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Synthesis of doxorubicin-PLGA loaded chitosan stabilized (Mn, Zn)Fe_2O_4 nanoparticles: Biological activity and pH-responsive drug release

Wararat Montha [a], Weerakanya Maneeprakorn [b], Nattha Buatong [a], I-Ming Tang [c], Weeraphat Pon-On [a,*]

[a] Department of Physics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[b] National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand
[c] Department of Materials Science, Faculty of Science, Kasertsart University, Bangkok 10900, Thailand

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ABSTRACT

We have synthesized $Mn_{1-x}Zn_xFe_2O_4$ ((Mn, Zn) ferrite) magnetic nanoparticles (MNPs) having radius of 25 nm to act as platforms for delivering drugs. The $Mn_{0.9}Zn_{0.1}Fe_2O_4$ MNPs exhibit superparamagnetic behavior with large saturation magnetization (M_S). They were encapsulated in polymer so that they can be developed into PLGA-coated chitosan stabilized (Mn, Zn) MNPs, i.e., DOX-PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$ which can serve as an effective carrier of the anti-cancer drug doxorubicin (DOX) whose release would be controlled by the pH in the environment surrounding the cancer tumor. The structure of the as-prepared particles is of a magnetic core-encapsulated by polymer shell layer of around 50 nm thick. At a pH of 4.0, the DOX release within the first 5 h is fast (around 57%). It becomes slower (around 46% over the next 25 h) when the pH is increased to 7.4. The DOX-PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$ (for concentrations lower than 125 μg mL^{-1}) shows lower toxicity against HeLa cells using DOX only. When the DOX-PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$ is increased to 250 μg mL^{-1}, the DOX-PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$ shows greater anti-cancer activity and has satisfactory therapeutic effect. The slow sustained release of the DOX by the drug loaded particles when they are in the physiological pH environment (7.4) of normal tissues and mild toxicity of DOX against cancer cell at low concentration point to the DOX loaded PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$ being safely used for treating cancer. The higher dosage of DOX needed to kill the cancer cells will be released when the synthesized carriers are subject to the pH stimuli surrounding these cells.

1. Introduction

The possible use of nanoparticles as platforms for delivery of therapeutic drugs has recently emerged [1–11]. Much of the research on these drug delivery systems (DDSs) have been for the treatment of cancer. The research is centered on overcoming the main obstacle to the treatment of cancer by chemotherapy, i.e., the destruction of healthy tissue by the therapeutic agents. To overcome this, nanoparticle delivery systems have been developed which have a specific (size) distribution of anticancer agents and which have a mechanism to trigger the release of the therapeutic agents so that the drug release would only lead to an accumulation of the agents at the tumor sites. The typical materials in the platforms used primarily for drug delivery are either polymer or inorganic materials or combinations of these two materials. These materials are then mixed with the anti-cancer drug in a way such that they will be released in response to the physiological environment surrounding a tumor (internal stimuli).

During the past ten years, great efforts had been devoted to synthesizing stimuli-sensitive nanoparticles that would release the agents when certain values of a stimulus based on some physiological parameters (internal stimuli) such as pH, glucose or enzyme [12–20] were encountered. Special attention has been paid to the development of pH responsive nano-particle materials since the pH surrounding different types of tissues are different. The pH in the environment surrounding cancer cells is around 6.5 while the value around acidic organelles is around 4.0 to 5.0 [12,18,22]. As the drugs are released from the DDSs into the acidic region, the toxicity of the drug in the other parts of the body will be lessen.

In addition to external stimuli, external stimuli such as light, magnetic field, heat and ultrasound have been employed to make the particles more useful for applications in the biomedical fields [11–13,21]. Use of an externally applied magnetic field gradient to increase the accumulation of drugs in the target region, one must have magnetic nanoparticles (MNPs). The magnetic materials for these DDSs must be biodegradable and be able to release the drugs in response to internal stimuli (endo/lysomal condition). A material which is the most commonly used in biomedical applications, is the superparamagnetic iron oxide nanoparticles (SPIONs) [23–29]. In the present study, we have

* Corresponding author.
E-mail address: fsciwpp@ku.ac.th (W. Pon-On).

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chosen Mn and Zn substituted ferrite ($Mn_{1-x}Zn_xFe_2O_4$) nanoparticles since they can be easily made to have a small size and the high magnetization needed for them to respond to an external magnetic field [29,30]. Furthermore, Li et al. [30] reported that Mn–Zn ferrite embedded in a polymer matrix exhibited low Tc (Curie temperature) close to the body temperature which would be useful for clinical viability and safety.

To achieve the required pH sensitive controlled release properties, we have pick chitosan (CS) as the agent for triggering the release of the therapeutic agents. There is widespread interest for its potential application in DDSs [31–41] due to them being biodegradable and being a biocompatible cationic polymer with low toxicity. Javid et al. [35], Unsoy et al. [36], Deng et al. [37] and Hu et al. [38] reported that chitosan-coated super paramagnetic iron oxide and silica nanoparticles showed better doxorubicin (DOX) drug-loading, pH controlled release, enhanced cancer cell uptake and inhibited cancer cell growth after exposure to the doxorubicin. To improve the effectiveness of this drug, we have coated the chitosan stabilized (Mn, Zn) MNPs with Poly (lactic-co-glycolic acid) (PLGA). PLGA is a FDA approved biocompatible and bio-degradable polymer [42–44]. For PLGA application, Sivakumar et al. [45] studied on SPION and curcumin encapsulated HER2 targeted PLGA and found that they displayed promising potential anticancer activity by destroying the pancreatic cancer cells. Functionalizing PLGA by chitosan nanoparticles have been widely used for the delivery carrier of various chemo-therapeutic agents to the target site [39–44]. Utilizing the above interesting properties, we aim to synthesize the anti-cancer drug doxorubicin (DOX) into the PLGA-coated chitosan stabilized (Mn, Zn) ferrite nanoparticles (DOX-PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$). We have characterized the physicochemical properties and biological effects of the (DOX-PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$) particles and compared them with those of the free drug. To further investigate the drug delivery application, we studied their pH stimuli controlled release to see whether they would be an effective doxorubicin (DOX) anti-cancer drug carrier, i.e., released the doxorubicin (DOX) at the target sites.

2. Materials and methods

2.1. Materials

Ferric chloride ($FeCl_3$ anhydrous), manganese nitrate ($Mn(NO_3)4H_2O$) and zinc nitrate ($(Zn(NO_3))_2\ 6H_2O$) were obtained from UNIVAR (Australia). The sodium dodecyl sulfate (SDS), Na_2SO_4, citric acid, and ethylene glycol (EG) were obtained from Fluka (Switzerland). Glutaraldehyde (UNILAB) and Poly (lactic-co-glycolic acid) (PLGA) 85/15 with molecular weight of approximately 190,000–240,000, Chitosan (CS, deacetylation degree 90%, Mv 3.8×10^5) with 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and other biological reagents were purchased from Sigma-Aldrich (USA).

2.2. Fabrication of the PLGA@CS@(Mn, Zn) ferrite nanoparticles

The PLGA-coated chitosan stabilized (Mn, Zn) ferrite nanoparticles were synthesized in a two-step process based on the electric attraction between the positively charged ($-NH_3^+$) ions on surface of the chitosan and the negative charged carboxyl group ($-COO^-$) radicals of the PLGA. First, chitosan coated citric acid functionalized (Mn, Zn) ferrite nanoparticles are made. The chains on the chitosan are needed for the formation of the layer which would be sensitive to the pH stimuli. To stabilize surface and to increase the drug adsorption by the colloidal CS@(Mn, Zn) ferrite nanoparticles, Poly (lactic-co-glycolic acid) (PLGA) was coated onto them to form the core–shell (PLGA@CS@(Mn, Zn) ferrite nanoparticles. The inclusion of PLGA assisted in making the outer shell degradable.

2.2.1. Preparation of chitosan-stabilized (Mn, Zn)Fe_3O_4 nanoparticles

Magnetic $Mn_{1-x}Zn_xFe_2O_4$ nanoparticles were made using the chemical co-precipitation method with x value adjusted from 0.1 to 0.9. Typically, 50 mL of mixed solution containing manganese nitrate ($Mn(NO_3)\ 4H_2O$), zinc nitrate ($(Zn(NO_3))_2\ 6H_2O$) and ferric chloride in their respective stoichiometry was first prepared. 10 mL EG and 5 mL HCl (0.2 M) were then added into the mixture under vigorous stirring. The solution were then stirred for an additional 30 min at room temperature. Then, in a 250 mL three-neck round-bottom flask equipped with a magnetic stirring bar, 3 g NaOH and 0.54 g SDS were dissolved in 25 mL of distilled water at 80 °C for 30 min. The precursor solution was injected into the flask slowly and the stirring was continued for another 30 min under N_2 at 80 °C. The precipitate was collected by exposing the final solution to them to an external magnetic field. The concentrated precipitates were physically removed from the solutions and washed three times with ethanol and deionized water. The rinses were then freeze dried to obtain the nanoparticles.

We used the MNPs having the highest magnetic moments as determined by our VSM measurement (Fig. 3). Our next step was the fabrication of the chitosan-stabilized (Mn, Zn)Fe_2O_4 particles (CS@$Mn_{1-x}Zn_xFe_2O_4$) and the detailed procedures combined with previous reports [20,30,40]. We selected x = 0.1, i.e., $Mn_{0.9}Zn_{0.1}Fe_2O_4$ as the magnetic precursor. Surface modification of these magnetic nanoparticles was carried out by first treating it with citric acid. Citric acid (CA) (0.05 M) and the $Mn_{0.9}Zn_{0.1}Fe_2O_4$ NMPs were mixed together in a three-neck round-bottom flask having a magnetic stirring bar inside. The mixture was stirred for 1.5 h at 85 °C under N_2 atmosphere. At the end of reaction, the carboxylic functional magnetic particles were washed with deionized water for three times. The synthesized nanoparticles were freeze-dried and stored at −20 °C for further studies.

The chitosan stabilization was achieved by dispersing the CA-modified $Mn_{0.9}Zn_{0.1}Fe_2O_4$ nanoparticles into 15 mL of chitosan solution (CS) (1 g of chitosan dissolved in 100 mL of 2.5 wt.% acetic acid). This was achieved by rapidly stirring the mixture using a magnetic stirrer for 30 min at room temperature. At the end of the mixing, 3 mL glutaraldehyde solution (GD, 25 wt.%) was added in the solution and stirred for an additional 4 h. Finally, the CS-stabilized magnetic nanoparticles were collected by magnetic bar and washed three times with ethanol and deionized water, respectively. The obtained products were by freeze dying and stored at −20 °C for further studies.

2.2.2. Preparation of PLGA-coated chitosan stabilized (Mn, Zn)Fe_3O_4 nanoparticles and doxorubicin drug loading (DOX-PLGA@CS@(Mn, Zn) ferrite)

50 mg CS-modified $Mn_{0.9}Zn_{0.1}Fe_2O_4$ nanoparticles were added to a solution of PLGA (50 mg in 0.5 mL chloroform) and doxorubicin (1 mg/mL). This was mixed with a magnetic stirrer for 4 h. The mixture was then stirred continuously overnight at 4 °C so that the organic solvent (chloroform) would evaporate. The DOX encapsulated PLGA@CS@$Mn_{0.9}Zn_{0.1}Fe_2O_4$ nanoparticles were collected by centrifugation with the supernatant being collected in order to determine the concentration of drug remaining in it could be determined. The resulting products were freeze-dried and stored in a refrigerator at 4 °C for further investigations.

2.3. Physicochemical characterization of the PLGA-coated chitosan stabilized (Mn, Zn)Fe_3O_4 nanoparticles

The crystal structures of the synthesized powders were determined by powder X-ray diffraction (XRD) (Bruker diffractometer, Model D8 Advance) using the Cu Ka radiation and operating at 40 kV with 40 mA current. The XRD patterns were scanned from 2θ = 20°–70° at a scanning speed of 1 s per step with an increment of 0.037° per step. For the FT-IR absorption measurements of the with and without DOX-loaded PLGA@CS@(Mn, Zn) ferrite, the powders were mixed with KBr and pressed into pellets under a pressure of 10 tons for 1 min. The pellets were analyzed using a FT-IR spectrophotometer (Spectrum GX,