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Polymer nanoparticles composed with gallic acid grafted chitosan and bioactive peptides combined antioxidant, anticancer activities and improved delivery property for labile polyphenols

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ABSTRACT

Polymer nanoparticles assembled from gallic acid (GA) grafted chitosan (CS, GA-g-CS for GA grafted CS) and caseinophosphopeptides (CPP) were developed to deliver (–)-epigallocatechin-3-gallate (EGCG) as novel functional foods. The contents of GA in GA-g-CS copolymers were in the range of 26.5 ± 1.0–126.0 ± 1.1 mg/g, with the increase of molar ratio of GA to glucosamine in CS. Compared with CS, GA-g-CS possessed much higher solubility under neutral and alkaline environments. Spherical and physicochemical stable nanoparticles assembled from GA-g-CS and CPP were obtained with particle size around 300 nm and zeta potential of less than +30 mV. The GA-g-CS-CPP nanoparticles showed strong antioxidant activity and cytotoxicity against Caco-2 colon cancer cells. The EGCG-loaded GA-g-CS-CPP nanoparticles (84–90% for encapsulation efficiency) showed improved delivery property, controlling release of EGCG under simulated gastrointestinal environments, preventing its degradation under neutral and alkaline environments, and amplifying its anticancer activity against Caco-2 cells.

1. Introduction

Over the last decade, significant attentions have been paid to polymer nanoparticles as oral delivery systems for nutraceuticals to improve their bioavailability as novel functional foods (Acosta, 2009; Braithwaite et al., 2014; Ting, Jiang, Ho, & Huang, 2014). Polymer conjugates with biological activities synthesized through grafting antioxidant agents to the polymer molecular chains have drawn increasing attention for

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Abbreviations: CPP, caseinophosphopeptide; DLS, dynamic light scattering; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDC-HCl, 1-ethyl-3-(3′-dimethylaminopropyl-carbodiimide) hydrochloride; EGCG, (–)-epigallocatechin-3-gallate; ET, electron transfer; GA-g-CS, gallic acid grafted chitosan; GI, gastrointestinal; HAT, hydrogen atom transfer; HOBt·H_2O, 1-hydroxybenzotriazole monohydrate; LSD, least significant difference; PBS, phosphate buffer saline; PDI, polydispersity index; ROS, reactive oxygen species; SD, standard deviation; SGF, simulated gastric fluid; SIF, the simulated intestinal fluid; TEM, transmission electron microscopy

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their unique functions (Cirillo et al., 2010; Piras, Dessy, Dinucci, & Chiellini, 2011). Oxidative damage, related to over-production of reactive oxygen species (ROS), is always involved in the initiation and progression of many diseases and disorders, such as carcinogenesis and evolution of cancer (Fang, Seki, & Maeda, 2009; Kim et al., 2011; Miller, Albers, Pralle, Isacoff, & Chang, 2005). On the other hand, the bioactive polymer conjugates have also been paid a great attention as novel delivery systems for drugs or nutraceuticals (Ko et al., 2014; Williams, Lepene, Thatcher, & Long, 2009), because the carrier matrix materials are the major content compared with their payload content in most delivery systems. In addition, many labile drug or nutraceutical molecules are oxidation-sensitive, which is the most common cause for their deterioration during storage and/or transport to the required target site in the body (Janesirisakule, Sinthusake, & Wanichwecharungruang, 2013).

EGCG, the most abundant tea catechin in green tea, is known as a strong natural antioxidant. A lot of epidemiological and preclinical studies have demonstrated that EGCG can reduce the risk of cancer, which is mainly attributed to its inhibitory effects on enzyme activities and signal transduction pathways, resulting in the suppression of cell proliferation and enhancement of apoptosis (Yang, Wang, Lu, & Picinich, 2009). However, EGCG is very unstable in plasma and intestinal juice neutral and alkaline environments, leading to its very low bioavailability (Yoshino, Suzuki, Sasaki, Miyase, & Sano, 1999).

One of the main functions of the carrier system is to deliver their payload to the desired target sites, reducing nontarget (systemic) exposure and increasing the exposure concentrations and/or duration per administered dose at the target site(s). Chitosan (CS) and its derivatives are widely used in fabrication of promising vehicle for oral delivery of therapeutics or nutraceuticals to increase their bioavailability due to their excellent mucoadhesive and absorption-enhancing properties (Chen et al., 2013; Chiu et al., 2010). However, CS based nanoparticles swell highly, even burst break, in stomach acid, caused by the strong repulsion among the highly protonated amino-groups (Gamboa & Leong, 2013). In addition, owing to its semicrystalline nature and multiple H-bond forming groups, CS is insoluble in water (when pH > 6.4), which limits adopting CS for nutraceuticals delivery. Distinct chemical modifications such as glycol CS, PEGylated CS, thiolated CS, quaternary CS, carboxymethylated CS have been synthesized to improve their solubility in neutral and alkaline pH (Al-Hilal, Alam, & Byun, 2013). Despite that CS based antioxidant polymer conjugates have been developed in previous studies (Curcio et al., 2009; Spizzirri et al., 2010), the influence of antioxidant agent grafting on the physicochemical properties of CS and the nutraceuticals delivery properties of the CS based nanoparticle carriers were scarcely addressed.

Herein, gallic acid (GA)-grafted-CS (GA-g-CS) conjugates were synthesized using a chemical implanting method developed in our previous study (Xie, Hu, Wang, & Zeng, 2014). GA is a naturally occurring phenolic acid with high antioxidant activity. The phenolics-polysaccharide conjugates occur widely in natural food, which are formed through the covalent linkage between phenolics and cell wall structural components, such as cellulose, hemicellulose, lignin, pectin and rod-shaped structural proteins (Arranz, Silvan, & Saura-Calixto, 2010). In present study, GA was conjugated to CS forming amide bond which could easily be hydrolyzed in the gastrointestinal tract and the human colon. The GA-g-CS conjugate could be safe and extensive toxicology studies will still be needed before it can be widely used in the food industry.

The grafting reaction was confirmed and characterized by thin-layer chromatography, UV–vis spectroscopy and Fourier transform-infrared (FT-IR) spectroscopy. The solubility of the GA-g-CS under pH 7.0 and pH 8.4 was characterized and compared with that of CS. Novel polymer nanoparticles composed with GA-g-CS and caseinophosphopeptides (CPP) were prepared and characterized using transmission electron microscopy (TEM), dynamic light scattering (DLS) and electrophoretic mobility (ζ-potential) measurements. CPP is a group of anionic polypeptides, which are released from the N-terminus polar region during the tryptic digestion of milk casein proteins. The antioxidant activities of the GA-g-CS-CPP nanoparticles were evaluated using DPPH radicals assay and β-carotene-linoleic acid assay. The oral delivery properties of the GA-g-CS-CPP nanoparticles for phytochemicals were characterized through determining their encapsulation efficiency and release profile of EGCG, stabilization effects on EGCG in simulated gastrointestinal (GI) environment, and their anticancer activities against intestinal cancer cells loading with/without EGCG.

2. Materials and methods

2.1. Materials

CS (Average molecular weight, ~1.5×10^5; Degree of deacetylation, ≥90.0%), 1-ethyl-3-(3′-dimethylaminopropyl-carbodiimide) hydrochloride (EDC-HCl) and Folin–Ciocalteu reagent were purchased from Kayon Biological Technology Co., Ltd. (Shanghai, China). GA·H_2O, 1-hydroxybenzotriazole monohydrate (HOBt·H_2O) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). CPP were prepared and identified with HPLC-MS-MS as described in our previous report (Hu, Wang, Li, Zeng, & Huang, 2011). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), EGCG (purity > 98%), β-carotene, linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Synthesis and characterization of GA-g-CS

2.2.1. Synthesis of GA-g-CS

The synthesis was performed based on the one-pot method as reported (Xie, Hu, Wang, & Zeng 2014) with some modifications. CS (0.303 g, 1.85 mmol) was stirred in deionized water (30 mL) with HOBt (0.282 g, 1.85 mmol) overnight until a clear solution was obtained. GA (0.311 g, 1.85 mmol) was introduced into the CS solution followed by the dropwise addition of an alcoholic solution of EDC (0.355 g, 1.85 mmol, 2 mL). GA-g-CSs with different substitutions of GA were prepared by changing the ratio between glucosamine in CS and GA (1:0.1, 1:1, 1:3, and 1:5). The reaction was carried out for 24 h in ambient temperature and atmosphere in the dark. The resultant liquid was poured into dialysis bags (MWCO 8000–14,000 Da), dialyzed against deionized water for 6 days with four changes of water each day. The resulting solutions were firstly tested by