Preparation of natural polysaccharide nanosphere carrier and its application in antitumor

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Abstract. With the rapid development of nanotechnology, more and more polymer materials have been excavated for the preparation of nano drug microspheres for the treatment of malignant tumors. Due to its wide range of sources, low price, good biocompatibility and degradability, natural polysaccharides are widely used in the research of nano-microsphere carriers to achieve sustained release control of embedded drugs. In this paper, we mainly introduce the preparation methods of chitosan, sodium alginate and chitosan-sodium alginate nanospheres and their application research in anti-tumor, and look forward to their future development.

1. Introduction
In today's world, malignant tumors have become one of the chief culprits that seriously threaten human health. According to statistics, there were approximately 1 685 210 newly diagnosed malignant tumors in the United States in 2016, of which approximately 595 690 tumor patients died [1]. At present, the main methods for treating tumors include surgery, radiotherapy, and chemotherapy. However, these methods also kill normal cells while treating tumors, causing serious side effects to the body and tissues. In recent years, with the development of nanotechnology and smart materials, some nanomaterials have been developed as carriers of anticancer drugs. Nanomaterials have a large specific surface area and can increase the loading of drugs. When the nanomaterial is combined with the drug, on the one hand, the stability of the anticancer drug can be effectively enhanced, on the other hand, the sustained release of the drug can be realized, and the circulation time of the drug in the body can be prolonged, thereby reducing the toxic and side effects on the normal body and tissues. It provides a new method for the treatment of malignant tumors. Although a large number of studies on nano drug carriers have been reported, there are still problems such as how drugs are distributed in the body, the drug loading is optimized, and the drug release is controlled. With its ultra-micro volume, nano-microspheres are easier to pass through the interstitial space and are distributed in the body to transport the drug to the target site for drug delivery and targeted drug delivery. This technology has
improved the performance of drugs, solved many problems that currently affect the efficacy of drugs, and has received more and more attention on drug release [2].

Natural polysaccharides are biomacromolecules synthesized by organisms with high biocompatibility, bioactivity, homogeneity and bioadhesive properties, present in most higher plants, microorganisms (bacteria and fungi), lichen algae and Animal body [3]. As a kind of degradable nanocarrier, natural polysaccharide nanospheres are widely used in the carrier of protein and gene drugs for the treatment of malignant tumors because of their wide sources and low prices. Compared with traditional drug carriers, natural polysaccharide nanospheres have the advantages of large specific surface area, high stability, targeting and sustained release drug control [4, 5].

2. Preparation of natural polysaccharide nanospheres
Chitosan is obtained by deacetylation of chitin. Chitosan contains free amino groups, is weakly alkaline, insoluble in water and organic solvents, but soluble in acidic solutions. Due to its good biocompatibility, biodegradability, non-toxicity and easy to be absorbed, it is often used to prepare nanospheres that meet specific needs [6]. Alginate is also a natural polysaccharide polymer compound, mainly derived from brown algae, which is passed by β - D-mannuronic acid (M) and α - L-guluronic acid (G) residues: 4 formed by glycosidic bonds. Sodium alginate is a water-soluble anionic polysaccharide, and chitosan is a cationic linear polysaccharide [7]. The negatively charged sodium alginate is mixed with positively charged chitosan as a carrier material. It can improve the problem of loose porosity of sodium alginate gelation and delay the release of drugs. Compared with the monomeric natural polysaccharide nanocarriers, Li Sheng used the adsorption method to prepare chitosan composite microspheres with denser particle size and better sustained release effect. The experimental results show that the average release rate of the microspheres within 24 h is 71.3 % [8]. At present, the commonly used methods for preparing nano microspheres include ion crosslinking, emulsion cross-linking, precipitation, spray drying, reverse microemulsion, self-assembly, electrostatic complex, solid-liquid phase separation, ultrasonic and Electrostatic adsorption method.

2.1 Ion cross-linking
Under acidic conditions, the free amino group in the chitosan molecule can be protonated in combination with hydrogen ions, so that the surface has a positive charge, while some polyanions such as polyphosphate ions have a negative charge in the aqueous solution, and the control is constant. Under the condition, the free amino group of chitosan and the polyanion are cross-linked by electrostatic attraction to obtain chitosan nanospheres. Ion cross-linking is the most commonly used method for preparing chitosan nanospheres. Asila used ion crosslinking method to prepare paclitaxel nanofibers embedded in paclitaxel (PCX) with a particle size between 380 and 420 nm. The encapsulation efficiency of the nanosphere was 77.1 %, and the drug release rate was 45.1 % [9].

2.2 Emulsified cross-linking
The emulsification cross-linking method that based on the properties of drugs and natural polymer materials, is forming into an emulsion of O/W, W/O and so on, then a crosslinking agent is added to make the reactive groups in the crosslinking agent and the amino group or the alcohol group in the material. A condensation reaction takes place to produce microspheres. Zhang Wenjun used chitosan acetic acid solution as the water phase, and used surfactant-containing emulsifier as W/O type emulsifier formed in oil phase. It was centrifuged and dried under the cross-linking effect of glutaraldehyde. Glycan microspheres [10]. However, glutaraldehyde may react with some drugs to invalidate the drug, which is toxic and irritating. The use of new chemical crosslinkers such as glyceraldehyde can effectively solve this problem. The sodium alginate nanospheres prepared by Gursoy using emulsification technology can realize different control effects of drug release by loading drugs with different viscosities [11].
2.3 Precipitation
The precipitation method was first proposed in 1996 to prepare chitosan microspheres using sodium sulfate as a precipitant [12]. To the acetic acid solution of chitosan, Tween - 80 was added as a dispersing agent, and the sodium sulfate solution was dropped into the stirred chitosan solution, sonicated, and the formation of fine particles was determined by the turbidity of the solution. The method can prepare nanometer microspheres of different particle sizes by selecting chitosan of different molecular weight, adjusting stirring speed and ultrasonic intensity.

2.4 Spray drying
Under normal circumstances, the human stomach acid pH value is about 2, and most of the peptide drugs enter the stomach and are decomposed and inactivated. The sodium alginate microspheres loaded with L-lactate dehydrogenase developed by spray drying technology have well protected the protein and peptide drugs from the inactivation of different barriers in the gastrointestinal tract and prevented the loss of enzyme activity [13]. Li Sheng mixed chitosan with inorganic nano-components, Fe₃O₄ and graphene oxide (GO), and prepared uniform microspheres embedded with doxorubicin (DOX) with diameter of 100 - 1100 μm by spray drying method, and effectively controlled the release of Fe₃O₄ and DOX [14].

2.5 Reverse microemulsion
When the nanoparticle is prepared by the inverse microemulsion method, the reaction is carried out in the water core of the emulsion, and the outer layer is the oil phase, and the formed particle size is limited to the water core, so that the size of the microemulsion droplet can be changed to achieve the The control of the particle size produces nano-microspheres with uniform dispersion and uniform morphology. The average diameter of porous carbon nanospheres prepared by Wang Jiasheng using reverse microemulsion method is 20 nm and the pore diameter is 0.7 nm, which provides a simple and convenient method for controllable synthesis of ultra-small pore CNs with potential application value [15].

2.6 Self-assembly method
Self-assembly means that macromolecules with certain structures are spontaneously assembled into nanoscale collectives with special shapes and sizes through special interactions between molecules, such as electrostatic interactions. Chitosan chemically modified by self-assembly method has self-cluster assembly characteristics in solution, and chitosan nanoparticles can be prepared under certain conditions. It was found that an amphiphilic graft copolymer consisting of a chitosan backbone and three arms (Cs - Ge - P) was used to prepare a polystyrene graft under the action of toluene disocyanate. The extremely high solubility of the chitosan-polystyrene graft unit (CSg - PS) demonstrates a significant increase in its hydrophilic properties and exhibits a strong ability to adsorb transition metal ions [16]. In addition, super paramagnetic carboxymethyl chitosan-sodium alginate nanospheres made by Jiang Jianfang through self-assembly method and electrostatic interaction can effectively improve the drug activity, slow down the release rate and increase circulation time of the drug in body, and then it enhances the therapeutic effect [17].

2.7 Solid-liquid phase separation
The solid-liquid phase separation method is simply a method of separating solids and liquids by utilizing the incompatibility of solid and liquid phases. Chitosan hollow nanospheres (CHNs) synthesized by Liu Mingxian through solid-liquid phase separation have diameters of 500 - 1000 nm, microsphere openings of 300 - 500 nm, and CHN specific surface area of 101.91 m²/g [18]. The methyl-thiotetrazole test demonstrated that the chitosan hollow nanospheres had low cytotoxicity, and 87 % of the curcumin drug was released from the nanospheres within 50 h. CHN trials have shown potential applications for cancer therapy during drug and gene delivery.
2.8 Electrostatic adsorption
The electrostatic adsorption method is a drug-loading and modification method that relies only on physical action. The principle can be simply summarized as the mutual attraction between different charged groups under the action of electrostatic attraction, so that the heterogeneous charges are combined with each other. Liu Tao designed a chitosan catechol low nanosphere (ACHN) carrier coated with sodium alginate by electrostatic adsorption method, which was well applied to the synergistic delivery of paclitaxel (PTX) and doxorubicin (DOX) and achieved good therapeutic effect [19].

3. Application of natural polysaccharide nanospheres in anti-tumor
Currently, cancer remains a major threat to human health. Chemotherapy, as the main method of treating cancer, has not made significant progress in the past 30 years. Most of the clinically used chemotherapeutic drugs have poor water solubility and stability, short circulation time in the body, and lack of tumor cell targeting. Problems such as multidrug resistance often fail to achieve the desired therapeutic effect and may even seriously damage normal human tissues [20]. The use of natural polysaccharides as a nano drug carrier material can well compensate for the defects of chemotherapy methods, and therefore, its application in anti-tumor is more and more extensive.

3.1 Application of Chitosan Nanospheres in Antitumor
Because chitosan has unique biological properties, researchers have loaded different anticancer drugs on chitosan nanocarriers, which are used for the treatment of different cancers depending on the drug. Paclitaxel (PTX) is a very effective treatment for cancer drugs. Cytotoxicity tests showed that PTX-loaded chitosan nanocarriers have strong anti-tumor effects [21]. Chitosan is limited in its application due to poor water solubility. Many scholars have prepared nanospheres by chitosan modification or its derivatives. Nano-microspheres prepared by Alupei on the basis of chitosan-maltose derivatives and magnetite show enhanced hydrophilicity, and prove its potential use as targeted delivery of anti-tumor drugs through cytotoxicity [22]. In addition, the modified chitosan nanoparticles can be increased from 132.8 nm to 172.7 nm, which greatly increases the drug loading and effectively increases the therapeutic effect of cancer [23].

3.2 Application of alginate nanospheres in anti-tumor
Sodium alginate is a water-soluble anionic polysaccharide extracted from brown algae. Similar to chitosan, it is excavated for anti-tumor treatment because of its good natural properties. In vitro drug release experiments found that loaded DOX superparamagnetic Fe₃O₄ sodium alginate nanospheres prepared by coprecipitation were much faster in acidic environments (pH 5.0) than in alkaline environments (pH 7.4) [24]. The study found that magnetic alginate microspheres are easier to control and target release anticancer drugs, reducing side effects and achieving higher results.

Sodium alginate nanospheres are potential drugs for the treatment of osteosarcoma, but they are currently only in the experimental stage and have not been used in clinical trials. The rate and specificity of drug delivery based on nanocarriers prepared by sodium alginate monomer is not very high. Many researchers pay more attention to the combination of other materials with sodium alginate to make up for monomer deficiency. Nanocarriers prepared by combining graphene oxide (GO) with sodium alginate can significantly improve drug delivery rate and targeting by the improved Hummers method [25]. With the rapid development of science and technology, marine polysaccharides are gradually being developed, and alginate polysaccharides will also be widely used in anti-tumor treatment.

3.3 Application of Chitosan-Sodium Alginate Nanospheres in Antitumor
In recent years, combination therapy has been widely used in the clinical treatment of cancer. It can reduce the number of doses and doses, alleviate drug resistance, reduce side effects, produce synergistic effects, and enhance the therapeutic effect of any drug on tumors. The polymeric
nanocarriers composed of sodium alginate and chitosan not only enhance the natural characteristics of the two, but also make up for the deficiency of the two. It was found that the sodium alginate-coated chitosan nanoparticles prepared by the ion gel method control the release rate and time of the drug at different pHs. Under the transmission microscope, Wang Qingjun found that compared with the rapid release of drugs in chitosan nanoparticles under acidic conditions, chitosan nanoparticles coated with sodium alginate showed a slower and smoother drug release process under acidic and alkaline conditions, releasing about 88.9% within 48 h [26]. In vitro cytotoxicity assay, polymerized nanoparticles composed of chitosan succinate and alginate can inhibit the proliferation of HT-29 tumor cells to induce apoptosis over a longer period of time, prolong the release of CP in the colon, and improve anti-tumor effects [27]. In addition, sodium alginate-chitosan nanoparticles prepared by ionic polymer gel method were stable at 4 °C for 5 months [28]. This greatly ensures the safety of anticancer drugs and ensures efficient drug loading.

4. Conclusion and Outlook
Natural polysaccharide has a wide range of applications in medicine and biomaterials due to its wide source, good biocompatibility and biodegradability. Taking natural polysaccharide as nano drug carrier material can make up for the defects of chemotherapy methods in treating cancer. However, the preparation of natural polysaccharide nanospheres is only limited to cell experiments, animal experiments and antibacterial experiments, which is still a long way from the goal of clinical trials and the promotion of marketization. Through this review, the development direction of natural polysaccharide nanospheres carrier research is proposed: (1) Adjusting the stability and biocompatibility of the polymer by polymerizing natural polysaccharide and other high molecular materials; (2) Innovating the preparation method of natural polysaccharide nano microsphere carrier, simplifying the preparation process and improving drug loading and targeting; (3) On the basis of orthogonal experiments, the sensitivity conditions of natural polysaccharide nanospheres are continuously improved to realize the efficient control of drug slow release; (4) In-depth study on the modification of natural polysaccharide materials and the preparation method of nanospheres of their derivatives to make up for the shortage of monomers. In recent years, although a large number of studies on natural polysaccharide nanospheres have been reported one after another, the microenvironment sensitivity, drug loading and targeted drug delivery capability need to be further improved. Therefore, the development of a natural polysaccharide nanosphere carrier with little environmental impact, high drug loading and high efficiency targeting has important research significance and research value.

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