Chitosan Nanoparticles as Drug Delivery Carriers for Biomedical Engineering

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Summary: Chitosan is a rather abundant material, which has been widely used in food industrial and bioengieering aspects, including in encapsulating active food ingredients, in enzyme immobilization, and as a carrier for drug delivery, due to its significant biological and chemical properties such as biodegradable, biocompatible,bioactive, and polycationic. This review discussed preparation and applications of chitosan nanoparticles in the biomedical engineering field, namely as a drug delivery carrier for biopharmaceuticals.

Introduction

Chitosan, the second-most abundant polysaccharide next to cellulose, is a natural, non-toxic and biodegradable polysaccharide, composed by β-(1-4)-linked N-glucosamine and N-acetyl-glucosamine residues [1, 2]. Chitosan is insoluble in water and organic solvents [3, 4], but soluble in dilute aqueous acidic solutions, due to the protonation of the glucosamine residues into the soluble form $R\text{-}NH_3^+$ [5]. The reactivity and polycationic character allow the production of a variety of formulations with different properties, ranging from hydrogels and fibres to nano/microparticles and membranes [6]. Altogether, these properties make of medical grade chitosan a versatile material with extensive application in the biomedical and biotechnological fields [7]. One of the most well-known biomedical applications is the development of drug delivery systems. In fact, papers related to drug delivery systems using chitosan are available by the hundreds [8-20]. Different types of chitosan-made drug carriers have been conceived for various administration routes, such as oral, nasal, transdermal, parenteral, vaginal, cervical, intrauterine and rectal. In this short review, preparation of chitosan nanoparticles and their application in biochemical engineering field were mainly discussed.

Preparation of Chitosan Nanoparticles

Chitosan nanoparticles are widely studied drug delivery systems. The use of chitosan nanoparticles offers many advantages, providing targeted delivery of drugs, improving the bioavailability and stability of the therapeutic agents against chemical/enzymatic degradation [21]. Different methods can be used to prepare chitosan nanoparticles. Main methods to prepare chitosan nanoparticles are as follows.

Emulsion Crosslinking

In this process, chitosan solution is emulsified in oil (w/o emulsion). The aqueous droplets are stabilized using a suitable surfactant. The emulsion is then reacted with an appropriate crosslinking agent such as glutaraldehyde, to stabilize the polysaccharide droplets. The nanoparticles are then washed and dried [22-24]. *Ohya et al.* [25] reported for the first time the preparation of chitosan nanoparticles containing 5-fluorouracil using w/o emulsion method followed by glutaraldehyde crosslinking. These pioneering studies demonstrated the feasibility of preparing chitosan nanoparticles that could bind and delivery drugs. Major drawbacks of this method are associated with the use of organic solvents and crosslinking agents, that may adversely affect the stability of proteins [26]. Moreover, glutaraldehyde crosslinked nanoparticles present negative effects on cell viability.

Emulsion-Droplet Coalescence

This method involves both emulsion crosslinking and precipitation [27]. A stable emulsion

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containing the aqueous chitosan solution along with the drug in oil is produced; a second emulsion, containing a NaOH solution, is produced in a similar way. When both emulsions are mixed under high-speed stir, droplets of each emulsion collide at random, coalesce, and finally precipitate as small size particles. Nanoparticles are obtained within the emulsion-droplets. Gadolinium-bound chitosan nanoparticles were prepared using this method [28].

Coacervation/Precipitation

Spraying the chitosan solution into sodium hydroxide, NaOH-methanol or ethanediamine alkaline solutions, using compressed air, originates coacervated droplets, forming the nanoparticles [29]. Separation and purification of the particles is finally achieved by centrifugation, followed by successive washing steps with hot and cold water. Berthold *et al.* [30] described a different approach: the formation of the particles is obtained after addition of sodium sulfate solution to chitosan solution, under mild agitation and continuous sonication for 30 min.

Ionotropic Gelation

This method is based on the electrostatic interactions between the chitosan amine group and a polyanion such as tripolyphosphate (TPP) [31-33]. The complexation between oppositely charged macromolecules as a way to prepare chitosan nanoparticles has attracted much attention, because the process is very simple and mild. In our group, chitosan nanoparticles, obtained by this method, were studied for enzyme immobilization. For ionic gelatation method, solution pH, TPP concentration, and chitosan concentration significantly affected the size of chitosan nanoparticles. The minimum particles size was about 42 ± 5 nm under the optimized conditions [34]. The negatively charged DNA can also form polyelectrolyte complexes with cationically charged chitosan, through ionic gelation, as demonstrated by Mansouri *et al.* [35].

Reverse Micelles

In this technique, the surfactant was dissolved in organic solvent to prepare reverse micelles. The aqueous phase containing the chitosan and drugs are added to this emulsion with constant vortexing and the nanoparticles forms in the core of the reverse micelles. An additional amount of water may be added to produce larger sized nanoparticles. The maximum amount of drug that can be dissolved in reverse micelles has to be determined for each case, by gradually increasing the amount of drug, until the clear microemulsion is transformed into a translucent solution [36].

Template Polymerization

In this technique, chitosan is firstly dissolved in an acrylic monomer solution under magnetic stirring. Due to the electrostatic interaction, the negatively charged acrylic monomers align along the chitosan molecules. After complete dissolution of chitosan, the polymerization is started by adding the initiator ($K_2S_2O_8$) under stirring at 70ºC. The complete polymerization leads to the appearance of an opalescent solution, indicating the nanoparticles formation. The nanoparticles solution are then filtered and dialysed to remove the residual monomers and initiator. The obtained nanoparticles are positively charged and present a size in the range of 50 to 400 nm [37, 38].

Molecular Self-Assembly

Molecular self-assembly is characterized by diffusion followed by specific association of molecules through non-covalent interactions, including electrostatic and/or hydrophobic associations. There are several reports on chitosan hydrophobical modification and nanoparticle formation through self-assembly in aquesous solution. Lee *et al*. [39] modified chitosan hydrophobically by making it contain 5.1 deoxycholic acid groups per 100 anhydroglucoses with 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide(EDC)-mediated coupling reaction. In another study [40, 41], chitosan has been coupled with linolenic acid through the EDC-mediated reaction. The average particle size of self-aggregates of hydrophobically modified chitosan in PBS solution (pH 7.4) was 210.8 nm with a unimodal size distribution ranging from 100 to 500 nm. A transmission electron microscopy study showed that the formation of the nearly spherical nanoparticles has enough structural integrity.

Application of Chitosan Nanoparticles as Delivery Carriers

Chitosan Nanoparticles as Carriers for Proteins

Nanometer-sized particles as protiens delivery carriers have attracted growing interest. By trapping proteins in a hydrated polymer-network, these nanoparticles minimize denaturation, simultaneously allowing a slow, continuous and

controlled release of the protein, maintaining an effective concentration for the necessary period of time [42-45]. Among nanosized systems, chitosan nanoparticles have been tested as carriers for proteins, peptides and oligosaccharides.

Oral Delivery

The oral route is considered the most convenient and comfortable means of drug administration, because of its non-invasive nature. It reduces the risk of infection, and do not require trained personnel. However, the bioavailability of orally administered proteins is usually poor, because of the hostile gastric and intestinal environments, and also the limited gastrointestinal mucosal permeability. It has been reported that chitosan nanoparticles could delivery the drug into the specific target, thereby decreasing drug doses [46]. Lin *et al*. [47] reported the preparation of nanoparticles composed of chitosan and poly-γ-glutamic acid (γ-PGA) for insulin delivery. The stability and functionality of nanoparticles *in vitro*, using Caco-2 cell monolayers, and *in vivo*, in a rat model were studied. The authors observed that the chitosan nanoparticles could transiently and reversibly open the tight junctions between Caco-2 cells, thus enhancing the paracellular permeability. Sonaje *et al.* [48] prepared self-assembled nanoparticls, by mixing γ-PGA with chitosan in the presence of $MgSO_4$ and TPP. The efficacy of nanoparticles for oral delivery and intestinal absorption of insulin was investigated in a diabetic rat model. The *in vitro* results showed that the mucoadhesive properties of nanoparticles are affected by the pH and additionally, the transport of insulin across Caco-2 cell monolayers is pH-dependent. In addition, oral administration of insulin-loaded nanoparticles demonstrated a significant hypoglycemic action for at least 10 hours, in diabetic rats.

Nasal Delivery

The nasal mucosa is an attractive route for the delivery of vaccines because it has a relatively large absorptive surface and low proteolytic activity [49-54]. Importantly, nasally administered vaccines can induce both local and systemic immune responses. However, most proteins are not well absorbed from the nasal cavity when administered as simple solutions. The major factors limiting the absorption of nasally administered proteins are the poor ability to cross the nasal epithelia, and the mucociliary clearance, which rapidly removes protein solutions from the absorption site [49, 50, 55]. Mucoadhesive, hydrophilic chitosan nanoparticles have received much attention to overcome these obstacles and deliver protein antigens via the nasal route, because they strongly attach the mucosa increasing mucin viscosity.

Amidi and colleagues [56] prepared and characterized protein loaded Trimethyl chitosan nanoparticles as a nasal delivery system. It was observed that trimethyl chitosan nanoparticles have a high loading efficiency and capacity up to 50%. The release studies showed that more than 70% of the protein remained associated with the nanoparticles for at least 3h of incubation in PBS (pH 7.4), at 37ºC. *In vivo* uptake studies indicated the transport of the protein across the nasal mucosa. Zhang *et al.* [57] used polyethylene glycol-grafted chitosan nanoparticles to improve the systemic absorption of insulin, following nasal administration. *In vitro* release studies showed an initial burst, followed by a slow release of insulin. The nasal delivery of insulin using chitosan-acetyl-L-cysteine nanoparticles was proposed by Wang *et al.* [58]. These authors observed that intranasal administration of modified chitosan-based nanoparticles in rats enhanced the absorption of insulin by the nasal mucosa, as compared with unmodified chitosan nanoparticles and control free insulin solution.

Colon Delivery

Colon targeted drug delivery is useful in improving the absorption of peptide drugs via the GI tract. Site specific drug delivery to the colon is of special interest for drugs instable in the upper part of the GI tract, because of the peptidase activity in the small intestine. The colon is thought to have lower enzymatic activity than other regions of the GI, hence a greater absorption efficiency in this region would be expected, as long as the proteins/peptides are released locally [59]. Due to negligible activity of brush-border membrane and much less activity of peptidases and pancreatic enzymes, the colon has been considered suitable for the delivery of peptides and proteins.

Bayat *et al.* [60] developed a nanoparticulate system using two new quarternized derivatives of chitosan, triethylchitosan (TEC) and dimethylethylchitosan (DMEC) nanoparticles, for insulin colon delivery. The three kinds of nanoparticles showed a positive charge that could facilitate insulin uptake, allowing a low bursting

effect and a steady release of insulin *in vitro*. DMEC nanoparticles and TEC nanoparticles had smaller particle size, higher insulin loading capacity and improved transport and absorption of insulin, as compared with chitosan nanoparticles. The blood glucose lowering effect of TEC nanoparticles and DMEC nanoparticles, after injection into ascending colon, was superior to that obtained with free insulin or chitosan nanoparticles. This study indicated that nanoparticles prepared from quaternized derivatives of chitosan might be a promising vehicle of administration of proteins and peptides via colon absorption.

Chitosan Nanoparticles as Carriers for DNA and SiRNA

Gene therapy is an emerging field in medical and pharmaceutical sciences, a very promising one due its potential for the treatment of a wide range of diseases. However, naked therapeutic genes are rapidly degraded by nucleases, showing poor and non-specific cellular uptake and also low transfection efficiency [61]. Hence, the development of safe and efficient gene carriers is primordial for the success of gene therapy. Gene delivery systems include viral vectors, cationic liposomes and polycationic complexes. In spite of high transfection efficiency, the immune and oncogenic responses associated to viral vectors limit their therapeutic applications *in vivo*. To overcome these limitations, non-viral delivery systems (cationic liposomes and cationic polymers) have been proposed as a safe alternative. Non-viral systems can form stable complexes with DNA, keeping it safe from nuclease degradation, and readily interact with cells membrane. Among non-viral vectors, chitosan and its derivatives are good delivery systems for DNA, antisense oligodeoxynucleotides and siRNA. Chitosan gathers a number of desirable characteristics: cationic charge, biodegradability, biocompatibility, low toxicity, mucoadhesivness and functional groups with targeting ability. On the other hand, its transfection efficiency is relatively low, compromising potential clinical application. Several studies have been carried out to elucidate the influence of the CS-based formulation parameters on the gene expression [61].

Duceppe *et al.* [62] used nanoparticles made of ultra low molecular weight chitosan/Hyaluronic acid (HA), as a novel potential carrier for gene delivery. Addition of HA to the nanoparticles formulation improved transfection rate from 0.7 to 25%. Peng and colleagues [63] demonstrated that mixtures of chitosan, DNA and poly(gamma-glutamic acid) (g-PGA) in aqueous media lead to the formation of ‘‘compounded nanoparticles’’, containing domains of chitosan/DNA and chitosan/g-PGA. Lee *et al.* [64] synthesized chitosan nanoparticles, by coacervation, to encapsulate siRNA in the presence of polyguluronate (PG), a block of guluronic acid residues present in the alginate backbone. Chitosan/(PG+siRNA) nanoparticles were the most efficient formulation to deliver siRNA into HEK 293FT and HeLa cells, as compared with nanoparticles without PG or with alginate replacing PG.

Chitosan Nanoparticles as Carriers for Low Molecular Weight Drugs

Among other drug delivery strategies, a great deal of attention has been directed to chitosan nanoparticles as promising systems able to improve drug bioavailability, modify pharmacokinetics and/or protect the encapsulated drug [65]. In fact, chitosan nanoparticles improve transmucosal permeability, enhance transport through the paracellular pathway, due to the good bio- and mucoadhesive properties, and induce structural reorganization of tight junction [66]. chitosan derivatives have been designed to improve the properties of native chitosan. Chemical modifications of chtiosan originate amphiphilicity, an important characteristic for the formation of self-assembled NPs, potentially suited for drug delivery applications. The hydrophobic cores of the nanoparticles may act as reservoirs for a variety of bioactive substances [67]. Chitosan-based nanoparticles as delivery systems of low molecular weight drugs have attracted attention for cancer and organ-specific therapy. Hu *et al.* [68] verified that the PEGylation of stearic acid-grafted chitosan oligosaccharide (CSO-SA) micelles reduce significantly the cellular uptake by macrophages, not affecting the internalization by normal and tumor liver cells. Park *et al.* [69] synthesized self-assembled nanoparticles made of N-acetyl histidine-conjugated glycol chitosan, a promising system for intracytoplasmic delivery of paclitaxel. You *et al.* [70] developed the strategy for paclitaxel delivery. Micelles made of stearic acid and chitosan hold multiple hydrophobic “minor cores” near the surface, which improved the micelles internalization into cancer cells and accumulation of the drug in the cytoplasm. Jain and Jain [71] explored the utilization of HA grafted chitosan nanoparticles for the effective delivery of 5-FU to colon tumors. HA-chitosan-nanoparticles showed significantly

higher uptake by cancer cells, about 7.9 fold as compared to uncoupled nanoparticles. More recently, Motwani *et al.* [72] used mucoadhesive chitosan-sodium alginate (ALG) nanoparticles as a new vehicle for the prolonged topical ophthalmic delivery of antibiotic gatifloxacin.

Conclusions

This review summarized preparation of chitosan nanoparticles and their application in biochemical engineering field. From this review, it is concluded that chitosan nanoparticles have been used as drug delivery carriers. They have the potential for the development of novel encapsulation or delivery carriers. However, most of these studies are still at the laboratory level. Additional studies are necessary before the industrial application of it. We hope more chitosan nanoparicles carrier can be developed and applied in biochemical engineering field in the near future.

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