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Research paper

Facile fabrication of poly(acrylic acid) coated chitosan nanoparticles with improved stability in biological environments

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ABSTRACT

Chitosan is one of the most important and commonly used natural polysaccharides in drug delivery for its biocompatible and biodegradable properties. However, poor blood circulation of the chitosan nanoparticles due to their cationic nature is one of the major bottlenecks of chitosan-based drug delivery systems. To address this problem, a versatile platform based on poly(acrylic acid) (PAA) coated ionically cross-linked chitosan/tripolyphosphate nanoparticles (CTS/TPP-PAA NPs), is reported. The zeta potentials of CTS/TPP and CTS/TPP-PAA NPs are approximately 33 mV and −25 mV, respectively. CTS/TPP NPs quickly aggregate in PBS (phosphate buffered saline) and DMEM (Dulbecco's modified Eagle's medium). Conversely, CTS/TPP-PAA NPs exhibit excellent colloidal stability in plasma solution for more than 24 h. The PAA coating also endows CTS/TPP-PAA NPs with decreased protein adsorption capacity and improved buffering capacity. More importantly, the residual carboxyl and amino groups on CTS/TPP-PAA NPs provide abundant reactive sites for further functional modifications. Therefore, the CTS/TPP-PAA NPs reported here may be useful as an alternative drug delivery system.

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1. Introduction

Chitosan has been widely utilized for a variety of medical applications, such as cell imaging and drug delivery systems for peptides, genes and low-molecular-weight drugs [1]. As the only known naturally occurring polycationic polysaccharide, chitosan has versatile characteristics, such as bioavailability, biocompatibility, biodegradability, low immunogenicity, and high charge density [2]. Chitosan nanoparticles can be prepared by chitosan-drug conjugates, ionically or covalently cross-linked chitosan, or amphiphilic chitosan derivatives [3–10]. Among them, ionic cross-linking of chitosan using tripolyphosphate (TPP) as the crosslinker has numerous advantages, such as mild, convenient, non-toxic and organic solvent free preparation conditions [3,11,12].

Despite their biocompatibility and biodegradability, chitosan nanoparticles, like most of the positively charged nanoparticles, always interact strongly with serum components, which causes severe aggregation and rapid clearance from circulation and limits their *in vivo* application [13,14]. Therefore, tailoring the chitosan nanoparticles towards greater stability in biological environments is important for tumor targeting and intracellular drug delivery. The most frequently used method to overcome this drawback is hydrophilic modification of chitosan, using polymers and molecules such as β-cyclodextrin [15], PEG [7], and succinic anhydride [4,6], which enhance chitosan's solubility in slightly acid and neutral media and confer stability to the chitosan nanoparticles. However, hydrophilic modification of the chitosan backbone can compromise the hydrophobic and cationic integrity of the chitosan core, which can lead to a reduced hydrophobic drug loading capacity [16,17]. At the same time, methods of stabilizing chitosan nanoparticles against aggregation through surface decoration with hydrophilic polymers or macromolecules have rarely been reported [18].

In the present work, we aimed to develop a facile and versatile approach towards the formation of chitosan nanoparticles that were stable in biological media (Fig. 1) First, TPP cross-linked chitosan (CTS/TPP) nanoparticles were fabricated by the standard ionic gelation technique. Second, polyanion poly(acrylic acid) (PAA) was attached to the surface of chitosan nanoparticles through an electrostatic interaction between positively charged protonated chitosan chains and the negatively charged PAA polymer. Finally, the PAA polymer was fixed onto the surface of the CTS/TPP core using water-soluble carbodiimide (EDC) at room temperature. This chitosan-based nanoparticle has some interesting features: (1) the residual carboxyl and amino groups on

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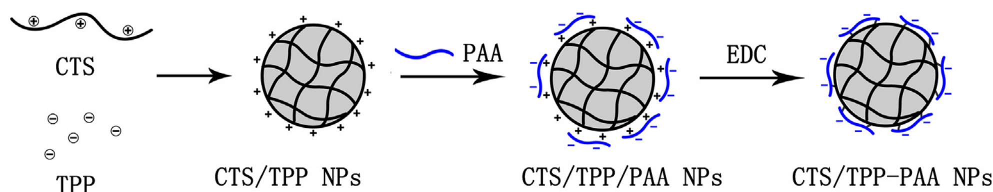

Fig. 1. Scheme for preparation procedure of chitosan-based nanoparticles.

PAA-CTS/TPP NPs provide abundant reactive sites for further functional modifications. (2) The post-modification of CTS/TPP NPs did not impair the integrity of the chitosan core, which is beneficial for increasing the drug loading capacity and gene condensing capacity. (3) The CTS/TPP-PAA NPs exhibited excellent colloidal stability in PBS and plasma solution. (4) The electrostatic modification of CTS/TPP nanoparticles is simple and versatile, and could easily be applied to a variety of chitosan nanoparticles and analogous nanoparticles.

2. Experimental

2.1. Materials

Chitosan was purchased from TCI Co. Ltd. (Japan), with deacetylation degrees of 75.0–85.0%. FITC-labeled chitosan (FITC-CTS) was synthesized according to our previous studies [10]. Poly(acrylic acid) (PAA, molecular weight of 1800 Da), folate (folic acid), sodium tripolyphosphate (TPP), fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) and 1-dimethylaminopropyl-et hylcarbodiimide hydrochloride (EDC) were all purchased from Aladdin (Shanghai, China) and used as received. The other chemical reagents used in this study were all commercially acquired from Tianjin Chemical Reagent Company (Tianjin, China) with analytical reagent grade.

The human liver carcinoma cell line (HepG2) was purchased from the Shanghai Institute of Biochemistry and Cell Biology cell bank. DMEM medium, FBS, trypsin and other biological reagents were all purchased from DingGuo Biotech. Co. (Shanghai, China).

2.2. Fabrication of chitosan nanoparticles

Fabrication of PAA coated chitosan nanoparticles were achieved in three steps, as illustrated in Fig. 1. TPP cross-linked chitosan nanoparticles (CTS/TPP NPs) were prepared based on the ionic gelation interaction between positively charged chitosan and negatively charged TPP according to a previously reported method [19,12,20,21]. Briefly, chitosan was dissolved overnight in aqueous acetic acid (1 wt%) at a concentration of 1 mg/mL, and the pH was adjusted to 6.0 using sodium hydroxide solution. Under intensive stirring, TPP solution (1 mg/mL) was added dropwise (1 drop per 1–2 s) to the CTS solution in an ice water bath. The solution was stirred for 24 h at room temperature before use.

Nanoparticles were ultra-centrifuged at 15,000 rpm at 4 °C for 1.5 h in an ultracentrifuge (Sigma 3K30, UK) and redispersed using a probe ultrasonic processor (Scientz-IID, Ningbo Scientz Biotech., Ningbo, China) with 10 W output power, 25 kHz ultrasonic frequency for 5 min.

Poly(acrylic acid) coated chitosan nanoparticles (CTS/TPP/PAA NPs) were prepared based on an electrostatic interaction between chitosan and poly(acrylic acid), according to the layer-by-layer (LBL) method [22]. Briefly, CTS/TPP NPs were ultra-centrifuged at 15,000 rpm at 4 °C for 1.5 h in an ultracentrifuge (Sigma 3K30, UK) and redispersed in an aqueous solution of 10 mg/mL PAA (molecular weight 1800 Da, pH = 6.0) using the same procedure mentioned above, during which the PAA was electrostatically attached to the CTS/TPP NPs. The nanoparticles were then separated from the PAA solution and redispersed in MilliQ water (Millipore Milli-Q purification system, MA, USA) using the same procedure mentioned above.

Poly(acrylic acid) conjugated chitosan nanoparticles (CTS/TPP-PAA NPs) were prepared by conjugation between the PAA coating and CTS/TPP core using a water-soluble carbodiimide (EDC) as the cross-linker, according to a previously described method [23,24]. Briefly, an EDC aqueous solution (10 mg/mL) was mixed with the CTS/TPP/PAA NPs (1 mg/mL) with sonication and reacted at room temperature overnight to fix the PAA coating via the amide bond between the carboxyl group of PAA and the amino group on the chitosan nanoparticles surface. Subsequently, nanoparticles were collected by centrifugation at 15,000 rpm for 1.5 h. The supernatants were discarded and the pellet was redispersed in MilliQ water.

2.3. Evaluation of nanoparticles stability

2.3.1. Size measurement of nanoparticles

The size, zeta potential of nanoparticles were determined using a Nano-ZS 90 Nanosizer (Malvern Instruments Ltd., Worcestershire, UK) equipped with 4 mW He Ne laser at a scattering angle of 90° at 532 nm at 25 °C. Polydispersity index (PDI) was also measured to determine particle size distribution. The typical nanoparticle concentration used was 0.1 mg/mL in MilliQ water or PBS. The solutions were filtered using a 0.45 μm filter prior to analysis. All the measurements were repeated three times, and an average value was reported.

The stability of nanoparticles under simulated physiological conditions was monitored by size measurements at different time points. Chitosan nanoparticles were ultra-centrifuged at 15,000 rpm for 1.5 h, and the obtained pellets were redispersed in MilliQ water, PBS (pH 7.4, 10 mM, I = 150 mM) and DMEM cell culture medium with 10% FBS, respectively. The nanoparticles were incubated at 37 °C under agitation (100 rpm).

The size and morphology of nanoparticles were measured by transmission electron microscopy (TEM) on a Tecnai G2 F20 S-Twin electron microscopy center (FEI Company, America). To prepare the TEM samples, the sample solution was dropped onto a carbon-coated copper grid, stained with 2% (w/v) phosphotungstic acid aqueous solution, and dried slowly in air. The acceleration voltage was 100 kV.

2.3.2. BSA absorption assay

The interaction between bovine serum albumin (BSA) and the chitosan nanoparticles was investigated according to the literature procedure [25]. Approximately 0.4 mL of a dispersion of CTS/TPP and CTS/TPP-PAA nanoparticles (0.5 mg/mL) was suspended in 0.2 mL of aqueous solution with FITC-BSA at an initial concentration of 0.05 mg/mL. The samples were then shaken for 1.5 h at 37 °C. Nanoparticles were separated from unbound FITC-BSA by ultracentrifugation at 15,000 rpm and 4 °C for 1.5 h. The fluorescence intensity of the supernatant was measured using a

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