Y, Liu F, Zhang Z (2007) Heparin/chitosan nanoparticle carriers prepared by polyelectrolyte complexation. J Biomed Mater Res A 83(3):806–812
107. Erbacher P, Zou S, Bettinger T, Steffan A-M, Remy J-S (1998) Chitosan-based vector/DNA complexes for gene delivery: biophysical characteristics and transfection ability. Pharm Res 15(9):1332–1339
108. Lee D, Zhang W, Shirley SA, Kong X, Hellermann GR, Lockey RF, Mohapatra SS (2007) Thiolated chitosan/DNA nanocomplexes exhibit enhanced and sustained gene delivery. Pharm Res 24(1):157–167
109. Talaei F, Azizi E, Dinarvand R, Atyabi F (2011) Thiolatedchitosan nanoparticles as a delivery system for antisense therapy: evaluation against EGFR in T47D breast cancer cells. Int J Nanomed 6:1963–1975
110. Varkouhi AK, Verheul RJ, Schiffelers RM, Lammers T, Storm G, Hennink WE (2010) Gene silencing activity of siRNA polyplexes based on thiolated N, N. N-trimethylatedchitosan Bioconjug Chem 21(12):2339–2346
111. Martien R, Loretz B, Sandbichler AM, Schnurch AB (2008) Thiolated chitosan nanoparticles: transfection study in the Caco-2 differentiated cell culture. Nanotechnology 19(4):1–9
112. Martien R, Loretz B, Thaler M, Majzoob S, Bernkop-Schnuerch A (2007) Chitosan-thioglycolic acid conjugate: an alternative carrier for oral nonviral gene delivery? J Biomed Mater Res A 82A(1):1–9
113. Zhao X, Li Z, Pan H, Liu W, Lv M, Leung F, Lu WW (2013) Enhanced gene delivery by chitosan-disulfide-conjugated LMW-PEI for facilitating osteogenic differentiation. Acta Biomater 9(5):6694–6703
114. Jeong JH, Kim SW, Park TG (2007) Molecular design of functional polymers for gene therapy. Prog Polym Sci 32(11):1239–1274
115. MacLaughlin FC, Mumper RJ, Wang J, Tagliaferri JM, Gill I, Hinchcliffe M, Rolland AP (1998) Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. J Control Release 56(1):259–272
116. Murata J, Ohya Y, Ouchi T (1996) Possibility of application of quaternary chitosan having pendant galactose residues as gene delivery tool. Carbohydr Polym 29(1):69–74
117. Murata J, Ohya Y, Ouchi T (1997) Design of quaternary chitosan conjugate having antennary galactose residues asa gene delivery tool. Carbohydr Polym 32(2):105–109
118. Abdelhady HG, Allen S, Davies MC, Roberts CJ, Tendler SJ, Williams PM (2003) Direct real-time molecular scale visualisation of the degradation of condensed DNAcomplexes exposed to DNase I. Nucleic Acids Res 31(14):4001–4005

119. Schaffer DV, Lauffenburger DA (1998) Optimization of cell surface binding enhances efficiency and specificity of molecular conjugate gene delivery. J Biol Chem 273(43):28004–28009
120. Lee KY, Kwon IC, Jo WH (2005) Jeong, Complex formation between plasmid DNA and self-aggregates of deoxycholic acid-modified chitosan. Polymer 46(19):8107–8112
121. Miao J, Zhang XG, Hong Y, Rao YF, Li Q, Xie XJ, Wo JE, Li MW (2012) Inhibition on hepatitis B viruse-gene expression of 10–23 DNAzyme delivered by novel chitosan oligosaccharide-stearic acid micelles. Carbohydr Polym 87(2):1342–1347
122. Han L, Zhao J, Zhang X, Cao WP, Hu XX, Zou GZ, Duan XL, Liang XJ (2012) Enhanced siRNA delivery and silencing gold-chitosan nanosystem with surface charge-reversal polymer assembly and good biocompatibility. ACS Nano 6(8):7340–7351
123. Liao ZX, Ho YC, Chen HL, Peng SF, Hsiao CW, Sung HW (2010) Enhancement of efficiencies of the cellular uptake and gene silencing of chitosan/siRNA complexes *via* the inclusion of a negatively charged poly(gammaglutamicacid). Biomaterials 31(33):8780–8788
124. Liu LX, Song CN, Song LP, Zhang HL, Dong X, Leng XG (2009) Effects of alkylated-chitosan-DNA nanoparticles on the function of macrophages. J Mater Sci Mater Med 20(4):943–948
125. Bao JB, Song CX (2009) Chitosan chip and application to evaluate DNA loading on the surface of the metal. Biomed Mater 4(1):1–5
126. Pimpha N, Rattanonchai U, Surassmo S, Opanasopit P, Rattanarungchai C, Sunintaboon P (2008) Preparation of PMMA/acid-modified chitosan core-shell nanoparticles and their potential as gene carriers. Colloid Polym Sci 286(8–9):907–916
127. Satoh T, Kakimoto S, Kano H, Nakatani M, Shinkai S, Nagasaki T (2007) In vitro gene delivery to HepG2 cells using galactosylated 6-amino-6-deoxychitosan as a DNA carrier. Carbohydr Res 342(11):1427–1433
128. Schatz C, Bionaz A, Lucas JM, Pichot C, Viton C, Domard A, Delair T (2005) Formation of polyelectrolyte complex particles from self-complexation of N-sulfated chitosan. Biomacromol 6(3):1642–1647
129. Pal K, Behera B, Roy S, Ray SS, Thakur G (2013) Chitosan based delivery systems on a length scale: Nano to macro. Soft Mater 11(2):125–142
130. Akbuga J, Ozbas-Turan S, Erdogan N (2004) Plasmid-DNAloaded chitosan microspheres for in vitro IL-2 expression. Eur J Pharm Biopharm 58(3):501–507
131. Vimal S, Taju G, Nambi KSN, Majeed SA, Babu VS, Ravi M, Hameed ASS (2012) Synthesis and characterization of CS/TPP nanoparticles for oral delivery of gene in fi sh. Aquaculture 358:14–22
132. Jiang H, Wu H, Xu YL, Wang JZ, Zeng Y (2011) Preparationof galactosylated chitosan/tripolyphosphate nanoparticles and application as a gene carrier for targetingSMMC7721 cells. J Biosci Bioeng 111(6):719–724
133. Fabregas A, Minarro M, Garcia-Montoya E, Perez-Lozano P, Carrillo C, Sarrate R, Sanchez N, Tico JR, Sune-Negre JM (2013) Impact of physical parameters on particle size and reaction yield when using the ionic gelation method to obtain cationic polymeric chitosan-tripolyphosphatenanoparticles. Int J Pharm 446(1):199–204
134. Nasti A, Zaki NM, de Leonardis P, Ungphaiboon S, Sansongsak P, Rimoli MG, Tirelli N (2009) Chitosan/TPPand Chitosan/TPP-hyaluronic acid nanoparticles: Systematicoptimisation of the preparative process and preliminary biological evaluation. Pharm Res 26(8):1918–1930
135. Bozkır A, Saka OM (2004) Chitosan nanoparticles for plasmid DNA delivery: effect of chitosan molecular structure on formulation and release characteristics. Drug Deliv 11(2):107–112
136. Conner SD, Schmid SL (2003) Regulated portals of entry into the cell. Nature 422(6927):37–44
137. Kadiyala I, Loo YH, Roy K, Rice J, Leong KW (2010) Transport of chitosan-DNA nanoparticles in human intestinal M-cell model versus normal intestinal enterocytes. Eur J Pharm Sci 39(1):103–109

138. Manaspon C, Viravaidya-Pasuwat K, Pimpha N (2012) Preparation of folate-conjugated pluronic F127/chitosan core-shell nanoparticles encapsulating doxorubicin for breast cancer treatment. J Nanomater 2012:1–11
139. Zhu HY, Liu F, Guo J, Xue JP, Qian ZY, Gu YQ (2011) Folate-modified chitosan micelles with enhanced tumor targeting evaluated by near infrared imaging system. Carbohydr Polym 86(3):1118–1129
140. Morris VB, Pillai CKS, Sharma CP (2011) Folic acidconjugateddepolymerized quaternized chitosan as potential targeted gene delivery vector. Polym Int 60(7):1097–1106
141. Galbiati A, Tabolacci C, Della Rocca BM, Mattioli P, Beninati S, Paradossi G, Desideri A (2011) Targetingtumor cells through chitosan-folate modified microcapsules loaded with camptothecin. Bioconjug Chem 22(6):1066–1072
142. Zheng Y, Cai Z, Song XR, Yu B, Bi YQ, Chen QH, Zhao D, Xu JP, Hou SX (2009) Receptor mediated gene delivery by folate conjugated N-trimethyl chitosan invitro. Int J Pharm 382(1):262–269
143. Jiang HL, Xu CX, Kim YK, Arote R, Jere D, Lim HT, Cho MH, Cho CS (2009) The suppression of lung tumorigenesis by aerosol-delivered folate-chitosan-graftpolyethylenimine/Akt1 shRNA complexes through the Akt signaling pathway. Biomaterials 30(29):5844–5852
144. Fernandes JC, Wang HJ, Jreyssaty C, Benderdour M, Lavigne P, Qiu XP, Winnik FM, Zhang XL, Dai KR, Shi Q (2008) Bone-protective effects of nonviral gene therapy with folate-chitosan DNA nanoparticle containing interleukin-1 receptor antagonist gene in rats with adjuvant-induced arthritis. Mol Ther 16(7):1243–1251
145. Ganta S, Devalapally H, Shahiwala A, Amiji M (2008) A review of stimuli-responsive nanocarriers for drug and gene delivery. J Control Release 126(3):187–204
146. Alvarenga ES (2011) Characterization and properties of chitosan. In: Biotechnology of biopolymers; Elnashar, M., Ed.;Intech, Crotia, pp 91–108
147. Huang M, Fong CW, Khor E, Lim LY (2005) Transfectionefficiency of chitosan vectors: effect of polymer molecularweight and degree of deacetylation. J Control Release 106(3):391–406
148. Koping-Hoggard M, Tubulekas I, Guan H, Edwards K, Nilsson M, Varum KM, Artursson P (2001) Chitosan as anonviral gene delivery system. Structure-property relationshipsand characteristics compared with polyethyleniminein vitro and after lung administration in vivo. GeneTher 8(14):1108–1121
149. Moreira C, Oliveira H, Pires LR, Simoes S, Barbosa MA, Pego AP (2009) Improving chitosan-mediated genetransfer by the introduction of intracellular bufferingmoieties into the chitosan backbone. Acta Biomater 5(8):2995–3006
150. Chang KL, Higuchi Y, Kawakami S, Yamashita F, Hashida M (2010) Efficient gene transfection by histidine-modified chitosan through enhancement of endosomal escape. Bioconjug Chem 21(6):1087–1095
151. Zaki NM, Nasti A, Tirelli N (2011) Nanocarriers for cytoplasmicdelivery: cellular uptake and intracellular fate ofchitosan and hyaluronic acid-coated chitosan nanoparticlesin a phagocytic cell model. Macromol Biosci 11(12):1747–1760
152. Thibault M, Astolfi M, Tran-Khanh N, Lavertu M, Darras V, Merzouki A, Buschmann MD (2011) Excess polycationmediates efficient chitosan-based gene transfer bypromoting lysosomal release of the polyplexes. Biomaterials 32(20):4639–4646
153. Chang KL, Higuchi Y, Kawakami S, Yamashita F, Hashida M (2011) Development of lysine-histidine Dendron modified chitosan for improving transfection efficiencyin HEK293 cells. J Control Release 156(2):195–202
154. Dehousse V, Garbacki N, Colige A, Evrard B (2010) Developmentof pH-responsive nanocarriers using trimethylchitosansand methacrylic acid copolymer for siRNAdelivery. Biomaterials 31(7):1839–1849
155. HerasAlarcón C, Pennadam S, Alexander C (2005) Stimuliresponsive polymers for biomedical applications. Chem Soc Rev 34(3):276–285
156. Bao HQ, Li L, Gan LH, Ping YA, Li J, Ravi P (2010) Thermo- and pH-responsive association behavior of dualhydrophilic graft chitosan terpolymer synthesized *via*atrpand click chemistry. Macromol 43(13):5679–5687

157. McMahon SS, Nikolskaya N, Choileain SN, Hennessy N, O'Brien T, Strappe PM, Gorelov A, Rochev Y (2011) Thermosensitive hydrogel for prolonged delivery of lentiviralvector expressing neurotrophin-3 in vitro. J. GeneMed. 13(11):591–601
158. Hastings CL, Kelly HM, Murphy MJ, Barry FP, O'Brien FJ, Duffy GP (2012) Development of a thermoresponsivechitosan gel combined with human mesenchymalstem cells and desferrioxamine as a multimodalpro-angiogenic therapeutic for the treatment of criticallimb ischaemia. J Control Release 161(1):73–80
159. Cheng YH, Yang SH, Lin FH (2011) Thermosensitive chitosan-gelatin-glycerol phosphate hydrogel as a controlledrelease system of ferulic acid for nucleus pulposus regeneration. Biomaterials 32(29):6953–6961
160. Loretz B, Thaler M, Bernkop-Schnurch A (2007) Role ofsulfhydryl groups in transfection? A case study with Chitosan-NAC nanoparticles. Bioconjug Chem 18(4):1028–1035
161. Schmitz T, Bravo-Osuna I, Vauthier C, Ponchel G, Loretz B, Bernkop-Schnurch A (2007) Development and invitro evaluation of a thiomer-based nanoparticulate genedelivery system. Biomaterials 28(3):524–531
162. Jia LJ, Li ZY, Zhang DR, Zhang Q, Shen JY, Guo HJ, Tian XN, Liu GP, Zheng DD, Qi LS (2013) Redox-responsive catiomer based on PEG-ss-chitosanoligosaccharide-ss-polyethylenimine copolymer for effectivegene delivery. Polym Chem 4(1):156–165
163. Mitra S, Gaur U, Ghosh P, Maitra A (2001) Tumour targeteddelivery of encapsulated dextran-doxorubicin conjugateusing chitosan nanoparticles as carrier. J Control Release 74(1):317–323
164. Tian Q, Zhang C-N, Wang X-H, Wang W, Huang W, Cha R-T, Wang C-H, Yuan Z, Liu M, Wan H-Y (2010) Glycyrrhetinic acid-modified chitosan/poly(ethyleneglycol) nanoparticles for liver-targeted delivery. Biomaterials 31(17):4748–4756
165. Dai H, Jiang X, Tan GC, Chen Y, Torbenson M, Leong KW, Mao H-Q (2006) Chitosan-DNA nanoparticlesdelivered by intrabiliary infusion enhance liver-targetedgene delivery. Int J Nanomed 1(4):507–522
166. Chen Q, Wang X, Chen F, Zhang Q, Dong B, Yang H, Liu G, Zhu Y (2011) Functionalization of upconvertedluminescent $NaYF_4$:Yb/Er nanocrystals by folic acid-chitosan conjugates for targeted lung cancer cell imaging. J Mater Chem 21(21):7661–7667
167. Issa MM, Köping-Höggård M, Tømmeraas K, Vårum KM, Christensen BE, Strand SP, Artursson P (2006) Targeted gene delivery with trisaccharide-substituted chitosanoligomers in vitro and after lung administration invivo. J Control Release 115(1):103–112
168. Wang X, Chi N, Tang X (2008) Preparation of estradiol chitosannanoparticles for improving nasal absorption and braintargeting. Eur J Pharm Biopharm 70(3):735–740
169. Aktas Y, Yemisci M, Andrieux K, Gürsoy RN, Alonso MJ, Fernandez-Megia E, Novoa-Carballal R, Quiñoá E, Riguera R, Sargon MF (2005) Development andbrain delivery of chitosan-PEG nanoparticles functionalizedwith the monoclonal antibody OX26. Bioconjug Chem 16(6):1503–1511
170. Hughes JA, Rao GA (2005) Targeted polymers for gene delivery. Expert Opin Drug Deliv 2(1):145–157
171. Talvitie E, Leppiniemi J, Mikhailov A, Hytonen VP, Kellomaki M (2012) Peptide-functionalized chitosan-DNAnanoparticles for cellular targeting. Carbohydr Polym 89(3):948–954
172. Wang M, Hu H, Sun Y, Qiu L, Zhang J, Guan G, Zhao X, Qiao M, Cheng L, Cheng L (2013) A pH-sensitivegene delivery system based on folic acid-PEG-chitosan–PAMAM-plasmid DNA complexes for cancer cell targeting. Biomaterials 34(38):10120–10132
173. Zhang H, Mardyani S, Chan WC, Kumacheva E (2006) Design of biocompatible chitosan microgels for targetedpH-mediated intracellular release of cancer therapeutics. Biomacromol 7(5):1568–1572
174. Park K, Kim J-H, Nam YS, Lee S, Nam HY, Kim K, Park JH, Kim I-S, Choi K, Kim SY(2007) Effect ofpolymer molecular weight on the tumor targeting characteristicsof self-assembled glycol chitosan nanoparticles. J Control Release 122(3):305–314

175. Raghuwanshi D, Mishra V, Das D, Kaur K, Suresh MR (2012) Dendritic cell targeted chitosan nanoparticles fornasal DNA immunization against SARS CoV nucleocapsidprotein. Mol Pharm 9(4):946–956
176. Min KH, Park K, Kim Y-S, Bae SM, Lee S, Jo HG, Park R-W, Kim I-S, Jeong SY, Kim K (2008) Hydrophobically modified glycol chitosan nanoparticles-encapsulatedcamptothecin enhance the drug stability and tumortargeting in cancer therapy. J Control Release 127(3):208–218
177. Han HD, Mangala LS, Lee JW, Shahzad MM, Kim HS, Shen D, Nam EJ, Mora EM, Stone RL, Lu C (2010) Targeted gene silencing using RGD-labeledchitosan nanoparticles. Clin Cancer Res 16(15):3910–3922
178. Dufes C, Muller J-M, Couet W, Olivier J-C, Uchegbu IF, Schätzlein AG (2004) Anticancer drug deliverywith transferrin targeted polymeric chitosan vesicles. Pharm Res 21(1):101–107
179. Sun Y, Chen Z-L, Yang X-X, Huang P, X-P Zhou, Du X-X (2009) Magnetic chitosan nanoparticles as a drugdelivery system for targeting photodynamic therapy. Nanotechnology 20(13):135102
180. Nam T, Park S, Lee S-Y, Park K, Choi K, Song IC, Han MH, Leary JJ, Yuk SA, Kwon IC (2010) Tumortargeting chitosan nanoparticles for dual-modality optical/MR cancer imaging. Bioconjug Chem 21(4):578–582
181. Wang C, Pham PT (2008) Polymers for viral gene delivery.Expert Opin. Drug Deliv 5 (4):385–401
182. Lameiro MH, Malpique R, Silva AC, Alves PM, Melo E (2006) Encapsulation of adenoviral vectors into chitosan–bile salt microparticles for mucosal vaccination. J Biotechnol 126(2):152–162
183. Taira K, Kataoka K, Niidome T (2005) Non-viral gene therapy: gene design and delivery. Springer, Tokyo