Chitosan Nanoparticles : A Promising System for Drug Delivery

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Summary

Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Their sub-micron size not only suitable for parenteral application, but also applicable for mucosal routes of administration, i.e., oral, nasal, and ocular mucosa, which are non-invasive route. The application for mucosal delivery also facilitated by chitosan absorption enhancing effect. Furthermore, chitosan nanoparticles also showed to be a good adjuvant for vaccines. Therefore, the objectives of this review are to summarize the available preparation techniques involved chitosan nanoparticles, the application of explored chitosan nanoparticles, and the mechanism of cell entry.

Introduction

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task.

One approach is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles. The idea of using submicron drug delivery systems for drug targeting was conceived and developed after Paul Ehrlich originally proposed the idea of tiny drug-loaded magic bullets over a hundred year ago (Kumar and Banker, 1996). Among these carriers, liposomes and micro/nanoparticles have been the most extensively investigated. Liposomes present some technological limitations including poor reproducibility and stability, and low drug entrapment efficiency. Nevertheless, several low molecular weight drugs are now commercially available which employ this technology. Polymeric nanoparticles, which possess a better reproducibility and stability profiles than liposomes, have been proposed as alternative drug carriers that overcome many of these problems.

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers. There are two types of nanoparticles depending on the preparation process:

nanospheres and nanocapsules (Allemann *et al*., 1993). Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces (Figure 1). Nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed onto their exterior (Figure 1).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.

Nanoparticles not only have potential as drug delivery carriers as they offer non-invasive routes of administration such as oral, nasal and ocular routes, but also show to be good adjuvant for vaccines. Despite these advantages, there is no ideal nanoparticle system available. Most of nanoparticles prepared from water-insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to the drug stability. Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force.

Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles (Table 1) such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. In the last two decades, chitosan nanoparticles (chitosan NP) have been extensively developed and explored for pharmaceutical applications. Thus, this review focuses on the chitosan nanoparticle preparation technology, applications and mechanism of cell entry.

Chitosan

Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some microorganisms, yeast and fungi (Illum, 1998). The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose. These units combined by β-(1,4) glycosidic linkages, forming a long chain linear polymer. Although chitin is insoluble in most solvents, chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric, and citric acid (LeHoux and Grondin, 1993; Peniston and Johnson, 1980). It is insoluble in phosphoric and sulfuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation. Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation.

Preparation method

Over the past 30 years, chitosan NP preparation technique has been developed based on chitosan microparticles technology. There are at least four methods available: ionotropic gelation, microemulsion, emulsification solvent

diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods offer

Table 1. Criteria for ideal polymeric carriers for nanoparticles & nanoparticle delivery systems

Polymeric carriers
- Easy to synthesize and characterize
- Inexpensive
- Biocompatible
- Biodegradable
- Non-immunogenic
- Non-toxic
- Water soluble

Nanoparticle delivery systems
- Simple and inexpensive to manufacture and scale-up
- No heat, high shear forces or organic solvents involved in their preparation process
- Reproducible and stable
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides
- Ability to lyophilize
- Stable after administration
- Non-toxic

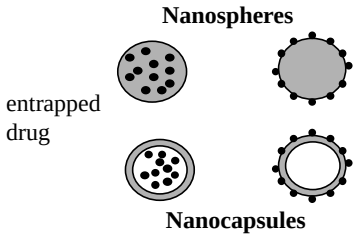

Figure 1. Various types of drug-loaded nanoparticles. Adapted from Allemann *et al.* (1993)

many advantages such as simple and mild preparation method without the use of organic solvent or high shear force. Thus, they would be applicable to a broad categories of drugs including macromolecules which notorious as labile drugs.

In general, the factors found to affect nanoparticles formation including particle size and surface charge are molecular weight and degree of deacetylation of chitosan. The entrapment efficiency is found to be dependent on the pKa and solubility of entrapped drugs. The drug is mostly found to be associated with chitosan via electrostatic interaction, hydrogen bonding, and hydrophobic interaction.

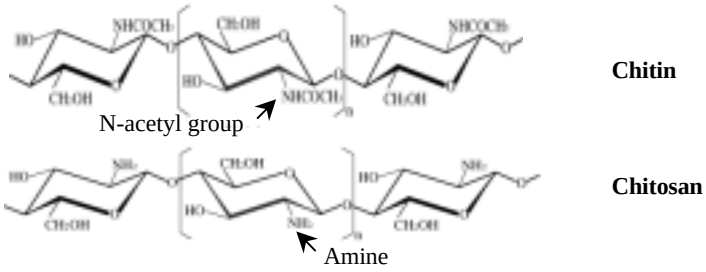

Figure 2. Chitin and chitosan

Ionotropic gelation

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo *et al*.,(1997b) and has been widely examined and developed (Janes *et al*., 2001; Pan *et al*., 2002). The mechanism of chitosan NP formation is based on electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate (Bodmeier *et al*., 1989; Xu and Du, 2003). This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer (Calvo *et al*., 1997a).

Microemulsion method

Chitosan NP prepared by microemulsion technique was first developed by Maitra *et al*.(1999). This technique is based on formation of chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, chitoan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of chitosan conjugate with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with $CaCl_2$ and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilyzation. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alter the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

Emulsification solvent diffusion method

El-Shabouri reported chitosan NP prepared by emulsion solvent diffusion method, (El-Shabouri, 2002) which originally developed by Niwa *et al*. employing PLGA (Niwa *et al*., 1993). This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring, follow by high pressure homogenization. The emulsion is then diluted with a large amount of water to vercome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles. This method is suitable for hydrophobic drug and showed high

percentage of drug entrapment. The major drawbacks of this method include harsh processing conditions (e.g., the use of organic solvents) and the high shear forces used during nanoparticle preparation.

Polyelectrolyte complex (PEC)

Polyelectrolyte complex or self assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature (Erbacher *et al*., 1998). The complexes size can be varied from 50 nm to 700 nm.

Applications of chitosan nanoparticles

Parenteral administration

Nano-sized particles can be administered intravenously because the diameter of the smallest blood capillary is approximately 4 μm. The biodistribution of nanoparticles can vary depending on the size, surface charge and hydrophobicity of the administered particles (Mueller, 1991; Tabata and Ikada, 1988). Particles greater than 100 nm in diameter are rapidly taken up by the reticuloendothelial system (RES) in the liver, spleen, lung and bone marrow, while smaller-sized particles tend to have a prolonged circulation time. Negatively-charged particles are eliminated faster than positively-charged or neutral particles (Tabata and Ikada, 1988). In general, opsonins (serum proteins that bind to substrates leading to their being taken up by the RES) prefer to adsorb on hydrophobic rather than hydrophilic surfaces. The creation of a hydrophilic coating (such as polyethylene glycol (PEG) or a nonionic surfactant) on hydrophobic carriers significantly improves their circulation time (Gref *et al*., 1996; Kreuter, 1994). Together, these data suggest that generating nanoparticles with a hydrophlilic but neutral surface charge is a viable approach to reduce macrophage phagocytosis and thereby improve the therapeutic efficacy of loaded drug particles.

The most promising drugs that have been extensively studied for delivery by this route are anticancer agents. Following intravenous injection, many nanoparticle systems including chitosan NP exhibited a marked tendency to accumulate in a number of tumors (Brasseur *et al*., 1980; Kreuter, 1994). One possible reason for the phenomenon may involve the leakiness of tumor vasculature (Gerlowski and Jain, 1986; Sadzuka *et al*., 1998). Doxorubicin loaded chitosan NP showed regression in tumor growth and enhance survival rate of tumor-implanted rats after IV administration. In addition, chitosan NP less

than 100 nm in size have been developed which showed to be RES evading and circulate in the blood for considerable amount of time.

Delivery of antiinfectives such as antibacterial, antiviral, antifungal and antiparasitic drugs, is another common use of nanoparticles (Bender *et al.*, 1996; Page-Clisson *et al*., 1998; Soma *et al*., 2000). The low therapeutic index of antifungal drugs, short half-life of antivirals and the limited ability of antibiotics to penetrate infected cells in intracellular compartments make them ideal candidates for nanoparticle delivery. Thus, it has been suggested that nanoparticles should improve the therapeutic efficacy while decreasing the toxic side effects of these drugs. In theory, chitosan NP are very attractive carrier system for these drugs as they offer many advantages such as hydrophilic surface particles, nano-size of less than 100 nm. However, to the best of my knowledge, chitosan NP as a tool to deliver these drugs have not yet been examined. In addition, intramuscular and subcutaneous administrations are also possible delivery routes of nanoparticles because of the decrease possibility of irritation at the injection sites,(Little and Parkhouse, 1962) protection of the loaded-drug from enzyme degradation, and the possibility of prolonged drug plasma levels.

Peroral administration

The idea that nanoparticles might protect labile drugs from enzymatic degradation in the gastrointestinal tract (GIT) leads to the development of nanoparticles as oral delivery systems for macromolecules, proteins and polynucleotides. This approach was extensively studied after a report that blood glucose levels were reduced in diabetic rats following the oral administration of insulin nanoparticles (Damge *et al*., 1990). Limiting nano-sized particles to less than 500 nm in diameter seems to be a key factor in permitting their transport through the intestinal mucosa most probably through an endocytotic mechanism (Jani *et al*., 1990). However, besides the enzymes, mucus layer, which hamper diffusion of drug molecules and nanoparticles (Norris *et al*,. 1998), and the epithelial absorption barriers are main hurdles against gastrointestinal protein drug absorption. Therefore, drug bioavailability can be improved by controlling the particle size along with prolonging the residence time of drug carrier systems in GIT (Takeuchi *et al*., 2001). Among polymeric nanoparticles, chitosan NP showed to be attractive carriers for oral delivery vehicle as they promote absorption of drug.

The absorption promoting effect of chitosan has been extensively studied by several research groups and found to be due to a combination of mucoadhesion and transient opening of tight junctions in the mucosal cell membrane which have been verified both *in vitro* and *in vivo* (Artusson *et al*., 1994; Aspden *et al*., 1996). The mucoadhesive properties of chitosan are due to an interaction between positively charged chitosan and negatively charge of mucin which provide a prolonged contact time between the drug and the absorptive surface, and thereby promoting the absorption (Soane *et al*., 1999). Chitosan mucoadhesion is also supported by the evidence that chitosan increases significantly the half time of its clearance (Soane *et al*., 1999). Furthermore, *in vitro* studies in Caco-2 cells have shown that chitosan is able to induce a transient

opening of tight junctions thus increasing membrane permeability particularly to polar drugs, including peptides and proteins (Dodane *et al*., 1999; Luessen *et al*., 1996). Recent studies have shown that only protonated soluble chitosan, in its uncoiled configuration, can trigger the opening of the tight junctions, thereby, facilitating the paracellular transport of hydrophilic compounds (Artusson *et al*., 1994). This property implies that chitosan would be effective as an absorption enhancer only in a limited area of the intestinal lumen where the pH values are below or close to its pKa. Although chitosan was able to open up the tight junctions, the uptake of particle > 50 nm could not be explained by a widening of the intercellular spaces (Jung *et al*., 2000). Mechanism of chitosan NP transport across GIT is most probably through adsorptive endocytosis. Electrostatic interaction between positively charged chitosan and negatively charged sialic acid of mucin causes association of chitosan NP to the mucus layer and subsequently internalization via endocytosis (Behrens *et al*., 2002; Huang *et al*., 2002). Chitosan NP internalization was found to be higher in the jejunum and ileum than in duodenum (Behrens *et al*., 2002).

The ability of chitosan to enhance hydrophilic compounds transport across mucosal epithelial membrane depends on the chemical compositions and molecular weights of chitosan. A high degree of deacetylation (>65%) and/or high molecular weights appears to be necessary to increase epithelial permeability (Schipper *et al*., 1996). As the degree of deacetylation increases, the charge density increases, and thereby improving drug transportation. Similarly, it was shown that the molecular weight has some importance in that a molecular weight of at least 100 kDa was needed to obtain the optimal effect. Although, the difference in chemical composition and molecular weight of chitosan enhance the drug transport in a different way, they have very similar mechanism at the cellular level (Schipper *et al*., 1997).

Pan *et al.* reported that hypoglycemic effect was observed in induced diabetic rats after orally administration of chitosan nanoparticles (Pan *et al*., 2002). Furthermore, chitosan can be employed as a coating material for liposomes, micro/nanocapsules to enhance their residence time, thereby improving drug bioavailability (Takeuchi *et al*., 2001; Vila *et al*., 2002).

In addition to being used as an oral delivery carrier, chitosan NP could also be applied to other mucous membrane systems. Pulmonary and nasal routes are considered as promising routes to deliver peptides and proteins since they possess very large surface areas and manifest less intracellular and extracellular enzymatic degradation (Fernandez-Urrusuno *et al*., 1999). Thus, nasal drug delivery may not need protection against enzymatic degradation by formulating as nanoparticles as oral drug delivery. It may be administered as solution or powder with absorption enhancing agent to slow down mucociliary clearance process and thereby prolong the contact time between the formulation and nasal tissue. Recently, chitosan was demonstrated to promote the nasal absorption of insulin in rats and sheep. However, the insulin-chitosan powder, chitosan blended with insulin using pestle and mortar, showed to have bioavailability greater than chitosan NP containing insulin (Dyer *et al*., 2002).

Non-viral gene delivery vectors

Although viruses can efficiently transfer genes into cells, concerns such as host immune response, residual pathogenicity, and potential induction of neoplastic growth following insertional mutagenesis have led to the exploration of non-viral gene transfer systems (Chong and Vile, 1996; Otto *et al*., 1994). These latter delivery systems are generally considered to be safer since they are typically less immunogenic and lack mutational potential.

There are usually considered to be five primary barriers that must be overcome for successful gene delivery: *in vivo* stability, cell entry, endosome escape, intracellular trafficking and nuclear entry. Cationic polymers and lipids have both shown promise as gene delivery agents since their polycationic nature produces particles that reduce one or more of these barriers. For example, by collapsing DNA into particles of reduced negative or increased positive charge, binding to the cell surface and enhanced endocytosis may be promoted (Boussif *et al*., 1995; Haensler and Szoka, 1993; Labat-Moleur *et al*., 1996; Mislick and Baldeschwieler, 1996). In many cases, cationic polymers seem to produce more stable complexes thus offering more protection during cellular trafficking than cationic lipids (Hwang and Davis, 2001; Pollard *et al*., 1998).

Among cationic polymers, PEI is particularly promising as a vector given its relatively high level of transfection in a number of target organs by various delivery routes (Boussif *et al*., 1995; Lemkine and Demeneix, 2001). The high charge density of PEI is thought to be a key factor that contributes to its high transfection efficiency. Unfortunately, the polycationic nature of PEI also appears to be the main origin of its marked toxicity, a property it shares with many other polycations (e.g. polylysine). This toxicity has severely limited its use as a gene delivery vector *in vivo*. On the contrary, chitosan is a cationic polymer with extremely low toxicity. It showed significantly lower toxicity than poly-L-lysine and PEI (Erbacher *et al*., 1998; Roy *et al*., 1997). Additionally, it enhances the transport of drug across cell membrane as discussed earlier.

Chitosan as a promising gene delivery vector was first proposed by Mumper (Mumper *et al*., 1995). Chitosan mediates efficient *in vitro* gene transfer at nitrogen to phosphate (N/P) ratio of 3 and 5. At these ratios, small chitosan-DNA complexes can be prepared in the range of 50-100 nm with a positively surface charge of approximately +30 mV. Sato *et al.* found that *in vitro* chitosan-mediated transfection depends on the cell type, serum concentration, pH and molecular weight of chitosan (Ishii *et al*., 2001; Sato *et al*., 2001). Hela cells were efficiently transfected by this system even in the presence of 10% serum. In contrast, chitosan have not been able to transfect HepG2 human hepatoma cells and BNL CL2 murine hepatocytes (Artusson *et al*., 1994). The transfection efficiency was found to be higher at pH 6.9 than that at pH 7.6. This can be explained by that at below pH 7, amine groups of chitosan are protonated which facilitate the binding between complexes and negatively charged cell surface. Transfection efficiency meditated by chitosan of high molecular weight, >100 kDa, is less than that of low molecular weight, 15 and 52 kDa.

Although chitosan successfully transfected cells in vitro, the transfection efficiency showed to be lower than that of other cationic polymer vehicles such as polyethylenimine (MacLaughlin *et al*., 1998). This leads to developing of chitosan NP system to increase transfection efficiency. So far, two approaches have been developed. First, by increasing chitosan solubility as it is well known that only soluble protonated chitosan can cause transient opening of tight junctions. Therefore, trimethyl chitosan (TMC), quaterinzed chitosan, was proposed by Guang Liu *et al*. as a vehicle that promoting transmembrane transport of gene by possessing better solubility than chitosan. The results showed that this vector provided transfection efficiency greater than chitosan and proved to be nontoxic (Guang Liu and De Yao, 2002). Another approach is attachment of cell targeting ligands to the chitosan particles. Park *et al.* developed liver targeted delivery system by preparing galactosylated-chitosan-graft-dextran DNA complexes, as galactose is known as liver targeted delivery (Park *et al*., 2000). Similarly, Mao *et al*. prepared transferrin-chitosan-DNA nanoparticles as a targeted drug delivery (Mao *et al*., 2001). Transferrin can be taken up by receptor-mediated endocytosis mechanism as transferrin receptor found on many mammalian cells. Unfortunately, they showed transfection efficiency less than expected. However, when KNOB (C-terminal globular domain of fiber protein) conjugated to the chitosan, the transfection efficiency in Hela cells can be improved by 130 fold.

The mechanism of cationic polymer-mediated have been investigated but still remained unclear. Few researcher groups have made efforts to correlate the transfection efficiency with the physico-chemical properties of polymeric nanoparticles, but not succeeded. Others have found that the size, morphology and charge of many DNA gene delivery complexes do not generally predict *in vitro* or *in vivo* transfection efficiency (Hwang and Davis, 2001).

Delivery of vaccines

Nanoparticles often exhibit significant adjuvant effects in parenteral vaccine delivery since they may be readily taken up by antigent presenting cells (Kreuter, 1995). Moreover, oral and nasal delivery of nanoparticles are thought to have the potential to provide mucosal protective immune responses, one of the most desired goals of modern vaccinology. The submicron size of nanoparticles allows them to be taken up by M-cells, in mucosa associated lymphoid tissue (MALT) i.e. gut-associated, nasal-associated and bronchus-associated lymphoid tissue, (Illum *et al*., 2001; van der Lubben *et al*., 2001) initiating sites of vigorous immunological responses. Immunoglobulin A (IgA), a major immunoglobulin at mucosal surface, and the generation of B-cell expressing IgA occur primarily in MALT. The B-cell then leave the MALT and reach systemic circulation where they clonally expand and mature into IgA plasma cells. Therefore, providing not only protective IgA at the pathogen entered sites, but also systemic immunity.

There are two main administration routes for mucosal vaccine delivery, oral and nasal. The main targeted for oral delivery vaccine are Peyer's patches. By incorporating vaccine into nanoparticles systems, the vaccine is protected against enzymatic degradation on its way to the mucosal tissue and efficiently taken up by

M-cells. In contrast to oral administration, nasal administered vaccines have to be transported over a very small distance, remain only about 15 minutes in the nasal cavity, and are not exposed to low pH values and degradative enzymes. Thus, nasally delivery vaccines may not necessary formulated as nanoparticles as discussed earlier. It may be administered as solution or powder with absorption enhancing agent to slow down mucociliary clearance process and thereby prolong the contact time between the formulation and nasal tissue.

Among the polymers used to form vaccine nanoparticles, chitosan is one of the most recently explored and extensively studied as prospective vaccine carriers (Illum *et al*., 2001; van der Lubben *et al*., 2001). Its absorption promoting effect is believed to improve mucosal immune response. The mechanism of action of chitosan in improving transport of drug across mucosal membrane can be explained by the same theory as discuss earlier in peroral administration section. Illum *et al.* successfully developed chitosan vaccines containing influenza, pertussis and diphtheria antigens for nasal delivery. They demonstrated that these vaccines produced a significant antibody level in mice, both serum and secretory IgA (Illum *et al*., 2001). Despite the potential carrier for mucosal delivery vaccine, chitosan has also been reported to act as an adjuvant for systemic vaccine delivery such as increasing the accumulation and activation of macropharges and polymorphonuclear cells. Activation of macropharges is initiated after uptake of chitosan (Murata *et al*., 1999; Seferian and Martinez, 2000).

Furthermore, chitosan has also been widely explored as the application for DNA mucosal vaccines. For instance, a chitosan-based DNA flu vaccine has been developed by Illum *et al*.(2001). This system showed high antibody level in mice after intranasal administration. Plasmid pCMVArah2 encoding peanut allergen gene were reported successfully incorporated into chitosan NP with good antigen expression and good protection after oral administration in mice (Roy *et al*., 1999).

Ocular administration

Nanoparticles have been found to be potential carriers for ocular delivery following the observation that various types of nanoparticles tend to adhere to the ocular epithelial surface (Wood *et al*., 1985). The resulting prolonged residence time of nanoparticles leads to a much slower elimination rate compared to conventional ophthalmologic formulations, thereby improving drug bioavailability. As a consequence, nanoparticles have been developed for targeted ophthalmic delivery of anti-inflammatory, antiallergic and beta-blocker drugs (De Campos *et al*., 2001; Zimmer *et al*., 1995).

Among mucoadhesive polymers explored now, chitosan has attracted a great deal of attention as an ophthalmic drug delivery carrier because of its absorption promoting effect. Chitosan not only enhance cornea contact time through its mucoadhesion mediated by electrostatic interaction between its positively charged and mucin negatively charged, its ability to transient opening tight junction is believed to improve drug bioavailability. Felt *et al.* found that chitosan solutions prolonged the cornea resident time of antibiotic in rabbits (Felt *et al*.,

1999). The same effects were also observed employing chitosan NP as demonstrated by De Campos *et al.* that chitosan NP remained attached to the rabbits' cornea and conjunctiva for at least 24 hr (De Campos *et al*., 2001). Chitosan also shown to be a low toxic material, ophthalmic formulation based on chitosan exhibited an excellent tolerance after applied chitosan onto the rabbit's corneal surface (Felt *et al*., 1999). Beside employing chitosan NP to improve drug transport via ocular, chitosan-coated nanoparticles can also be utilized as it exhibited ability to enhance the corneal penetration (Calvo *et al*., 1997c). In addition, De Campos *et al.* found that after ocular administration of chitosan NP in rabbits, most of drug were found in extraocular tissue, cornea and conjunctiva, while negligible drug were found in intraocular tissues, iris/ciliary body and aqueous humor. Together, these results suggested that chitosan NP showed to be attractive material for ocular drug delivery vehicle with potential application at extraocular level.

Conclusions

Chitosan NP showed to be prospective drug delivery carriers as they offer many advantages. First, chitosan is considered as a safe material as it is natural polymer that possesses biocompatible and biodegradable properties. Second, it is water-soluble polymers which is an ideal property for drug delivery carriers, therefore, simple and mild preparation methods can be applied. This renders chitosan NP as promising drug delivery carriers that are suitable for a broad category of drugs including macromolecules and labile drugs. Third, chitosan is available in a wide range of molecular weights and is easily chemically modified by coupling with ligands providing flexibility in formulation development. Forth, chitosan provides absorption promoting effect that prolongs the contact time between substrate and cell membrane. In addition, their nano-sized facilitates the drug uptake through the cell membrane. Together, the absorption enhancing effect and nano-sized particles exhibited ability to improve drug bioavailability. Fifth, chitosan NP offer versatile routes of administration, especially non-invasive routes, i.e. peroral, nasal, and ocular mucosa, which are preferable routes administration. Furthermore, chitosan NP demonstrated to be good adjuvant for vaccine delivery.

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