BIODEGRADABLE GELATIN DECORATED Fe_3O_4 NANOPARTICLES FOR PACLITAXEL DELIVERY

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ABSTRACT

The objective of this study is to prepare biodegradable iron oxide nanoparticles with gelatin (GEL) for paclitaxel (PTX) delivery. In detail, Fe_3O_4 nanoparticles were prepared and then coated them with GEL (Fe_3O_4@GEL) conjugate by co–precipitation method. Furthermore, the formation of Fe_3O_4@GEL was demonstrated by Fourier transform infrared (FT–IR) and powder X–ray diffraction (XRD). The superparamagnetic property of Fe_3O_4@GEL was also showed by hysteresis loop analysis, the saturation magnetization reached 20.36 emu.g^{-1}. In addition, size and morphology of Fe_3O_4@GEL nanoparticles were determined by transmission electron microscopy (TEM). The results indicated that Fe_3O_4@GEL nanoparticles were spherical shape with average diameter of 10 nm. Especially, PTX was effectively loaded into the coated magnetic nanoparticles, 86.7 ± 3.2 % for drug loading efficiency and slowly released up to 5 days. These results suggest that the potential applications of Fe_3O_4@GEL nanoparticles in the development of stable drug delivery systems for cancer therapy.

Keywords: superparamagnetic iron oxide, gelatin, paclitaxel, drug delivery.

1. INTRODUCTION

Iron oxide nanoparticles (Fe_3O_4 and γ–Fe_2O_3 NPs), one of the most prominent properties of magnetic nanoparticles (MNPs), has been commonly used in biomedical applications such as *in vivo* magnetic resonance imagining, magnetic–mediated hyperthermia for cancer treatment and tissue–specific delivery of therapeutic agents [1, 2]. Moreover, if the size of magnetic structure is small enough MNPs may have superparamagnetic properties, becoming magnetized in the presence of a magnetic field and showing no magnetization in the absence of magnetic field [3, 4]. However, MNPs tend to aggregate and form a larger cluster because of magnetic dipole–dipole attractions between NPs, which may limit their potential biomedical applications [5]. To overcome this drawback, MNPs were often surface modified with many materials such as silica, carbon, and biopolymers. These modifications are not only improving the chemical stability but also increase the biocompatibility of MNPs [6].

Gelatin (GEL), a protein derived from collagen, possesses numerous useful features such as high solubility, biodegradability, biocompatibility and pH–induced surface charge. It can be

widely used to bind with drug or poly(ethylene glycol) due to its multifunctional groups, like $–NH_2$ and –COOH [7, 8]. Furthermore, fibronections, are large glycoprotein found at cell surface, in extracellular matrices and in blood plasma, have binding sites for a number of other macromolecules, including GEL [9]. Moreover, tumor cell phagocytosis can be significantly enhanced in the presence of GEL [10]. There is some research using GEL–coated MNPs for delivery of chemo– and bio–therapeutic agents. Babita Gaihre and co–workers developed GEL A– and B–coated MNPs as potential nanocarriers for magnetic doxorubicin (DOX) targeting. The results demonstrated that electrostatic interactions between DOX and the coated MNPs played a crucial role in the encapsulation efficiency of the DOX, and pH–responsive drug release of DOX–loaded particles [11]. In addition, Erxi Che reported a magnetic targeted drug delivery system based on magnetic mesoporous silica NPs (MMSN), which were surface coated by GEL layer, for sustained release of paclitaxel (PTX). The results showed that the biodistribution of the GEL–coated MMSN were altered by external magnet, and therefore the higher concentration of these nanocarriers detected in tumor tissues than normal tissues. According to the result of tumor reduction study, the tumor growth of S180 tumor–bearing mice treated with the PTX–loaded carriers were significantly delayed without obvious body weight loss [12]. These results suggested that the GEL–coated MMSN can be used as promising drug carriers for effective delivery of anticancer drugs in the treatment of cancer.

In this study, we report the preparation and characterization of magnetite nanoparticles coated with GEL for PTX delivery system. GEL as polymeric outer layers were prepared and coated on Fe_3O_4 NPs (Fe_3O_4@GEL NPs) which was prepared by the co–precipitation method. The obtained samples were then characterized by transmission electron microscopy (TEM), Fourier transform infrared spectra (FT–IR), powder X–ray diffraction (XRD), and vibration sample magnetometer (VSM). Especially, either drug loading efficiency or drug release behavior of PTX–loaded Fe_3O_4@GEL NPs were also evaluated. This study is expected to improve the stability of magnetic NPs for controlled delivery systems in cancer therapy.

2. MATERIALS AND METHODS

2.1. Materials

GEL type A was obtained from Sigma–Aldrich (St. Louis, MO, USA). Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$, 97%), iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$, 99%) and tetrahydrofuran (THF) were purchased from Merck (Germany). Ammonium Hydroxide (28–30%) was obtained from Tianjin Bodi Chemical Co., Ltd. (China). PTX was supplied by Samyang Corporation (Seoul, Korea). All chemicals and solvents were of highest analytical grade and used without further purification.

2.2. Preparation of Fe_3O_4 and Fe_3O_4@GEL MNPs

Fe_3O_4 NPs were prepared by the chemical co–precipitation method as described previously with some modifications. Initially, an 80 mL mixture of 0.2 M of $FeCl_3.6H_2O$ and 0.1 M of $FeCl_2.4H_2O$ (the molar ratio of Fe^{2+}/ Fe^{3+} = 1:2) was added into the three–necked flask and constantly stirred under nitrogen. NH_4OH solution (10 w/w%) was injected into the mixture and the reaction was maintained at room temperature under vigorously stirring for 1 h until pH reach to 10. The color of the solution changed to dark black. Thereafter, the precipitate was isolated by using a super magnet bar and rinsed with deionized water (deH_2O) several times, sonicated and then freeze–dried to obtain Fe_3O_4 NPs.

Fe_3O_4@GEL MNPs were formed by adding Fe_3O_4 solution (0.4 g of Fe_3O_4 dissolved in 25 mL of deH_2O) drop–wise into GEL solution (0.1 g of GEL dissolved in 20 mL of deH_2O) at room temperature under ultra–sonication for 1 h. During this process, GEL was adsorbed onto the surface of Fe_3O_4 NPs, and the obtained substance was dialyzed by dialysis membrane (MWCO 12–14 kDa, Spectrum Laboratories, Inc., USA) against deH_2O for 36 hours at room temperature. The deH_2O was changed 5–6 times a day and the resulting solution was then lyophilized to obtain Fe_3O_4@GEL.

2.3. Characterization

The size and morphology of Fe_3O_4@GEL MNPs were confirmed by TEM (JEM–1400 TEM; JEOL, Tokyo, Japan) at National Key Lab for Polymer & Composite, HCMUT–VNUHCM. For the purpose of investigating the presence of GEL on the surface of Fe_3O_4 NPs, FTIR analysis (Nicolet Nexus 5700 FTIR, Thermo Electron Corporation, Waltham, MA, USA) of GEL, bare Fe_3O_4 and Fe_3O_4@GEL NPs was carried out with KBr pellets in 400–4000 cm^{-1} range at Department of Agricultural Chemistry, ICT–VAST. The sizes and crystalline structures of Fe_3O_4 and Fe_3O_4@GEL were assessed by Rigaku D/Max–2550 V diffractometer with CuKα radiation (λ = 0.15405 nm, 40 kV, 40 mA) at a scanning speed of 4°/min in the 2θ range from 30° to 70°, at Faculty of Basic Science, The College of Accounting and Finance. Moreover, the magnetization curves of these MNPs were recorded at –15 to 15 kOe at room temperature using EV11 vibrating sample magnetometer (EV11 VSM, USA) at Department of New Materials and Nano structured Materials, HCMIP–VAST.

2.4. PTX loaded Fe_3O_4@GEL MNPs, PTX loading contents and *in vitro* PTX release

In order to prepare PTX–loaded Fe_3O_4@GEL MNPs, 10 mg PTX was dissolved in methanol and 100 mg Fe_3O_4@GEL MNPs was dissolved in deionized water. The PTX solution and Fe_3O_4@GEL solution were mixed together, sonicated for 60 min for 24 h, and then dialyzed with deH_2O to remove free drug and methanol. The resulting solution was freeze–dried to obtain the PTX–loaded Fe_3O_4@GEL MNPs. The PTX loading contents in Fe_3O_4@GEL MNPs were analyzed using a Shimadzu LC–20A Prominence system (Shimadzu, Kyoto, Japan). The injected volume was 10 μL, and the mobile phase (acetonitrile/water = 60:40 v/v) was delivered at 1.00 mL/min. A reverse–phase Fortis C18 column (150 × 4.6 mm i.d., pore size 5 μm; Fortis Technologies Ltd., Cheshire, UK) was used, and column effluent was monitored with a UV detector at 227 nm. The calibration curve for quantification of PTX in Fe_3O_4@GEL MNPs was found to be linear over the standard PTX concentration range of 0–20,000 ng/mL with a high correlation coefficient of R^2 = 0.998. The following equations were used to calculate the drug loading efficiency (DLE) and drug loading content (DLC):

DLE (%) = weight of PTX in Fe_3O_4@GEL MNPs/ weight of PTX feed initially × 100

DLC (%) = weight of PTX in Fe_3O_4@GEL MNPs/ weight of Fe_3O_4@GEL and PTX × 100

In vitro release of PTX from Fe_3O_4@GEL MNPs was performed in phosphate buffer saline (PBS) containing 0.5 wt% Tween–80 (0.01 m, pH 7.4) at 37 °C using a dialysis method. One milliliter of this suspension (PTX content, 0.3 mg/mL) was transferred into a dialysis bag (MWCO = 12–14 kDa) and then immersed into 14 mL fresh medium at 37 °C. The samples were placed in an orbital shaker bath, which was maintained at 37 °C and horizontally shaken at 100 rpm. At predetermined time intervals, 14 mL of the released medium was withdrawn, filtered (pore size = 0.20 μm), and replaced with an equal amount of fresh medium. Following

lyophilization of the collected medium, the amount of PTX released from Fe_3O_4@GEL MNPs was determined using high performance liquid chromatography (HPLC).

3. RESULT AND DISCUSSION

After preparation of Fe_3O_4 NPs by the chemical co–precipitation, the obtained nanoparticles were coated with GEL, which was then characterized by TEM, FT–IR, XRD, and VSM. TEM image of Fe_3O_4@GEL NPs (a) and its particle size distribution (b) are shown in Figure 1. The Fe_3O_4@GEL NPs were nearly spherical in shape with average diameter of 10 nm. Besides, these MNPs still maintained the morphological property of Fe_3O_4 particles without aggregation or fusion. These results implied that GEL would be a promising surface–hydrophilic layer for improving the dispersibility of MNPs.

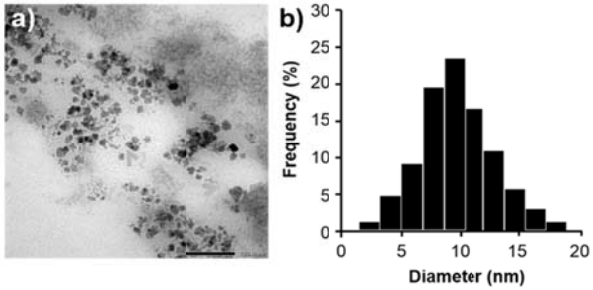

Figure 1. (a) TEM image and (b) particle size distribution of Fe_3O_4@GEL.

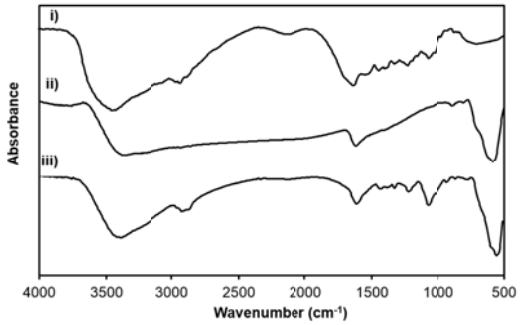

Figure 2. FT–IR spectra of (i) GEL, (ii) Fe_3O_4, and (iii) Fe_3O_4@GEL.

Fourier–transform infrared (FT–IR) spectra of (i) GEL, (ii) Fe_3O_4 and (iii) Fe_3O_4@GEL NPs are shown in Figure 2. The spectrum of GEL show vibration bands at 3285 cm^{-1} (N–H stretch coupled with hydrogen bonding), 3085 cm^{-1} (alkenyl C–H stretch), 2956 cm^{-1} (CH_2 asymmetrical stretching), 1631 cm^{-1} (C=O stretch/HB coupled with COO–), 1533 cm^{-1}(N–H bend coupled with CN stretch), 1444 cm^{-1} (CH_2 bend), 1240 cm^{-1} (NH bend), and 1078 cm^{-1} (C–O stretch). Additionally, the characteristic peaks of Fe_3O_4 at 571 cm^{-1} and 578 cm^{-1} could be obtained and the presence of Fe_3O_4 particles were identified by the O–H stretching vibration at 3416 cm^{-1} and 3420 cm^{-1}, which were detected in both Figure 2ii and iii. As compared with the spectrum of Fe_3O_4, there were a strong shift of CH_2 asymmetrical stretching (2917 cm^{-1}) of Fe_3O_4@GEL because of the existence of GEL. The band appearing at 1100 cm^{-1} is related to the C–O stretching of GEL. Additionally, the peaks at around 1240 cm^{-1} and 1444 cm^{-1} corresponds

to the NH and CH_2 bend of GEL. In order words, all those characteristic bands of GEL are presented in the spectrum for Fe_3O_4@GEL either. These results confirmed that GEL was successfully attached onto the surface of Fe_3O_4 NPs.

As shown in Figure 3a, the characteristic adsorption peaks for Fe_3O_4 NPs marked by their indices ((220), (311), (400), (422), (511) and (440)) could be observed in the X–ray diffraction patterns of either (i) Fe_3O_4 NPs or (ii) Fe_3O_4@GEL. These six diffraction peaks are the standard pattern for crystalline magnetite with spinal structure. The insignificant effect of the outer–modifiers on the core of samples were also indicated by XRD data, Fe_3O_4 NPs still maintained their structure after polymeric coating.

Magnetization curves of (i) Fe_3O_4 and (ii) Fe_3O_4@GEL are shown in Figure 3b. The size plays a critical role in its magnetic properties. If the size is small enough, such nanostructures have superparamagnetic properties. The saturation magnetization values (Ms) of Fe_3O_4 NPs and Fe_3O_4@GEL were 69.01 emu.g^{-1} and 20.36 emu.g^{-1}, respectively. These results demonstrated that both structures are superparamagnetic which allow for rapid and easy separation of a number of MNPs in a reaction mixture. More importantly, lower Ms of the coated Fe_3O_4 is the result of the non–layer coated on Fe_3O_4 NPs, GEL. As a result, after coating, Fe_3O_4@GEL NPs exhibited good magnetic separation ability.

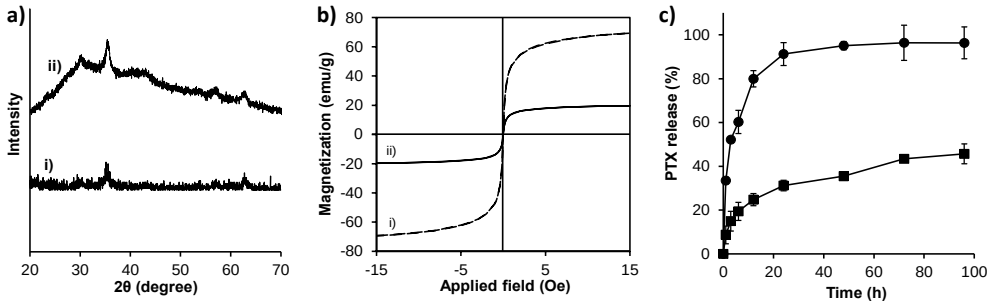

Figure 3. (a) XRD pattern and (b) hysteresis loops of (i) Fe_3O_4 and (ii) Fe_3O_4 @GEL NPs and (c) *In vitro* release profiles of free PTX (circle) and PTX from PTX–loaded Fe_3O_4@GEL (square).

DLE is an important property in drug–loaded nanocarriers and directly affects the therapeutic effect of the system. The higher encapsulation capacity NPs have, the larger number of drug are released at the tumor site. In this study, the DLE of Fe_3O_4@GEL was found to be 86.7 ± 3.2 %. The result demonstrated that Fe_3O_4@GEL with the high DLE have the potential to be delivered more efficiently to tumor tissues. *In vitro* release profiles of free PTX and PTX from PTX–loaded Fe_3O_4@GEL were performed in order to evaluate the stability and release behavior of Fe_3O_4@GEL. As shown in Figure 3c, the prepared Fe_3O_4@GEL showed a long term stable drug release profile up to 5 days. The cumulative release amount of PTX in the initial 3 h was around 10 % as compared with 34% of free PTX. The initial release of PTX could be explained by the PTX molecules, which were absorbed into the outer GEL layer of Fe_3O_4@GEL. Within the first 24 h, 31 % PTX was released from Fe_3O_4@GEL, which was significantly smaller than this amount of free PTX, approximately 91 %. And for the last 5 days, total release amount of PTX from Fe_3O_4@GEL was around 44 %, compared with around 95 % of free PTX. The release behaviors of free PTX and PTX in the Fe_3O_4@GEL were significantly different. These results together confirmed that Fe_3O_4@GEL may serve as stable NPs for controlled drug delivery system.

4. CONCLUSION

In this study, GEL have been successfully coated on Fe_3O_4 NPs with 10 nm in size and high saturation magnetization. The PTX–loaded Fe_3O_4@GEL showed a steady and sustained release profile *in vitro* up to 5 days. These results suggest that the PTX–loaded Fe_3O_4@GEL NPs may serve as stable delivery systems with dual therapeutic effects (hyperthermia combined with chemotherapy) for cancer therapy.

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