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Fabrication and study of curcumin loaded nanoparticles based on folate-chitosan for breast cancer therapy application

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ABSTRACT

Purpose of this article is developing novel, inexpensive curcumin loaded chitosan nanoparticles with targeting ability. Curcumin loaded folate-modified-chitosan-nanoparticles (NPs) have been synthesized and fabricated via self-assembling process. Chemical structures of modified chains, nanoparticle size in dry and wet state, zeta potential, morphology of NPs, physical state of curcumin in NPs, drug release profile and cytotoxicity of NPs were investigated by FTIR, FE-SEM, DLS and XRD, UV–vis spectrophotometer, and MTT assay against L929 and MCF7 cell lines, respectively. Results show nanoparticle size in dry state varied in range of 119–127 nm and curcumin was loaded into nanoparticles with 96.47% efficacy. Drug release studies showed by decreasing pH of release medium from 7.4 to 5, release rate of curcumin from NPs increased, which shows pH responsive capacity of folate-modified chitosan nanoparticles. Cell viability studies confirmed that curcumin loaded NPs have good potential as a drug delivery system for breast cancer therapy.

1. Introduction

Cancer is a multifaceted disease which is characterized by a high degree of adaptability and flexibility (Dominietto, Tsinoremas, & Capobianco, 2015). By understanding the biology of cancer, the development of chemotherapy has been advanced to targeted therapy through growth factor receptors instead of identifying the toxic agents (Shukla, Meeran, & Katiyar, 2014). Despite these improvements, a significant number of metastasis cancers cannot respond to therapeutic agents owing to drug resistance. The development of drug resistance causes a significant failure in the treatment of metastasis cancer (Ferrara & Adamis, 2016). Nanoparticles that are capable of carrying drugs and can minimize side effects of conventional techniques can be used to diminish drug resistance in cancer therapy (Tong & Kohane, 2016).

Curcumin is really effectual against many cancer cells such as breast cancer. Extensive studies showed that curcumin possesses chemopreventive properties, mainly due to its ability to arrest cell cycle and induce apoptosis in cancer cells. Action mechanisms of curcumin are diverse and have not been elucidated thus far. By regulating multiple important cellular signaling pathways, curcumin is known to activate cell death signals and induce apoptosis in precancerous or cancer cells without affecting normal cells, thereby inhibiting tumor progression (Chen, Wang, & Chen, 2014). Regardless of its good properties, it has poor solvability in the aqueous system, thus it is not widely exploited for cancer treatment. In addition, curcumin faces other problems such as low rapid metabolism and poor availability (Gao et al., 2015).

Polymeric nanoparticles have been widely used for biomedical applications especially for drug delivery and tissue engineering (Sahu et al., 2010). Among these applications, drug delivery by nanoparticles with specific internalization into a target cell has been taken into account (Sheikh et al., 2009; Singh & Lillard, 2009).

Among these, chitosan based nanoparticles have captured a lot of interest due their unique characteristics, for instance, they are biodegradable, biocompatible, positively charged with the ability to increase cell membrane permeability and cellular uptake of nanoparticles (Croisier & Jérôme, 2013). Some research has been reported on encapsulation of curcumin into chitosan based nanoparticles. However, in these researches mostly hydrophilic chitosan (Anitha, Deepagan et al., 2011; Anitha, Maya et al., 2011; Mofazzali-jahromi, Pirestani, Al-musawi, & Fasihi-rammandi, 2016) was used while due to the hydrophobic nature of curcumin, drug loading efficiency might be reduced.

Nanoparticles based on hydrophobic modified chitosan can efficiently encapsulate various hydrophobic anticancer drugs into

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their hydrophobic domains. Compared to a free drug formulation, Chitosan-based nanoparticles showed prolonged blood circulation time in vivo and improved therapeutic efficacy (Nitta & Numata, 2013).

In order to induce amphiphilic properties, hydrophobic moieties such as fluorescein isothiocyanate, hexanoyl and so on can be introduced into chitosan chains. Hydrophobic modifications of chitosan promote self-assembly into nanoparticles and increased interactions with lipid structures, such as cell membranes. Chitosan contains primary amino groups ($-NH_2$) in the main backbone, so self-assembled nanoparticles can be simply prepared by modifying chitosan with a variety of biocompatible substances such as folate (Larsson et al., 2013).

It has been reported that folate (FA), a vitamin with very low solubility in water, can increase targeting capacity. Most mammalian cells obtain their normal folate requirement through low affinity reduced folate carrier or proton-coupled folate transporter; however, accessible folate receptors are normally expressed in significant numbers mostly on cancer cells. Folate conjugates display no affinity for the reduced folate carrier or proton coupled folate transporter, and they bind to folate receptors (FRs) on the cancerous cell types with high affinity (Kd ≈ 10–9 M) and enter FR-expressing cells by receptor-mediated endocytosis (Lu, Sega, Leamon, & Low, 2004; Sudimack & Lee, 2000; Xia & Low, 2010). Therefore, folate groups introduced to polymer chains can increase the targeting capacity of nanocarriers due to high affinity to its receptor which are over expressed in some cancerous tissues, while it is highly restricted in normal tissues (Nicolas, Mura, Brambilla, Mackiewicz, & Couvreur, 2013)

The degree of substitution of FA groups affects the final properties of the nanoparticles such as size, morphology, release profile, loading efficiency (LE) and loading capacity (LC) (Yu et al., 2013).

In this work we have prepared curcumin loaded folate modified chitosan NPs with amphiphilic characteristics and the effect of molecular weight and degree of folate (FA) substitution on physico-chemical properties of modified chitosan nanoparticles and curcumin encapsulation were investigated. This study aimed to develop chitosan nanoparticles, with targeting potential to cancer cells, containing curcumin, an inexpensive drug with less side effects in comparison with anti-cancer drugs.

2. Experimental

2.1. Materials

Chitosan ($\bar{M}_v \cong 400$ kDa, DD = 89%) (Fluka CID:50494), hydrogen peroxide 30% (Merck CID:108597), acetic acid (Merck CID:100063), hydrochloric acid (Merck CID:100317), sodium hydroxide (Merck CID:106462), dimethyl sulfoxide (DMSO) (Merck CID:802912), sodium acetate (Merck CID:106268), *N*-hydroxySuccinimide (NHS) (Merck CID:804518), *N*,*N*-dicyclohexylcarbodiimide (DCC) (Merck CID:802954), trypan blue (Merck CID:111732) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Merck CID:111714), Dulbecco·s modified eagle medium (DMEM) (GIBCO CID:30030), foetal bovine serum (FBS) (GIBCO CID:10437010) and PBS tablets (GIBCO CID:003002), folic acid (FA) (Roth CID:2004190), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Alfa Aesar CID:B25057), curcumin (sigma CID: C1368)

2.2. Methods

2.2.1. Synthesis of folate modified chitosan

2.2.1.1. Chitosan degradation. Hydrogen peroxide was used for the degrading of chitosan, for reducing the chitosan molecular weight from 400 to 40 KD. To do so, 4.4 ml hydrogen peroxide was added to chitosan solution (2% (w/v) in 1.2 M HCl) and stirred for 1.5 h. By the drop-wise addition of NaOH (5 M), pH was raised to 7. The resulting precipitate was removed by vacuum filter and washed with ethanol and dried over night at ambient temperature (Esfandiarpour-Boroujeni, Bagheri-Khoulenjani, & Mirzadeh, 2016).

2.2.1.2. Preparation of folic acid modified chitosan. In order to introduce folate groups into chitosan, NHS (0.1 g) and DCC (0.2 g) were added to the folic acid solution in 10 ml DMSO and stirred for 12 at ambient temperature with a molar ratio of FA:NHS:DCC = 1:2:2. 1% (w/v) and chitosan solution (7 ml) in the buffer (pH 4.7) was added to the resulting solution. EDC was added to the mixture in different amounts (FA/EDC molar ratio: 1/1) and was stirred for 16 h at room temperature. Diluted aqueous NaOH was added to the mixture drop wise to raise the pH to 9.0 and the mixture was dialyzed against PBS (pH 7.4) and then against water for 24 h. Finally, FA modified chitosan was dried over night at ambient temperature (Esfandiarpour-Boroujeni et al., 2016).

2.2.2. Preparation of nanoparticles

FA modified chitosan was suspended in distilled water at a concentration of 0.1 mg/ml at 37 °C for 23 h, and stirred for 1 h at 37 °C. Then it was sonicated using a probe ultrasonicator (Hielscher UP 100H) at 20 W for 9 min until an optically clear solution was obtained.

2.2.2.1. Preparation of curcumin-loaded nanoparticles (FCC – curcumin). 5 ml curcumin solution (ratio of drug to polymer: 5% and 10%) was added to 5 ml solution of modified chitosan to reach the final concentration of 0.1 mg/ml, and then suspended at 37 °C for 23 h. The final solution was stirred for 1 h at 37 °C and sonicated using a probe type sonifier at 20 W for 9 min. The excess curcumin, which was not incorporated into the nanoparticles, was removed by centrifuging at 25155 g for 15 min. The excess amount of curcumin was measured by a UV–vis spectrophotometer at λ_{max} of 440 nm. Centrifuging was repeated until the intensity of absorption at 440 nm of the filtrate was zero.

2.2.3. FTIR and XRD studies

Chemical composition of chitosan before and after interaction with folic acid and nanoparticles was analyzed by FTIR (Bruker FRA model 106/5). The KBr tablet with KBr: polymer ratio of 100:1 was fabricated. The FTIR spectrum was obtained in frequency range of 400–4000 cm^{-1}.

To study the curcumin physical state inside the NPs, X-ray Diffraction (XRD) (EQuniox 3000, Inel) was applied using radiation Cu Kα (40 kV, λ = 15.4 nm, and 30 mA) at room temperature. Relative intensity was recorded against 2θ in the range of 5–80°.

2.2.4. Measurement of substitution degree (DS) of folate group

The UV/vis spectrophotometer (Analytik Jena, Germany) was used to determine the degree of substitution of FA into chitosan chains at λ_{max} of 363 nm. Briefly, 0.02 wt.% solution of chitosan–FA conjugate in water and DMSO (1/1: v/v) was prepared, and its absorption was recorded using a UV/vis spectrophotometer. DS was calculated using Eq. (1):

$$DS = \frac{C/M_{FA}}{(m - c)/M_{chitosan}} \quad (1)$$

where c is the FA content obtained from the calibration curve based on the absorption intensity; m is the mass of polymer; M_{FA} is the FA molecular weight and $M_{chitosan}$ is the molecular weight of chitosan monomers.