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Synthesis, characterization and cellular cytotoxicity evaluation of a new magnetic nanoparticle carrier co-functionalized with amine and folic acid

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ABSTRACT

In the present work, for the first time, co-functionalizing the surface of a core-shell Fe_3O_4@$nSiO_2$ nanoparticle was performed for transporting doxorubicin anticancer drug (DOX). This core-shell nanoparticle (NP) is composed of superparamagnetic iron oxide core synthesized by solvothermal method and a nonporous silica ($nSiO_2$) layer shell. The nanocomposite shell was co-functionalized with amine group (NH) and folic acid (FA). The characterization results confirmed the superparamagnetic property of Fe_3O_4@$nSiO_2$ nanoparticle with the average particle size of 75 nm. In addition, the successful co-functionalizing the surface of NPs was confirmed with FTIR analysis. Dox loading on the nanocomposite was performed at the optimum adsorption condition with the entrapment efficiency of 81.5%. In vitro release study showed a very slow release at pH = 7.4 while a relative rapid release within 10 h and a sustained release until 144 h was observed at pH = 5.5. Finally, the in vitro cytotoxicity assay showed that cellular cytotoxicity of DOX-loaded Fe_3O_4@$nSiO_2$-NHFA on the breast cancer cell lines (MDA-MB-231) is increased considerably in comparison to free DOX. This confirms the improvement of the synthesized nanocomposite structure. All results confirmed that this new synthesized nanoparticle with an appropriate size and cellular cytotoxicity is a suitable candida for drug-delivery vehicles.

1. Introduction

Cancer is one of the most common diseases in recent years, which is usually subjected to chemotherapy as the most prevalent method of cancer treatment [1,2]. Doxorubicin hydrochloride (DOX), as an anthracycline ring antibiotic [3], is a widely used anticancer agent for the treatment of several forms of cancers [4,5]. A major problem of this drug treatment such as other chemotherapeutic agent is their cytotoxicity that leads to the attacks of drugs to the healthy and normal cells [6]. Therefore, selective targeting of chemotherapeutic agents to the tumor could mitigate this side effect [6]. Recently, by growth of nanotechnology, new opportunities to deliver small-molecule drugs and biological agents to the target sites have been provided [7]. Magnetite nanoparticle is one of the best nanomaterials proposed for the loco regional cancer treatment [6]. In the magnetically drug delivery system, coated magnetic nanoparticles loaded with anti-cancer drugs are injected into the patient body via the human circulatory system. To locate the drug loaded carriers at the tumor site, an external magnetic field is used. Drug can be released from the carriers by enzymatic activity or changing the physiological conditions such as temperature or pH or osmolality [6].

Several magnetic materials have been under investigation, but iron oxide is the most commonly used magnetic material due to its biodegradable and biocompatible properties [8]. In biomedical applications, most magnetic nanoparticles are made of either magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) [9]. Fe_3O_4 nanoparticles have several limitations including spontaneous aggregation, low binding capacity of target molecule, and oxidation ability by exposing in the air [9]. Coating the surface of the magnetic

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nanoparticles can solve these problems. Modification the surface of the nanoparticles can protect the core from harmful environments such as oxygen, acids or bases [9]. Coating the surface of Fe_3O_4 nanoparticles with silica can prevent its agglomeration specially in aqueous media [10]. In addition, silica particles have hydrophilic surfaces due to hydroxyl groups [4].

Recently, the modification of core-shell nanoparticle surfaces with small molecules such as folate [11] has been proposed as an alternative targeting strategy with major biomedical advances. In a recent report, Wang et al. designed a folic acid–modified magnetic mesoporous core–shell nanocomposite with a diameter of 350–400 nm [12]. Since, for drug delivery and blood injection, nanocomposite size should be in nanometeric range (<100 nm), investigating an appropriate carrier with an average size of <100 nm is a challenge. Moreover, multifunctional core/shell of Fe_3O_4 nanoparticles is a new option in nanocarrier design [13].

The main objective of this study is designing a new nanocarrier with small size (diameter< 100 nm), convenient access, simple conjugation chemistry, and presumed lack of immunogenicity for transporting Doxorubicin anticancer drug. Fe_3O_4 nanoparticle was selected as the core part of the carrier. Silica shell was applied to prevent the agglomeration of magnetite nanoparticles and amine and folic acid (FA) were used to modify the magnetic/silica core-shell nanocomposite surface. The main novelty of this study is co-functionalizing the surface of Fe_3O_4@$nSiO_2$ with the particle size lower than 100 nm by applying both amine and folic acid functional groups. Although there are some reports about Fe_3O4@$nSiO_2$ functionalized with folic acid [12], but the nanoparticle size is not appropriate for drug injection. Moreover, this is the first study that optimize the process conditions for achievement of maximum DOX loading and entrapment efficiency on the synthesized structure. The behavior of drug adsorption on the NPs from isotherm modeling point of view was investigated, too. Furthermore, the influence of afore mentioned structure was examined in in-vitro environments at a pH of 5.5 and 7.2. Additionally, the in vitro cytotoxicity of free DOX and DOX-loaded Fe_3O_4@$nSiO_2$–NHFA nanocomposites against MDA-MB-231 Cells was investigated.

2. Materials and methods

2.1. Materials

The study materials included iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate anhydrous (NaAc), ethylene glycol (EG), 1,2-ethylenediamine (ETH), ethanol, ammonia (25–28%), tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), ethyl-[3-(dimethylamino) propyl]-carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), folic acid (FA), Dimethyl sulfoxide (DMSO), toluene, phosphate buffer solution (pH = 7.4), acetic acid, and sodium hydroxide. All chemicals were purchased from Merck Company. Doxorubicin anticancer drug (Mw = 543.52 g/mol) was purchased from Sigma Company. Monolayer breast cancer cells (MDA-MB-231) were obtained from Pasteur Institute of Iran.

2.2. Synthesis of magnetic Fe_3O_4 nanoparticles

The magnetic nanoparticles (MNPs) were prepared in a solvothermal method [11]. 1 g of $FeCl_3 \cdot 6H_2O$ is dissolved in 30 mL of EG to make a clear solution, and followed by the addition of NaAc (3 g) and ETH (10 mL). The mixture was stirred vigorously for 30 min and then transferred to a Teflon-lined stainless-steel autoclave and sealed to be heated at 200 °C. After a reaction of 8 h, the autoclave was cooled to the room temperature. The resultant black magnetite particles were washed with acetone and water several times, before being dried at the room temperature.

2.3. Synthesis of Fe_3O_4@$nSiO_2$ nanocomposite

The core-shell structured Fe_3O_4@SiO_2 nanoparticles were synthesized by a modified Stober sol-gel process [14]. 0.1 g of Fe_3O_4 particles were treated using 50 mL of HCl (0.1 M) solution under ultra-sonication for 20 min. Subsequently, the treated particles were collected by a magnet, washed with deionized water, and then dispersed in a mixture solution of ethanol (80 mL), deionized water (20 mL) and concentrated aqueous ammonia (1.0 mL) (28 wt%). Then, 0.1 g of TEOS was added drop wise to the solution. After being stirred at room temperature for 6 h, the product was collected with a magnet and washed several times with ethanol and water before being dried at 60 °C for 6 h.

2.4. Surface modification of Fe_3O_4@$nSiO_2$

To perform the surface modification of Fe_3O_4@$nSiO_2$ with the folic acid, it is necessary to prepare the folate–APTES conjugate in advance. APTES is the most commonly used for amino-functionalization of silica-based materials. Attachment of APTES molecules was performed by silanization reaction [15].

As such, 1 mg of folic acid and 5 μL of APTES were mixed in 1 mL of DMSO which contained 30 mg of NHS and 50 mg of EDC and then stirred for 2 h [16]. NHS and EDC are commonly used as an activating reagent for carboxylic acids [17]. Then, the folate–APTES conjugate was added to a flask containing 4 mL of toluene and 1 mL of Fe_3O_4@$nSiO_2$ solution (20 mg nanoparticles suspended in 1 mL DMSO) and stirred for 20 h at room temperature. Finally, the functionalized sample was centrifuged and washed with toluene before dispersion in the ethanol [16].

In the other words, folic acid grafting has been considered as a functionalization procedure based on silanization and carbodiimide activation. At first, surface cleaning and hydroxyls exposition have been performed in order to activate the surface for further functionalization. Due to the silica based of the surface, it is easy to expose a significant amount of hydroxyl groups by simple water based washings. An amino silane was grafted to expose hydroxyl groups. Finally, folic acid was attached to expose amino groups via carboodimide activation of its carboxylic group (see Scheme 1).

2.5. Drug loading

Some important parameters on the drug loading and entrapment efficiency like the influence of nanocomposite mass, the pH of drug solution, solution volume, contact time and the initial concentration of the drug were optimized in a batch system. One factorial experiment was applied for this aim.

In these experiments, a certain amount of adsorbent (Fe_3O_4@$nSiO_2$–NHFA nanocomposite) (5 and 10 mg) was transferred to the reactor containing the specific volume of doxorubicin aqueous solution (10, 20, 30 and 50 ml) by determined initial concentration (10, 20, 30 and 50 ppm) at the constant pH (2, 6, 10). After stirring for a specific time (12, 24, 48 and 72 h), the DOX-loaded sample was magnetically collected and washed. The supernatants were collected for the UV absorption measurement to determine the amount of DOX loaded and entrapment efficiency. The wavelength of $\lambda = 488$ nm was applied for DOX detection in UV visible test.

The drug loading content and entrapment efficiency were determined by the following formula [17]: