Colloids and Surfaces B: Biointerfaces 117 (2014) 406–413

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Doxorubicin-conjugated core–shell magnetite nanoparticles as dual-targeting carriers for anticancer drug delivery

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 8 February 2014
Accepted 2 March 2014
Available online 12 March 2014

Keywords:
Dual-targeting
Nanocarrier
Magnetite nanoparticles
Cancer targeting
Doxorubicin
Conjugation

ABSTRACT

The present study reports the successful synthesis of core–shell nanostructures composed of magnetite nanoparticles (Fe_3O_4-NPs) conjugated to the anticancer drug doxorubicin, intended for dual targeting of the drug to the tumor sites via a combination of the magnetic attraction and the pH-sensitive cleavage of the drug–particle linkages along with a longer circulation time and reduced side effects. To improve the carrier biocompatibility, the prepared nanocarrier was, finally coated by chitosan. FT-IR analysis confirmed the synthesis of functionalized Fe_3O_4-NPs, doxorubicin-conjugated Fe_3O_4-NPs, and chitosan-coated nanocarriers. Scanning electron microscopy (SEM) indicated the formation of spherical nanostructures with the final average particle size of around 50 nm. The vibrating sample magnetometer (VSM) analysis showed that the saturation magnetization value (M_s) of carrier was 6 emu/g. The drug release behavior from the nanocarriers was investigated both in acidic and neutral buffered solutions (pH values of 5.3 and 7.4, respectively) and showed two-fold increase in the extent of drug release at pH 5.3 compared to pH 7.4 during 7 days. The results showed that the dual-targeting nanocarriers responded successfully to the external magnetic field and pH. From the results obtained, it can be concluded that this methodology can be used to target and improve therapeutic efficacy of the anticancer drugs.

1. Introduction

In recent years, there has been an increasing impetus for targeting therapeutic agents to specific cells of the diseased site with the aim to improve their efficiency and/or minimize the undesirable side effects [1]. Among the numerous approaches used for this purpose, targeting based on magnetic properties using magnetic nanoparticles, mainly magnetite (Fe_3O_4)-based nanoparticles, is widely considered as a promising targeted delivery system due to its distinct advantages, mainly including a well-documented biosafety, ease of preparation and handling, the possibility of controlling the characteristics of the nanocarriers, availability, affordability of the materials needed for this procedure, and more importantly, possibility of targeting the drug(s) of interest to the desired location within the host body by using an external magnetic field. Furthermore, core–shell magnetic nanoparticles have attracted much attention due to their multifunctional properties such as small size, superparamagnetism and low toxicity [2,3]. Silica-coated Fe_3O_4–nanoparticles are one of the most extensively used Fe_3O_4–nanoparticles which possess very high specific surface with abundant Si–OH or Si–NH_2 groups with ability to react with proper functional groups [4,5].

Besides the above mentioned advantages, there are a number of drawbacks against the widespread use of Fe_3O_4 nanoparticles for targeted drug delivery systems. Firstly, due to the high surface-area-to-volume ratio characteristic of such nanoparticles, they tend to aggregate and form clusters with low magnetization properties. Secondly, it is shown that a major part of the naked Fe_3O_4 nanoparticles are rapidly cleared from blood circulation by the reticular endothelial system (RES) before they could be able to reach the intended target site, thereby being localized in the RES organs, mainly liver [5,1]. To address these issues, one approach is to encapsulate Fe_3O_4 nanoparticles within

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http://dx.doi.org/10.1016/j.colsurfb.2014.03.001
0927-7765/

biodegradable and/or biocompatible polymers [6]. Additionally, polymer-encapsulated Fe_3O_4 nanoparticles can be designed in order to provide further diverse and desirable functionality to enable conjugation to the drug of interest [7]. It is believed that the use of a trigger alone as a targeting function cannot ensure complete localization of drug at the site of interest. Therefore, to enhance targeting characteristic of a carrier, it represents a well-established approach to use more than one trigger to direct carrier to the specific site. Such carriers are generally referred to as dual targeting drug delivery systems [8]. Zhang and Misra [7] synthesized a novel dual targeted magnetic carrier consisting of magnetic nanoparticles encapsulated within dextran-g-poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) co-polymer as a smart thermo-sensitive polymer showing high drug release rate for longer durations specially in acidic pH.

Chitosan, a naturally-derived co-polymer of N-acetylglucosamine and D-glucosamine, has been extensively studied as a biodegradable and biocompatible polymer in drug delivery systems, gene therapy, and membranes for ultrafiltration [9,10]. It seems that chitosan-encapsulated Fe_3O_4 nanoparticles would most likely improve magnetite nanoparticles characteristics in terms of biocompatibity and long circulation time. The amino groups on the chitosan structure can also be used for further functionalization with specific components, such as various drugs, targeting agents, or other functional groups. Thus, it seems to be a suitable polymer to modify the Fe_3O_4 nanoparticles [11]. Feng et al. [12] synthesized monodisperse chitosan/polyacrylic acid/Fe_3O_4 nanoparticles which could be used for magnetic resonance imaging (MRI). Donadel et al. [13] prepared iron oxide magnetic particles coated with chitosan intended for hyperthermia.

Recently, Shen et al. [14] prepared dual-drug delivery system in which doxorubicin and verapamil were physically loaded into chitosan coated magnetite nanoparticles. The resultant nanoparticles were entrapped into the poly (lactic acid-coglycolicacid) (PLGA) nanoparticles and used as near infrared (NIR) trigger drug delivery system. The anthracycline antibiotic doxorubicin is a widely used anticancer drug in clinical practice for the treatment of a variety of cancers like leukemia, ovarian, prostate, brain cancers, especially late stage breast cancer [15]. Although doxorubicin is one of the most widely used anticancer agents, its application is still limited by its deleterious side effects, including myelosuppression, gastrointestinal toxicity and, more importantly, cardiotoxicity. Drug targeting, therefore, represents an interesting incentive to prevent side effects and increase cytotoxicity of doxorubicin [15,16]. A number of approaches including chemical conjugation and/or physical entrapment have been employed to target doxorubicin using different carriers such as dendrimers, polymeric nanoparticles, polymer–drug conjugates and micelles [17–20,16]. Herein we report the synthesis and in vitro characterization of a dual targeted drug delivery system using a core–shell magnetite nanoparticulate system conjugated with doxorubicin via acid-cleavable imine linkage. To enhance the biocompatibility of the prepared dual targeted carrier and minimize undesirable side effects of doxorubicin and Fe_3O_4 nanoparticles, conjugated magnetite core–shell nanoparticles were encapsulated within chitosan.

2. Experimental

2.1. Materials and methods

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), tetraethylorthosilicate (TEOS), (3-aminopropyl)triethoxysilane(APTES) all were purchased locally from Merck and used as received. Chitosan of molecular weight in the range of 10^5–3×10^5 g/mol and degree of deacetylation ≥75% was provided from Sigma. Solvents of the highest grade commercially available (Merck) were purchased locally and used without further purification. Doxorubicin hydrochloride was purchased from Celonlabs (Andhra Pradesh, India).

2.2. Synthesis of spherical Fe_3O_4 nanoparticles

The Fe_3O_4 nanoparticles were synthesized in accordance with the method of Massart [21] consisting of co-precipitation of Fe(III) and Fe(II) with ammonia in an aqueous solution. In essence, iron(II) chloride (1.0 mmol) and iron(III) chloride (2.0 mmol) were dissolved in 45 mL deionized water. Then, 3 mL aqueous ammonia (25%) was added to the solution and stirred for 1 h under the N_2 flow. The black product was separated by an external magnet and washed several times with deionized water and dried at 60 °C under vacuum for 12 h.

2.3. Synthesis of Fe_3O_4 core–shell nanoparticles

Synthesis of Fe_3O_4 core–shell nanoparticles was adapted from the literature [22]. In a typical procedure, 2.0 mL TEOS was added to the suspension of Fe_3O_4 nanoparticles in water and stirred at 1500 rpm for 2 h at room temperature. The nanoparticles were, then, separated by an external magnet, washed with deionized water and ethanol, each for three times, and finally were dried under vacuum at 60 °C for 12 h.

2.4. Functionalization of Fe_3O_4 core–shell nanoparticles

For introduction of amine functional group on the surface of Fe_3O_4 core–shell nanoparticles, in a three-necked flask 1.0 g of Fe_3O_4 core–shell nanoparticles were dispersed in 50 mL of ethanol using an ultrasonic bath for 10 min. Then, 1.0 mL of APTES was added to the suspension, and the mixture stirred mechanically at 60 °C under N_2 flow for 6 h. The synthesized magnetite core–shell nanoparticles were separated using an external magnet and washed with deionized water and ethanol for several times, and finally dried at room temperature under vacuum for 12 h.

2.5. Drug conjugation to functionalized Fe_3O_4–nanoparticles

Conjugation of magnetite nanoparticles with doxorubicin was accomplished by reaction between the amine group of functionalized magnetite and the carbonyl group of the drug via Schiff base chemistry. The amino-functionalized Fe_3O_4–nanoparticles (100 mg) were dispersed in 15 mL of ethanol by high speed homogenization (Heighdolph, Germany, Silent Crusher M) at 8000 rpm for 2 min in ambient temperature after adding two drops of glacial acetic acid as a catalyst. Doxorubicin (10 mg) was added to the resulting colloidal dispersion and the reaction was carried out at room temperature for 48 h, thereby leading to the chemical conjugation of doxorubicin to the nanoparticles via imine linkage. Finally, the drug-conjugated core–shell magnetic nanoparticles were separated by an external magnet, washed several times with deionized water and ethanol, and dried under vacuum at 50 °C for 24 h. The drug conjugation efficiency was determined by two direct and indirect methods. Indirect method involved the analysis of the remained residual intact drug in the solution (free drug) by spectrophotometry at 480 nm. The conjugation efficiency was calculated using Eq. (1):

$$\text{Conjugation efficiency}\,\% = \frac{W_{\text{Feed drug}} - W_{\text{Free drug}}}{W_{\text{Feed drug}}} \times 100 \qquad (1)$$

where $W_{\text{feed drug}}$ and $W_{\text{free drug}}$ show the weight of drug used initially in the conjugation step and the total weight of drug found in the supernatant.