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Ultrasound-assisted synthesis of pH-responsive nanovector based on PEG/chitosan coated magnetite nanoparticles for 5-FU delivery

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ABSTRACT

pH-responsive magnetic carriers at the nanoscale are one of the most important agents for the targeted treatment of cancer. In this study, Fe_3O_4 nanoparticles were prepared by co-precipitation method and functionalized with three types of PEG using ultrasound waves. PEGlated particles were modified with chitosan shell through ultrasound-assisted double emulsion method. The prepared material which was used as a pH responsive carrier for pinpointed 5-FU delivery. The chemico-physical properties of prepared nanoparticles have been investigated. Results demonstrated that pure Fe_3O_4 had a mean diameter of 20 nm with the regular spherical shape which was increased after modification step depending on the type of PEG. 5-FU loading properties and releasing behaviors studies in different pHs which showed that 5-FU can be efficiently loaded in the Fe_3O_4@Cs-PEG. Also, in the case of release, the amount of 5-FU released at pH = 5.8 is noticeably higher compared to the released amount at pH = 7.4 in all three samples at any distinct time. For instance at pH = 7.4, 27% of the 5-FU was released from the Fe_3O_4@Cs-PEG2 during 48 h; as the pH decreases to 5.8, the cumulative amount of 5-FU released enhanced to 52%. The *in vitro* MTT assay results demonstrated that the cell viability decreases in all synthesized nanoparticles as the pH medium of MCF-7 culture became to 5.8. For example, cell viability of Fe_3O_4@Cs-sPEG decreased from 44 ± 2% to 36 ± 1.9% at a concentration of 5 (μg/ml) as the pH varied from 7.4 to 5.8.

1. Introduction

Magnetite nanoparticles are one of the most functional vectors in therapeutic and diagnostic approaches. They play an important and undeniable role in drug delivery systems because of great magnetization values and small size [1]. Compared to body cells, the nanoparticles have a much smaller size and can be entered into the cells, which by intracellular or extracellular interaction with genes, enzymes and receptors may lead to cell death [2,3]. Since the series of intracellular events that leads to the formation of cancer cells occur at the nanoscale, nanotechnology can be used to diagnose and treat them. Functional ability and capability to respond to the magnetic field make magnetic nanoparticles to be introduced as a useful carrier for the targeted diagnosis and treatment of cancer. Recently, great attention has been concentrated on Fe_3O_4 nanoparticles due to their great biocompatibility, drug targeting, and imaging [4,5]. However, these nanoparticles tend to accumulate a lot because of powerful magnetic dipole-dipole interactions. Therefore, in order to enhance the stability, they were usually modified by oxides, metallic nanoparticles, organosilanes, surfactants and polymers [6]. Surface-modification not only improves the stability of magnetic nanoparticles but also provides the conditions for grafting special targeting agents and stimuli-responsive polymer on the surface of the carrier for drug delivery systems. Magnetic nanovectors are formed from a magnetic core to ensure an appropriate response to the magnetic field and organic shell to provide the desired sensitive part towards environmental conditions. Moreover, the multi-valent system can form from grafting different polymers which may lead to significantly improved the efficiency of drug loading [7]. However, the permeability of the drug through the cell membrane in the drug loaded nanoparticles compared to the free drugs is lower.

Chitosan is one of the most biocompatible and biodegradable polymers with positively surface charge containing reactive groups (hydroxyl and amine) [8]. Because of the positively charged of chitosan, cellular uptake and cell adhesion of chitosan to negatively charged cell membranes are very high which is very favorable for the treatment of solid tumor [9]. Therefore, the carrier made of chitosan has been extensively used in pharmaceutical and biological applications. However, due to great mucoadhesive properties, the magnetic nanoparticles modified with chitosan in the process of blood circulation absorbed into normal cells [10]. Clearly, this process is absolutely inappropriate for a targeted drug delivery therapy. According to studies published so far, few studies have addressed this point to modify

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magnetic nanoparticles with chitosan.

Poly(ethylene glycol) (PEG) is a hydrophilic polymer which is widely used in *in vitro* and *in vivo* studies [11,12]. By modifying the surface of magnetic nanoparticles with PEG, reticuloendothelial system (RES), enzymatic degradation, and toxicity of particles decreases and water solubility and stability of nanoparticles significantly improves, which leads to an increase in the circulation half-life in the body. In addition to the above-mentioned points, many scientists have focused on new approaches to modify iron nanoparticles via chitosan [13].

In this study, chitosan was used as pH-responsive section and PEG was utilized to improve RES and circulation half-life of the functionalized Fe_3O_4 nanoparticles. Many methods have been proposed to modify Fe_3O_4 nanoparticles with chitosan including co-precipitation, polymer microgel template, and blending procedures. Unfortunately, these methods result in reaching particle size to the micrometer scale and losing their application in intracellular drug delivery [14]. To overcome this problem, a new approach was adopted so that as-synthesized Fe_3O_4 nanoparticles were first modified with different kinds of linear and star-shaped PEG via Michael addition.

Ultrasound, as a novel technology, can lead to agitate nanoparticles in a solution media and reduce the size of particles which provide greater controllability on the morphology of the nanoparticles, especially on the magnetic specimens such as Fe_3O_4 [15–17]. Aggregated magnetic nanoparticles can be broken apart and well dispersed in the media.

In order to physically coat pH-responsive chitosan and loading 5-FU to synthesized Fe_3O_4@PEG, W/O/W multiple emulsion was applied which ultrasound irradiation plays an important role in this process. The double emulsions are prepared by a two-step emulsification process using two surfactants: a hydrophobic one (Span) designed to stabilize the interface of w/o internal emulsion and a hydrophilic one (Tween) for the external interface of the oil globules for w/o/w emulsions. Synthesized Fe_3O_4@PEG in W/O state was dispersed ultrasonically and added to the aqueous solution contained chitosan, thus, long-term stable chitosan coated Fe_3O_4@PEG (Fe_3O_4@Cs-PEG) nanoparticles are prepared through ultrasound treatment.

The chemico-physical properties of Fe_3O_4@Cs-PEG were characterized by Fourier transform infrared (FT-IR), thermogravimetric analysis (TGA), vibrating sample magnetometer (VSM), transmission electron microscopy (TEM), scanning electron microscope (SEM), and dynamic laser light scattering (DLS). The ability of Fe_3O_4@PEG/Cs as carrier to load and release of 5-Fu was investigated by *in vitro* measuring.

2. Materials and methods

2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ammonium hydroxide (NH_4OH), 3-Aminopropyltriethoxysilane (APTES), polyethylene glycol (MW = 1000, 2000 $gmol^{-1}$), succinic anhydride (97%), dimethylaminopyridine (DMAP), span 60 (sorbitan monostearate), and tween 60 (polyoxyethylene sorbitan monostearate) were obtained from Merck Chemical Co. Chitosan (Mn = 3 kDa) from shrimp shell with 75% degree of deacetylation was purchased from Sigma. All other reagents and solvents were of analytical grade and directly used without any purification. It should be noted that linear PEG was purified by azeotropic distillation in toluene prior to use.

2.2. Methods

2.2.1. Preparation of sPEG from polyoxyethylene (20) sorbitanmonolaurate (tween-20)

Star shaped polyoxyethylene (sPEG) was synthesized according to the literature with some changes [18]. Briefly, 8 g of polyoxyethylene (20) sorbitan monostearatev were dissolved in 20 ml of THF in a round bottom flask and then 1 g of KOH was added as hydrolysis agent. After refluxing about 24 h, the solution was concentrated and added to a mixture of acidic water/hexane (1:1). The aqueous phase in which the sPEG is dissolved was separated by a separation funnel from hexane. Next, the aqueous phase was neutralized with HCl and extracted with dichloromethane. In order to ensure the purification of the sPEG, this process was repeated further three times with yield 86%.

2.2.2. Preparation of carboxyl terminated star shaped PEG (HO-sPEG-$(COOH)_3$)

The reaction procedure was done according to the reported literature [18]. Briefly, a solution containing 0.3 g (3 mmol) succinic anhydride and 0.4 ml (3 mmol) of triethylamine in 10 ml of THF was dropwise added to a stirred solution of 1 mmol of sPEG in 10 ml of anhydrous THF for 12 h at 75 °C. The solvent was evaporated by a rotary evaporator and the obtained dark yellow viscous liquid was dissolved in acidic water (pH = 3). Then, the carboxyl terminated sPEG was extracted by using CH_2Cl_2 for further three times. Finally, CH_2Cl_2 was evaporated under reduced pressure to obtain a pale yellow colored viscous liquid product with a yield around 72%.

2.2.3. Preparation of acrylated-sPEG-$(COOH)_3$

Acryloyl chloride (0.08 mL, 1 Equiv) was dropwise added to a stirred solution of OH-sPEG-$(COOH)_3$ (1.245 g, 1 mmol) and triethylamine (1 mmol) in 20 ml of CH_2Cl_2 for about an hour at −5 °C under nitrogen purging and then reaction continued for 12 h. The obtained solution was filtrated to remove precipitated triethylamine and the filtered solution was washed with diluted NaOH and then deionized water. The solution was concentrated and precipitation in diethyl ether with 68% yield.

2.2.4. Preparation of carboxylated linear PEG

In order to convert hydroxyl end groups of linear PEG (1000 and 2000) to carboxyl groups, dried PEG (1 mmol), succinic anhydride (0.1 g, 1 mmol), triethylamine (0.13 mL, 1 mmol), and dimethylaminopyridine (DMAP) (0.122 g, 1 mmol) were added to anhydrous dioxane (40 mL) and reacted for about 48 h at room temperature. The residue was dissolved in CH_2Cl_2 and the product was washed for further three times with acidic water at pH = 3 in order to eliminate unreacted succinic anhydride. Finally, the solid product of carboxylated PEG was gained via precipitation into diethyl ether with 83% yield [19].

2.2.5. Preparation of Fe_3O_4 magnetic nanoparticles

Naked Fe_3O_4 nanoparticles were synthesized according to our previously reported method by chemical co-precipitation of Fe^{2+} and Fe^{3+} ions with a molar ratio of 1:2 [19]. Briefly, 2.4 g of $FeCl_3{\cdot}6H_2O$ and 0.8 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 30 mL of deionized water under continuous N_2 purge at 70 °C and vigorous stirring followed by dropwise addition of 10 mL of $NH_3{\cdot}H_2O$ to the reaction mixture until the color of mixture turned to black and kept reacting for 90 min to complete the reaction. At the end, the synthesized Fe_3O_4 nanoparticles were separated by a magnet and washed by water and ethanol for further three times with 89.3% yield.

2.2.6. Preparation of amine-terminated Fe_3O_4 nanoparticles

The surface of Fe_3O_4 nanoparticles was aminated by using 3-aminopropyltriethoxysilane via a silanization reaction [19]. Briefly, a homogeneous suspension of Fe_3O_4 nanoparticles (3.7 g) in toluene (40 mL) were prepared by using ultrasound and, then, APTES (4.55 ml, 19.5 mmol) was added by syringe and reaction continued for 5 h at room temperature by vigorous mechanical stirring under the continuous N_2 purge. Next, the obtained aminated magnetic nanoparticles were washed with ethanol and CH_2Cl_2 with 82.4% yield.

2.2.7. Preparation of Fe_3O_4@PEG

Three types of acrylated PEG (sPEG, PEG 1000 and PEG 2000 g/