Abstract: Non-viral vectors, including lipid- or polymer-based systems, have attracted much attention to date as a gene delivery vehicle, due to safety issues with viral vectors. Chitosan, a naturally existing cationic polymer, has shown great potential as a gene delivery carrier, as it has low immunogenicity and toxicity, excellent transcellular transport ability, and is relatively easy to chemically modify. This review summarizes and discusses the general features of chitosan and its applications as a delivery carrier of DNA and RNA.

Keywords: chitosan, nanoparticle, gene delivery, targeting, siRNA.

Introduction

Gene delivery is used to introduce genetic material into cells in order to alleviate symptoms or prevent the occurrence of a particular disease. Appropriate carriers, typically viral or non-viral vectors, are essential for gene delivery, as it is difficult to move naked DNA, a negatively charged macromolecule, through negatively charged cell membranes. Several viral vectors such as retroviruses and adenoviruses have been frequently employed. However, critical safety issues have been raised in clinical uses. Therefore, non-viral vectors have been extensively investigated as alternatives. Several features required for non-viral gene delivery systems include (1) commercial availability and stability, (2) ease of bulk synthesis, (3) high binding efficiency, (4) ability to transflect most cells, and (5) lack of immunogenicity or biohazardous activity. Generally speaking, when cells are treated with DNA/vector complexes, the complexes adhere to the cell surface via either electrostatic interaction or receptor-mediated uptake. Endocytosis is the dominant mechanism for entry of the complexes, and the released DNA from the endosome enters into the cytosol in a partially de-condensed form. DNA finally reaches the nucleus and delivers genetic material.
nucleus via passive diffusion and nuclear membrane crossing, and the DNA is transcribed into mRNA, resulting in gene expression. A schematic description for transfection of eukaryotic cells with polymer/DNA complexes is shown in Figure 1.

Liposomes, self-closed colloidal particles in which bilayered membranes encapsulate a fraction of the medium, have been frequently used as a non-viral gene delivery system. Cationic liposomes are used as a gene carrier to reduce the net negative surface charge of DNA in an attempt to reduce charge-charge repulsion at the surface of cell membranes. However, the application of liposomes in vivo is limited due to poor biocompatibility and rapid degradation. An alternative approach to the development of non-viral vectors is the use of cationic polymers designed to complex with DNA. Polymer-based non-viral gene carriers have been frequently used due to the avoidance of potential immunogenicity and toxicity, the possibility of repeated administration, and the ease of establishing a good manufacturing practice (GMP). Simple mixing of DNA with poly(L-lysine) or DEAE-dextran resulted in the formation of polyelectrolyte complexes for transferring DNA into cells. But, these systems still showed a low transfection efficiency, cytotoxicity, and limited usefulness for systemic administration due to the rapid clearance following intravenous injection. New types of polymers such as poly(ethylene glycol)-b-poly(L-lysine), poly(L-lysine)-b-poly(D,L-lactic-co-glycolic acid), poly-N-(2-hydroxypropyl) methylacrylamide-b-poly(timethyloxacyctethyl methacrylate chloride), polyethylenimine-g-poly(vinyl pyrrolidone), and dendrimers have been synthesized and developed for gene delivery.

Although non-viral gene delivery systems have been dominated by synthetic polymer- or lipid-based gene carriers, natural polymers may provide a useful means of developing non-viral gene carriers due to their distinctive characteristics. For example, chitosan, a positively charged natural polysaccharide, has shown great potential as a gene delivery carrier. In this review, we discuss the general features of chitosan and its use as a delivery carrier of DNA and RNA.

General Properties of Chitosan

Chitin, composed of (1,4) linked N-acetyl-β-D-glucosamine, is the second most abundant natural polymer in the world, and is primarily obtained from shrimp and crab. When the degree of deacetylation (DD) of chitin reaches about 50%, it becomes soluble in aqueous acidic media and is called chitosan. Chitosan has a repeated structure of (1,4) linked β-D-glucosamine, and has an apparent pK of 6.5. Traditionally, commercial products are composed of 80% β-D-glucosamine and 20% N-acetyl-β-D-glucosamine (Figure 2). Chitosan is generally soluble at pH below 6, and its solubility is usually tested by dissolving it in 1% or 0.1 M acetic acid. The solution properties of chitosan depend on its molecular weight, degree of deacetylation, and distribution of acetyl groups in the main chain, as the deacetylation is usually carried out in the solid state and generates an irregular structure due to the semicrystalline feature of chitosan. H-NMR is the most convenient technique to measure the content and distribution of acetyl groups in chitosan. and 13C and 15N solid state NMR are also frequently used to investigate the distribution of acetyl groups in the chain (e.g., random or blockwise), which may influence the solubility and inter-chain interactions.

Chitosan is a cationic polymer and has been widely used in the areas of food, cosmetics, biomedical and pharmaceutical applications, etc. The extensive biological properties of chitosan enable various biomedical applications and include (1) biocompatibility and biodegradability, (2) binding capability to cells, (3) acceleration of wound healing, (4) haemostatic properties, (5) anti-bacterial and anti-fungal properties, and (6) anti-tumor properties. A large variety of useful forms such as beads, films, sponges, tubes, powders, and fibers, can be obtained from chitosan. Chitosan is degradable by enzymes such as lysozyme and chitosanase, and the degradation rate is also dependent on the temperature, ionic strength, and pH of the medium in vitro. In general the lower the degree of deacetylation of chitosan, the faster the degradation. Further, chitosan has been proven to be biodegradable when implanted into animals.

Chitosan/DNA Polyelectrolyte Complexes

Simple and direct mixing of chitosan and DNA generated nanoparticles by a coacervation process, which was useful for oral allergen-gene immunization. Oral administration of nanoparticles containing a dominant peanut allergen gene (pCMV4ah2) substantially reduced allergen-induced anaphylaxis, indicating a prophylactic use of the nanoparticles in treating food allergies. The molecular weight (MW) and degree of deacetylation (DD) are critical factors when using chitosan as a gene delivery carrier, because they affect DNA binding and release, and ultimately the in vitro and in vivo gene transfection efficiency. A549 cellular uptake of chitosan/pEGFP-C2 complexes was significantly reduced by decreasing the MW or DD of chitosan. The decreased DD also resulted in a decrease of overall luciferase expression...