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Paclitaxel loaded magnetic nanocomposites with folate modified chitosan/carboxymethyl surface; a vehicle for imaging and targeted drug delivery

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ABSTRACT

In this study, Paclitaxel (PTX) containing, bovine serum albumin (BSA) nanoparticles were fabricated via a simple approach. Folic acid (FA) was conjugated to chitosan (CS)/carboxymethyl cellulose (CMC) through an esterification reaction to produce BSA–CS–FA or BSA-CMC-FA conjugates. $NiFe_2O_4$ noncore (NFs) and PTX were loaded through a heat treatment and by a diffusion process. NFs-BSA–CS and NFs-BSA–CMC–FA with size of about 80 nm, showed superior transversal R_2 relaxation rate of 349 $(mM)^{-1}\,s^{-1}$ along with folate receptor-targeted and magnetically directed functions. NFs-BSA–CS–FA or NFs-BSA–CS–FA were found stable and biocompatible. Application of an external magnetic field effectively enhanced the PTX release from PTX-NFs-BSA–CS–FA or PTX-NFs-BSA–CS–FA and hence tumor inhibition rate. This study validate that NFs-BSA–CS–FA or NFs-BSA–CMC–FA and PTX-NFs-BSA–CS–FA or PTX-NFs-BSA–CS–FA are suitable systems for tumor diagnosis and therapy.

1. Introduction

Nanomedicine is rapidly developing targeted drug delivery systems. This is particularly striking in cancer therapy, to increase the localized delivery to the malignancy, while circumventing the side effects to normal tissues (Su et al., 2013). Various Inorganic (Huang et al., 2009; Puvvada et al., 2012a), composite (Venkatesan et al., 2011; Ganta et al., 2008; Hashida et al., 2014), polymeric vesicles (Thompson et al., 2012; Hoffman, 2008; Liu et al., 2014) and liposomes (Shahin et al., 2013) nanocarriers have been proposed for drug delivery systems (DDS). However, low drug-capacity and prolonged release profile were the key challenges in establishing concentrations of chemotherapeutics in nanocarriers designs (Puvvada et al., 2012b; Puras et al., 2014).

Metal oxide toxicity, nanoparticle's agglomeration and immune reactivity are crucial to DDS fabrications. To reduce such risks, natural biocompatible polymers are excellent choice for structuring nanocarriers and surface functionalization of the DDS.

Chitosan (CS) and carboxymethyl cellulose (CMC) are recognized for non-toxic, biocompatible, biodegradable and non-immunogenic nature. These are abundant natural biopolymers used as a surface-tethering agent (Chen et al., 2002). Biopolymers based drug carries are being extensively investigated in the pharmaceutical industry for their potential in the development of drug/gene delivery systems because of the, ease of purification, low cost, abundance and unusual ligand-binding properties (Nitta and Numata, 2013; Elgadir et al., 2016; Wen and Oh, 2014). Yang et al. recently demonstrated sustained release of doxorubicin from silica nanocarriers using biocompatible polymer for surface modification. Carboxymethylation of chitosan overcomes water insolubility issues, encouraging new solution-based research (Bhattacharya et al., 2011).

Paclitaxel, is extensively used for the treatment of various cancers, including ovarian cancer, malignant lymphoma,

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lymphoblastic leukemia and breast cancer (Rivkin et al., 2010; Xiao et al., 2009). However, the dose-limiting problems associated with *Paclitaxel* include its toxicity toward healthy tissues and poor solubility. In this perspective, a controlled and focused drug release system would, in principle, improve the pharmacology of drug thus, enhancing its therapeutic utility.

Magnetic nanocarriers (MNCs) with biocompatible surface coatings (making their colloidal dispersions more stable) are highly desired to better address precise clinical needs (Bansal et al., 2012; Johnson et al., 2015). Biocompatible coatings not only produce more hydrophilic nanostructures, but also develop diverse surface functional groups to inhibit aggregation, bind drug molecules and improve colloidal stability in biological environment (Wabler et al., 2014). These efficiently coated MNCs may also result in greater magnetic moments resulting in an enhanced MR contrast ability (Mikhaylova et al., 2004a, 2004b). Theranostic potential of MNCs permit synchronized diagnosis and multiple therapeutic functions together such as imaging, remotely controlled drug delivery and hyperthermia (Gobbo et al., 2015).

Many techniques have been developed for the modification of MNCs using bipolymers (Tong et al., 2010). These procedures, frequently named as "one-pot procedure or synthesis", have numerous benefits over stepwise modification methodologies, including a reduced agglomeration (Colombo et al., 2012). However when added during nucleation and growth of nanocrystals, these polymers can leave a significant influence on the morphology and crystal structure of the MNCs. Similarly chemical agents used for surface modifications convey hydrophilicity and stability to MNCs, but they may cause cytotoxicity when used beyond the threshold level (Chu et al., 2015). Hence biomacromolecules like proteins and carbohydrates are preferably chosen for surface functionalization of many of the recently emerging MNCs (Takara et al., 2014; Costo et al., 2015).

Subsequent conjugation of these MNCs with targeting ligands, such as folic acid and antibodies, would further enhance their tumor uptake. Folic acid (FA) has a high binding affinity to folate receptors (FR), because of its relatively low expression level in healthy tissues and overexpression in cancerous tissues. Overexpression of folate-receptor in the cancer cells makes folate a suitable targeting agent for such cancer cells. Moreover, folate-conjugated MNCs can selectively deliver chemotherapy drugs into cancer cells both in vitro and in vivo.

In this study, we combine the merits of $NiFe_2O_4$ and three biopolymers, bovine serum albumin (BSA), CS and CMC to fabricate nanoparticles for potential clinical use that addresses several important issues: biocompatibility, process formulation, specificity, detectability, *Paclitaxel* capacity and efficacy. We used BSA as matrix to laden *Paclitaxel* (PTX) and superparamagnetic $NiFe_2O_4$ nanocores (NFs). Furthermore, the surface of PTX loaded NFs were modified with FA for targeting tumor. It is hypothesized that nontoxicity, hydrophilicity, and cancer-specific capability of these biopolymers endow the magnetic cores with exceptional aqueous dispersibility as well as the "stealth" property, which prolongs the circulation time of the magnetic nanocores in blood stream.

This study, presents a simple approach to fabricate drug loaded nanoconjugates combining MR imaging properties of $NiFe_2O_4$, chemotherapeutic efficiency of PTX, and cancer cell targeting properties in a single construct.

2. Methods

2.1. Materials

Iron acetylacetonate (III), Nickel acetylacetonate (II), oleic acid, oleylamine, sodium citrate, tetrahydrofuran (THF), $NaHCO_3$, Paclitaxel, Bovine Serum Albumin (Mw ~66,000), Chitosan (low molecular weight, Brookfield viscosity 20,000cps), Carboxymethyl cellulose sodium salt, tripolyphosphate (TPP), acetic acid were obtained from Sigma Aldrich.

2.2. Preparation of PTX loaded NFs (PTX-NFs-BSA–CS–FA and PTX-NFs-BSA–CMC–FA)

2.2.1. Synthesis of nickel ferrite cores

Nickel Ferrite cores (NFs) were synthesized by thermal decomposition of Nickel acetylacetonate. For 26 nm cores, Nickel (II) acetylacetonate (0.2 mmol) and Iron (III) acetylacetonate (1 mmol), oleic acid (5 mL), oleylamine (5 mL) were mixed in a 100 mL two-neck flask and heated to 120 °C under vacuum with vigorous stirring for 30 min, and then the temperature was ramped to 200 °C and kept at the same temperature for 60 min under Ar atmosphere. The solution was further heated to 300 °C at a heating rate of 10 °C min^{-1} and kept at 300 °C for 60 min under stirring. The reaction mixture was cooled down to room temperature, precipitated by adding isopropanol (45 mL) and nanoparticles were collected by centrifugation (8000 rpm, 10 min).

For surface modification, tetrahydrofuran (16 mL) and NF cores (2 mg) were mixed in a 100 mL two-neck flask containing water (8 mL), sodium citrate (15 mg) and $NaHCO_3$ (33 mg). The reaction mixture was heated to 95 °C for 4 h under magnetic stirring. After that, it was cooled down to room temperature and collected by centrifugation (8000 rpm, 10 min). NF cores (NFs) were redispersed in water and then filtered through sterilized membrane filters for further use.

2.2.2. Preparation of CS–FA and CMC-FA conjugates

FA was conjugated to CS and CMC via an amidation and an esterification reaction respectively with some modifications (Du et al., 2010; Surh and Tannenbaum, 1994; Eisele et al., 2010; Chakraborty et al., 2016). In brief folic acid was dissolved in dimethyl sulfoxide (DMSO; 20 mL) and the solution was subjected to sonication for 45 min. The carboxylate group of FA was activated by the addition of *N*-hydroxy succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC). The solution was stirred at 30 °C in the dark and under nitrogen atmosphere for 30 min. Molar ratio of FA/NHS/EDAC was kept as 2:2:1.

After adding 1%w/v CS (in 1% acetic acid) or CMC (in Milli-Q water), the activated FA solution was stirred for another 20 h at 30 °C under nitrogen atmosphere. The solution was centrifuged at 6000 rpm for 30 min and filtered. Filtrate was purified by dialysis against phosphate buffer (PBS pH 7.4) to remove the unreacted FA. The conjugate was characterized by UV–vis and FTIR spectroscopy.

2.2.3. Preparation of BSA-CS–FA and BSA-CMC–FA nanoconjugates

BSA was synchronously conjugated to CS–FA and CMC-FA. In detail, CS–FA or CMC-FA conjugate and free BSA were dissolved together in Milli Q water keeping a molar ratio of 1:3 (BSA to CS or CMC) and pH to 8.0. The solution was centrifuged at 6000 rpm for 30 min to remove the unreacted polymer.

2.2.4. Preparation of PTX-NFs-BSA–CS–FA and PTX-NFs-BSA–CMC–FA nanoparticles

BSA–CS, BSA-CMC, BSA–CS–FA or BSA-CMC-FA, nanoconjugate were dissolved in 20 mL Milli Q- water; 5 mL NFs solution (NFs concentration: 2.0 ± 0.3 mg mL^{-1}) was added to the reaction medium (pH adjusted to 4.0), stirred in the dark for 10 hours, and then heated at 80 °C to produce NFs loaded conjugates termed as NFs-BSA–CS, NFs-BSA-CMC, NFs-BSA–CS–FA and NFs-BSA-CMC-FA.

PTX solution was added into NFs-BSA–CS–FA or NFs-BSA–CMC–FA solution at room temperature. Weight ratio of PTX to BSA was