Chitosan and its derivatives as vehicles for drug delivery

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ABSTRACT
Chitosan and its derivatives as vehicles for drug delivery can achieve the purpose of sustained release and controlled release for drugs, improve the stability of drugs, and reduce adverse drug reactions. So, the bioavailability of drugs can be enhanced. Therefore, chitosan and its derivatives have become a hotspot in the field of drug delivery. Their characteristics as drug delivery vectors were introduced, the types and applications were summarized. The development direction of chitosan and its derivatives in this field was also forecasted.

1. Introduction
Chitosan is a kind of natural polysaccharide cellulose, widely found in insect, crustacean shell and fungal cell wall, also known as soluble chitin, which is nontoxic, biocompatible, biodegradable, etc. (Riva et al., 2011). Its preparation is simple, source is rich, and the hydrophilicity is strong. Chitosan can be biodegradable by in vivo lysozyme, pepsin, and other enzymes. The degradation products are nontoxic and can be completely absorbed by the organisms. Moreover, chitosan has the ability to resist acid, anticoagulant, and ulcer. It can prevent or weaken the stinging reaction caused by drugs in the stomach. In addition, the chitosan matrix can form floating and gradually expand in acidic environment. These characteristics make chitosan an ideal drug delivery material (Sonia & Sharma, 2011).

The vehicle of nano-sized polymer particle for drug delivery and controlled release, because of its ultra-small size, reasonable in vivo distribution and efficient drug utilization, can pass the tissue epithelium. So, the targeted delivery and controlled release of drugs are more effective. Therefore, the nano-sized polymer particle becomes the ideal vehicle of embedded peptide, protein, nucleic acid, vaccine, and other bioactive macromolecules (Lee et al., 2010).

Chitosan has good film-forming property; the film has good biological compatibility and permeability. It has important development and research value in sustained-release drugs and targeted drug delivery. So, chitosan is often used as vehicle for the sustained release of drugs. Many drugs are administered through mouth, nose, and mucous membrane. The drugs are released slowly over a long period of time, so as to achieve the goal of sustained administration (Mengatto et al., 2012).

Chitosan has a unique polycationic property that can interact with sodium alginate (polyanion) by electrostatic interaction. The sodium alginate microcapsule surface is attached with a layer of polyelectrolyte membrane. Thereby, it can improve the stability of microcapsules and drug loading, and adjust the drug release rate. The higher the chitosan content is in the microcapsules, the stronger the sustained release effect of sodium alginate is (Lam et al., 2012). The sustained release effect in the buffer with pH 4 ~ 5 is significantly greater than that in the buffer with pH 1 ~ 2. The results of study can be used for the dosage form design of drugs with high gastric irritation.

Chitosan is a hydrophilic polymer, which can be used to prepare microspheres with different sizes. The combination of microspheres and drugs can avoid the use of organic solvents, and prevent the denaturation of antigenic proteins. Chitosan microspheres have porous structure, which makes antigen proteins not only adsorbed on the surface of microspheres, but also can be embedded into the inner surface of chitosan microspheres. Chitosan microspheres can also control the release of protein drugs and enhance the absorption of antigen protein (Saravana & Ramaswamy, 2011). In view of the stability of proteins, most researchers prepared chitosan blank microspheres, and then passively adsorbed antigen to microspheres.

Chitosan is a nontoxic and biocompatible polycation with low immunity. It is a good choice for gene delivery systems, because chitosan has cations that can bind effectively to DNA with anions and protect it from nuclease degradation. Chitosan particles loaded with DNA are stable in storage, so it as a vehicle of gene drugs is of great significance (Saranya et al., 2011).
Hydrogels have a 3-D network structure of cross-linked hydrophilic polymer, and have attracted much attention because of their potential uses in a variety of therapeutic and biomedical applications (Bhattarai et al., 2010).

Herein, chitosan and its derivatives as vehicles, such as nanoparticles, microcapsules, microspheres, hydrogels, and so on, for delivery of chemical drugs and genes or proteins were summarized and discussed.

2. Chitosans and its derivatives as vehicles to deliver chemical drugs

Chitosan nanoparticle as a novel drug vehicle has nontoxic-ity, good biocompatibility, biodegradability, improving drug stability, changing the route of administration, increasing drug absorption, improving the bioavailability of drugs, and other characteristics. So, it can achieve the role of controlled release and targeted therapy for drugs in vivo (Alishahi et al., 2011). According to the structural characteristics of chitosan and the nature of drug itself, drug-loaded chitosan nanoparticles can be prepared by a variety of methods. Chitosan has become the focus of research on targeted and slow/control-rolled release, and has broad application prospects. In addition, the surface modification of chitosan nanoparticles, which has the selectivity required by target organs, target tissues and target cells (Bhargava et al., 2015), can be carried out by using modern molecular design ideas and advanced synthesis techniques. It is also the development trend of future research.

Khodadust et al. (2014) produced different sized chitosan MNPs by in situ synthesis method. It showed that the chito-san MNPs had pH responsive release characteristics. Doxorubicin (DOX)-loaded chitosan MNPs were efficiently taken up by MCF-7 (MCF-7/S), and DOX was resistant to MCF-7 (MCF-7/1 μM) breast cancer cells, which increased the efficacy of drug and also maintained overcoming the resist-ance of DOX in MCF-7/DOX cells. So, chitosan MNPs, which were synthesized at various sizes, could be effectively used for the pH dependent release of DOX in cancer cells. Therefore, it can provide new insights in the development of pH responsive targeted DDSs to overcome the side effects of conventional chemotherapy.

Tamoxifen (Tam) has a broad spectrum of anticancer activ-ity, but it is limited in clinical application. Vivek et al. (2013) investigated the smart pH-responsive drug delivery system (DDS), which was based on chitosan nanoparticles for their potential in enabling more intelligent controlled release and enhancing chemotherapeutic efficiency of Tam. Tam was loaded onto chitosan-nanoparticles by forming complexes and it was released from the DDS much more rapidly at pH 4.0 and 6.0 than at pH 7.4, which is a desirable characteristic for tumor-targeted drug delivery. It was demonstrated that Tam-loaded chitosan nanoparticles increased intracellular concentration of Tam in human breast cancer MCF-7 cells and enhanced their anticancer efficiency by inducing apoptosis in a caspase-dependent manner, which indicated that drug-loaded nanoparticles could act as an efficient DDS importing Tam into target cancer cells.

Termsarasab et al. (2014) successfully synthesized chitosan oligosaccharide-arachidic acid (CSOAA) conjugate, which was used to develop self-assembled nanoparticles for DOX delivery. It indicated that DOX-loaded CSOAA-based nanoparticles exhibited the sustained and pH-dependent drug release profiles according to the result of in vitro release test. The CSOAA showed negligible cytotoxicity in FaDu (human head and neck cancer) cells. Cellular uptake of DOX in FaDu cells was higher in the nanoparticle-treated group compared to the free DOX group. The antitumor efficacy of DOX-loaded nanoparticles was also verified in FaDu tumor xenografted mouse model. These results suggested that synthesized amphiphilic CSOAA might be used to prepare the self-assembled nanoparticles for anti-cancer drug delivery.

Acyclovir (ACV) is one of the drugs of choice to treat epidermal, ocular, or systemic herpetic infections. However, its trans-mucosal limited absorption and the scarce contact time of the formulation with the mucosal surface (especially in the ocular mucosa) constitute a big limitation of the antiviral effi-ciency. The most effective way to solve the above problems is to increase the quantity and the residence time of the drug over the ocular surface. The chitosan nano- and micro-particles (MPs) could be used for topical drug delivery (Sezer & Cevher, 2012). In order to meet all these requirements, Calderon et al. (Calderon et al., 2013) developed MPs and nanoparticles containing ACV using cross-linked chitosan with tripolyphosphate owing to the biocompatibility, bio-adhesion ability and the potential power as penetration enhancer of this polymer. It indicated that the amounts of ACV effectively diffused in 24 h were 30, 430, and 80 μg for the ACV solution, MP and nanoparticle, respectively. It also showed that chitosan-based particles induced moderate irri-tation and mild tissue damage from slug mucosal irritation (SMI) results, what supposes that ACV-MP constitutes a promising alternative for further development of an antiviral formulation.

Shen et al. (2012) used the novel carboxymethyl chitosan-based folate/Fe3O4/CdTe nanoparticle to achieve targeted drug delivery and cell imaging. Grenha et al. (2010) develop the new chitosan/carrageenan nanoparticles to deliver drugs. Feng et al. (2013) utilized the chitosan/O-carboxymethyl chito-san nanoparticles to efficiently and safely deliver the oral anticancer drug. Zhou et al. (2013) prepared the galactosylated chitosan-polyacrylactone nanoparticles for hepatocyte-targeted curcumin delivery. Anitha et al. (2012) used the curcumin-loaded N,O-carboxymethyl chitosan nanoparticles to deliver cancer drug. Millotti et al. (2011) used chitosan-6-mercaptonicotinic acid nanoparticles to deliver oral peptide. Xing (2013) prepared the folate modified-chitosan nanoparticles for new tumor-targeting drug delivery.

Pengpong et al. (2014) designed and prepared a hydrophobic mucadhesive thiolated chitosan for hydrophobic drug delivery by conjugating p-coumaric acid (pCA) to increase hydrophobic compatibility with drug via π-π interaction and then covalently linking homocysteine thiolactone (HT) to the pCA-chitosan to increase the muoadhesive properties. A model hydrophobic drug, piperine (PIP), was encapsulated in pCA-HT-chitosan MPs via electrospray ionization with an encapsulation efficiency of over 80%. It showed that
a sustained release of PIP was >75% over 12 h between pH 1.2 and 6.4 from in vitro release experiments.

Chitosan microspheres show some unique functional advantages. For example, they have rich polysaccharide chains, which can be identified by specific cells or tissues. Chitosan microspheres can target the delivery of drugs to the lesion site for storage and release (Mei et al., 2009). Their surface can be grafted with functional groups, which can be flexibly loaded with different drugs by adsorption or wrapping (Varshosaz, 2007). Based on the above advantages, chitosan microspheres have become a hotspot in recent years.

Abdel Mouez et al. (2014) produced the microspheres using a spray-drying and precipitation techniques. It was demonstrated that the microspheres were spherical with size 21-53 μm suitable for nasal deposition. It indicated that the nasal microspheres exhibited a significantly higher bioavailability (58.6%) than nasal solution of verapamil hydrochloride (VRP) (47.8%) and oral VRP solution (13%) from the bioavailability study. Overall, the chitosan-based nasal VRP microspheres are promising to enhance VRP bioavailability by pass metabolism of the drug substance.

Chitosan microspheres have been extensively used as an ideal vehicle for ocular drug delivery. Umadevi et al. (2010) prepared the folate-decorated chitosan microspheres for sustained release of fluconazole (FLU). It showed that there was the greatest ex vivo drug permeation for the chitosan solutions alone. However, the poloxamer/chitosan formulation presented similar in vitro performance than the chitosan solution at 1.0%; both formulations showed sustained release and about 3.5-fold greater total amount of FLU permeated when compared with simple aqueous solutions of the drug. In a word, it was demonstrated that both the in situ gelling formulation evaluated and the chitosan solution are viable alternatives to enhance ocular bioavailability in the treatment of fungal keratitis.

Wang et al. (2013) developed a near infrared (NIR) triggered drug delivery platform, which was based on the chitosan-modified chemically reduced graphene oxide (CRGO) incorporated into a thermosensitive nanogel (CGN). CGN exhibited an NIR-induced thermal effect, which was similar to that of CRGO, it had reversible thermo-responsive characteristics at 37-42 °C and high DOX hydrochloride (DOX) loading capacity (48 wt%). The DOX-loaded CGN (DOX-CGN) released DOX faster at 42 °C than at 37 °C. It revealed that DOX expression was in the cytoplasm of cancer cells when incubated with DOX-CGN at 37 °C but in the nucleus at 42 °C by fluorescence images. Upon irradiation with NIR light (808 nm), a rapid and repetitive DOX release from the DOX-CGN was observed. Moreover, the cancer cells incubated with DOX-CGN and irradiated with NIR light displayed significantly greater cytotoxicity than without irradiation because of NIR-triggered increase in temperature leading to nuclear DOX release. Therefore, these results demonstrate CGN’s promising application for on-demand drug release by NIR light.

Chitosan as a natural polymer in the application of hydrogels, has attracted great attention in recent years. Because it has good biocompatibility, low toxicity, and can be degraded by enzymes in the human body, coupled with its unique physical characteristics (such as hydrophilicity, functional amino acid group and cationic net charge), this makes chitosan a perfect tool for intelligent drug delivery (Rani et al., 2010).

Chitosan-based thermosensitive solutions, which turn into semi-solid hydrogels upon injection at body temperature, have increasingly drawn attention over the last decades as an attractive new type of in situ forming depot (ISFD) DDS. Supper et al. (2014) investigated glucose-1-phosphate (G1-P) as an alternative gelling agent for improving the stability of chitosan-based ISFD solutions. It showed that a sustained release was over days to weeks for hydrophilic model compounds from in vitro release experiments, which demonstrated thereby that chitosan/G1-P might be suitable for the prolonged delivery of drugs. The inflammatory reaction observed in the tissue surrounding the hydrogel in rats was a typical foreign body reaction. It confirms the potential of chitosan/G1-P solutions as an injectable ready-to-use in situ forming hydrogel from these features.

Lee et al. (2011) prepared decanoic acid-modified glycol chitosan (DA-GCS) hydrogels containing tightly adsorbed palmitic acid-modified exendin-4 (Ex4-C16). GCS was conjugated with N-hydroxysuccinimide-activated DA in anhydrous 0.4% dimethylaminopyridine/dimethylsulfoxide at different feed ratios. DA-GCS hydrogels were formed by
physical self-assembly during dialysis vs. deionized water. The hypoglycemia caused by Ex4-C16-loaded DA-GCS hydrogels was evaluated by subcutaneous administration in type 2 diabetic db/db mice. The in vitro and in vivo release of Ex4-C16 from DA-GCS hydrogels was dramatically delayed compared with native Ex4 probably due to strong hydrophobic interactions. In particular, Ex4-C16 in DA-GCS hydrogels was found to be present around the injection site up to 10 days after subcutaneous administration, whereas Ex4 in DA-GCS hydrogels was cleared from injection sites in ~2 days in Institute of Cancer Research (ICR) mice. Finally, the hypoglycemia induced by Ex4-C16 DA-GCS hydrogels was maintained for >7 days. It demonstrates that Ex4-C16 DA-GCS hydrogels offer a potential delivery system for the long-term treatment of type 2 diabetes.

Cheng et al. (2014) developed an injectable thermosensitive chitosan/gelatin/glycerol phosphate (CS/G/GP) hydrogel as a sustained-release system of latanoprost for glaucoma treatment. The latanoprost-loaded CS/G/GP hydrogel can gel within 1 min at 37 °C, and it showed a good in vitro and in vivo biocompatibility. The results prove a sustained release of latanoprost from CS/G/GP hydrogel. A rabbit model of glaucoma was established by intravitreal injection of triamcinolone acetonide. After a single subconjunctival injection of latanoprost-loaded CS/G/GP hydrogel, intraocular pressure (IOP) was significantly decreased within 8 days and then remained at a normal level. It suggests that latanoprost-loaded CS/G/GP hydrogel might have a potential application in glaucoma therapy.

Yadollahi et al. (2015) synthesized antibacterial chitosan/silver bio-nanocomposite hydrogel beads for drug delivery. Kono & Teshirogi (2015) used the cyclodextrin-grafted chitosan hydrogels for controlled drug delivery. Yang et al. (2013) developed the pH-sensitive interpenetrating network hydrogels based on chitosan derivatives and alginate for oral drug delivery.

3. Chitosan and its derivatives as vehicles to deliver gene or protein

Chitosan is a good choice for gene delivery systems because it has the cation that can be effectively bound to an anion-containing DNA and protect DNA from nuclease degradation. Moreover, chitosan particles loaded with DNA are more stable at the time of storage, so it is of great significance to use chitosan as a vector for gene drug (Ducape & Tabrizian, 2010). Chitosan as a protein vehicle can protect protein drugs from enzyme degradation and can control the release of drugs to achieve the purpose of sustained or controlled release, which can help extend the biological activity of protein drugs in vivo (Lü et al., 2010).

The low solubility of chitosan has limited its gene transfection efficiency. The water solubility of chitosan can be improved by appropriate derivatization (Siafaka et al., 2015). Toh et al. (2011) reported the synthesis of different substitution degrees of succinated chitosans (CS-succ) to increase water solubility. It indicated that the plasmid DNA was readily entrapped at a CS-succ/DNA weight ratio of 20; the sizes and zeta potentials were between 110–140 nm and ±1–5 mV, and the polyplexes exhibited low cytotoxicity against HEK 293 T cells. CS-succ with 5 and 10% degrees of substitution showed improved transfection efficiency as compared with nascent chitosan.

Rajesh et al. (2012) synthesized and evaluated the LCS-g-PEI (lauryl CS graft polyethyleneimine) polymer as a potential vehicle of therapeutic molecules, such as the p53 gene and DOX. This polymer had lower interactions with blood components than the unmodified PEI. LCS-g-PEI buffered protons, which were protected DNA from nuclease attack, induced effective gene transfection efficiency in the C6 cell line. LCS-g-PEI, which had incorporated DOX, exhibited an enhanced release of this compound at pH 5. It demonstrated that LCS-g-PEI had efficacy in the enhancement of drug uptake and the promotion of gene expression in the C6 cell line. So, LCS-g-PEI shows great promise as a drug/gene vehicle with potential applications in cancer therapy.

Chitosan-disulfide-conjugated low molecular weight polyethyleneimine (LMW-PEI) (CS-ss-PEI) was prepared by conjugating LMW-PEI to chitosan through oxidation of thiols introduced for the formation of disulfide linkage (Zhao et al., 2013). With the increment in the LMW-PEI component, the copolymer showed increased DNA binding ability and formed denser nanocomplexes. CS-ss-PEI exhibited low cytotoxicity in COS-1, HepG2 and 293 T cells over the different weight ratios. The transfection efficiency of CS-ss-PEI4 was significantly higher than that of PEI25k and comparable with Lipofectamine in mediating luciferase expression. Its application for bone morphogenetic protein (BMP2) gene delivery was confirmed in C2C12 cells by BMP2 expression. Its application for bone morphogenetic protein (BMP2) gene delivery was confirmed in C2C12 cells by BMP2 expression. For inducing in vitro osteogenic differentiation, CS-ss-PEI4 mediated BMP2 gene delivery showed a stronger effect in MG-63 osteoblast cells and stem cells in terms of alkaline phosphatase activity and mineralization compared with PEI25k and Lipofectamine. Overall, it provided a potential gene delivery system for orthopedic-related disease.

The successful clinical translation of small interference RNA (siRNA)-based therapeutics requires efficient vehicle systems that can specifically deliver siRNA within the cytosol of the target cells. Although numerous polymeric nanocarriers forming ionic complexes with siRNA have been investigated for cancer therapy, their poor stability and lack of tumor targetability have hindered their in vivo applications (Forbes & Peppas, 2014). For chitosan, derivatization can improve its stability and targeting ability, for example, Hu et al. (2016) used the chitosan-based glycolipid-like nanocarrier for selective redox-responsive siRNA delivery.

Wassmer et al. (2013) investigated the release kinetics of chitosan MPs (CSMPs) in vitro, and assesses their biocompatibility and cytotoxicity on retinal cells in vitro and in vivo. The in vitro release kinetics were dependent on the protein encapsulated, with bovine serum albumin (BSA) showing higher release than tet-EGFP (enhanced green fluorescent protein fused to the transactivator of transcription peptide). CSMPs, which contained the encapsulated tet-EGFP, were tested for cellular toxicity in photoreceptor-derived 661W cells. They showed no signs of in vitro cell toxicity at a low concentration (up to 1 mg/mL), but they were associated
with cytotoxic effects at a higher concentration (10 mg/mL). In vivo, CSMPs, which were injected into the subretinal space, were found beneath the photoreceptor layer of the retina, and persisted for at least 8 weeks. Similar to the in vitro studies, the lower concentration of CSMPs was generally well tolerated, but the higher concentration resulted in cytotoxic effects and in reduced retinal function. In a word, it was suggested that CSMPs were effective long-term delivery agents to the retina, but the concentration of chitosan might affect cytotoxicity.

Liu et al. (2010) used the calcium-carboxymethyl chitosan hydrogel beads to deliver protein. Katas et al. (2013) developed the chitosan nanoparticles for protein/siRNA delivery. Ayensu et al. (2012) prepared the lyophilized chitosan wafers for protein drug delivery via the buccal mucosa.

4. Conclusion

Chitosan, as a biodegradable polymer with excellent properties, is a promising new excipient for pharmaceutical preparations. With the deepening of research on chitosan and its derivatives, the organic combination of chitosan bioactivities and drugs will promote the development of drug preparations, and be widely used in the new dosage forms of drugs. The development of chitosan vehicles that can control the release of drug will become one of the future research directions.

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