

Research Article

Heparin-Pluronic Coated Magnetic Nanoparticles for Doxorubicin Delivery

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Abstract

In this study, superparamagnetic Fe₃O₄ nanoparticles were surface coated with heparin-Pluronic (HP) for Doxorubicin (DOX) delivery. In detail, Fe₃O₄ nanoparticles were prepared and then coated with HP (Fe₃O₄@HP) conjugate by co-precipitation method. Furthermore, the formation of Fe₃O₄@HP was demonstrated by fourier transform infrared (FT-IR) and Powder X-ray diffraction (XRD). The superparamagnetic property of Fe₃O₄@HP was also showed by hysteresis loop analysis, the saturation magnetization reached 24.92 emu g⁻¹. In addition, sizes and morphologies of Fe₃O₄ and Fe₃O₄@HP nanoparticles were spherical shape with average diameter of 19 nm as compared with Fe₃O₄ nanoparticles (12 nm), were determined by transmission electron microscopy (TEM). Especially, DOX was effectively loaded into the coated magnetic nanoparticles, 66.9% ± 2.7% for drug loading efficiency and slowly released up to 120 h. These results suggest that the potential application of Fe₃O₄@HP nanoparticles in the development of stable drug delivery system for cancer therapy.

INTRODUCTION

Magnetic nanoparticles (MNPs) have been used as an effective agent that can be targeted to specific organs, tissues, and cells in biomedical applications, such as magnetic resonance imaging (MRI), drug delivery systems (DDS), hyperthermic treatment, and gene delivery [1]. Superparamagnetic iron oxide nanoparticles (SPIO NPs), in particular, are the primary choice for biological and biomedical applications due to their biocompatibility, chemical stability, and superparamagnetic behaviour. However, there are several inherent drawbacks of SPIO NPs including weak physiological stability, fast blood clearance from the circulation, and lack of target specificity, which may limit their potential clinical application [1,2].

Surface modification, one of the most outstanding approaches, have been studied for the purpose of overcoming disadvantages of SPIO NPs, such as amphiphilic molecules, bi-functional polymeric ligands, or biomolecules. Among different types of synthetic and natural macromolecules for stabilizing magnetic particles, natural macromolecules containing proteins and polysaccharides are more promising owing to their excellent biocompatibility and biodegradability. Particularly, heparin (Hep) has been attracting a great attention for improving dynamic stability of MNPs because of its high biocompatibility, relevant biodegradability, and low toxicity. Hep not only has a variety of biological functions

due to the specific interaction with protein in its domain [3], but also shows extra- and intra-cellular interactions that may enhance the bioactivity of drugs. Moreover, it has a variety of therapeutic activities including anticoagulant activity, inhibition of angiogenesis, and tumor development [4,5]. Lately, it was investigated that Hep showed anti-cancer activity in the progress of tumor by inducing apoptotic cell death [6]. For instance, Javid Amaneh and co-workers determined the anti-cancer effect of Doxorubicin (DOX), loaded Hep-based SPIO NPs (SPIO-DOX-HP) on the human cell lines of A2780. Cytotoxicity tests showed that SPIO-DOX-HP had higher cell toxicity than Hep alone [7]. In addition, Mohammad Fazilati reported the anti-neoplastic effect of Hep coated magnetic NPs on the human ovarian cancer cells of CP70. The result demonstrated that Hep-based MNPs have potential applications as anti-cancer drug delivery systems and also have an enhanced anticancer effect [8]. Considering the effectiveness of the modification of MNPs, Pluronic was also used in order to develop better MNPs for anti-cancer therapy. Pluronic, amphiphilic tri-block copolymers, shows drastic sensitization of multidrug-resistant cancer (MDR) tumors to various anti-cancer agents, and enhances the bioavailability of various drugs [9-11]. Shin young Park et al., developed DOX-loaded thiolated Pluronic decorated MNPs for A549 cell lines. DOX-loaded MNPs showed lower cytotoxicities against A549 cells compared to the individual DOX [12]. These experiments indicated the potential

properties of Hep and Pluronic for modification of the surface MNPs in the treatment of cancer.

In this study, we report the fabrication and characterization of magnetite nanoparticles coated with Hep-Pluronic (HP) for DOX delivery system. Fe_3O_4 NPs were prepared by co-precipitation method and further coated by polymeric outer layer HP. The obtained samples were characterized by powder X-ray diffraction (XRD), vibration sample magnetometer (VSM), transmission electron microscopy (TEM), and Fourier transform infrared spectra (FT-IR). Especially, either drug loading efficiency or drug release behaviour of DOX loaded Fe_3O_4 @HP NPs were also evaluated. This study is expected to improve the stability of magnetic NPs for controlled delivery systems in cancer therapy.

EXPERIMENTS

Materials

DOX, succinic anhydride, dimethylamino pyridine (DMAP), 4-morpholinoethanesulfonic acid (MES), 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC), N-hydroxysuccinimide (NHS), Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 97%), iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 99%), oleic acid (OA, 99%), and Pluronic F127 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hep sodium and triethylamine (TEA) were purchased from Acros Organics (Geel, Belgium). All chemicals and solvents were analytical grade and used without further purification.

Preparation of heparin-Pluronic (HP)

HP was prepared by utilizing cross-linking agent as previous report with minor modification. Briefly, DMAP (0.339 g, 3.0 mmol) and TEA (0.281 g, 3.0 mmol) were added into the mixture of succinic anhydride (0.334 g, 3.3 mmol) and Pluronic F127 (35 g, 2.8 mmol) dissolved in 400 mL of dioxane. The mixture was then stirred at 30°C for 24 h under nitrogen atmosphere. Carboxylated Pluronic was produced by evaporation, precipitation in cold diethyl ether, filter and drying overnight under vacuum. Next, the carboxylated Pluronic was conjugated to Hep, 12.5 g (1.0 mmol) of carboxylated Pluronic was dissolved in 140 mL of 0.5 M MES buffer (pH 4.75), immediately followed by addition of EDC (1.2 mmol) and NHS (0.6 mmol). After 15 min, the activated Pluronic solution was added into the Hep solution (2 g of Hep was dissolved into 80 mL of MES buffer (0.5 M)). The reaction was kept for 24 h under stirring at room temperature. The resulting solution was dialyzed using dialysis membrane (MWCO 50 kDa, Spectrum Laboratories, Inc., USA) against distilled water for 4 days at room temperature. Distilled water was changed 5-6 times a day and the resulting solution was then lyophilized to obtain HP conjugate.

Preparation of Fe_3O_4 and Fe_3O_4 @HP MNPs

Fe_3O_4 NPs were prepared by chemical co-precipitation method described previously with some modifications [13]. Initially, 80 mL mixture of 0.2 M of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.1 M of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (the molar ratio of $\text{Fe}^{2+} : \text{Fe}^{3+} = 1:2$) were 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 super magnet bar and then rinsed with deionized water several times.

In order to prepare Fe_3O_4 @HP MNPs, 300 mg of HP was dissolved in 50 mL of deionized water. Then, 154 mg of Fe_3O_4 dissolved in 50 mL of deionized water was added into the previous solution under ultra-sonicated at room temperature for 6 h. The HP conjugates were adsorbed on the surface of the nanoparticles during this process and the obtained substance was centrifuged and lyophilized for further use.

Characterization

The magnetization curves of the magnetite-polymer nanoparticles were measured with a vibrating sample magnetometer (EV11, USA) at room temperature from -15 to 15 kOe. The crystalline structure of the Fe_3O_4 and Fe_3O_4 @HP and their sizes were assessed using Rigaku D/Max-2550 V diffractometer at a scanning rate of 4°/min in the 2 θ range of 30-70° ($\lambda = 0.15405$ nm, 40 kV, 40 mA). FT-IR analysis of bare Fe_3O_4 and Fe_3O_4 @HP was carried out using the FT-IR spectrophotometer (Nicolet 5700, Thermo Electron Corporation, MA, USA) with KBr pellets in the range of 500-4000 cm^{-1} to investigate the presence of HP coating on the surface of Fe_3O_4 NPs. Morphology and size of the resulting magnetite nanoparticles were confirmed with the transmission electron microscope (TEM, JEM-1400, Tokyo, Japan).

DOX loaded Fe_3O_4 @HP MNPs, DOX loading contents and in vitro DOX release

In order to prepare DOX loaded Fe_3O_4 @HP MNPs, 10 mg of DOX and 100 mg Fe_3O_4 @HP MNPs were dissolved in 10 mL of deionized water. Next, the mixture was sonicated for 60 min for 24 h and then dialyzed by distilled water for removing free-drug. The resulting solution was freeze-dried for obtaining DOX loaded Fe_3O_4 @HP MNPs. The drug loading efficiency (DLE) and drug loading content (DLC) were quantified using a UV-Vis spectrophotometer (NIR-V670-JASCO, Japan) and presented by equation (1) and (2), respectively:

$$\text{DLE} = \frac{\text{weight of fed drug} - \text{weight of unloaded drug}}{\text{weight of fed drug}} \times 100 \quad (1)$$

$$\text{DLC} = \frac{\text{weight of fed drug} - \text{weight of unloaded drug}}{\text{weight of drug loaded } \text{Fe}_3\text{O}_4 \text{ @HP}} \times 100 \quad (2)$$

In vitro release of DOX from Fe_3O_4 @HP MNPs was performed in PBS buffer (0.01 M, pH 7.4) at 37°C using dialysis method. First, 1 mL of DOX loaded Fe_3O_4 @HP MNPs suspended in PBS (DOX content, 0.3 mg/mL) was transferred to dialysis bag (MWCO 12-14 kDa, Spectrum Laboratories, Inc., USA) and immersed it into 14 mL of 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 released DOX was determined using UV-Vis spectrophotometer.

RESULT AND DISCUSSION

After preparation of Fe₃O₄NPs by chemical co-precipitation, the obtained nanoparticles were coated with HP to form Fe₃O₄@HP, which was then characterized by XRD, VSM, TEM, and FT-IR. As shown in Figure 1a, the characteristic adsorption peaks for Fe₃O₄NPs marked by their indices ((220), (311), (400), (422), (511) and (440)) could be observed in the X-ray diffraction patterns of either Fe₃O₄NPs or Fe₃O₄@HP. These six diffraction peaks are the standard pattern for crystalline magnetite with spinal structure. The insignificant effects of the outer-modifiers on the core of samples were also indicated by XRD data, Fe₃O₄NPs still maintained their structure after polymeric coating. In addition, the crystallite sizes D (311) of Fe₃O₄ and Fe₃O₄@HP particles were 2.53 and 2.52 Å, while the particle sizes were 12 and 18 nm calculated by Debye-Scherrer method, respectively.

Magnetization curve of Fe₃O₄ and Fe₃O₄@HP is shown in Figure 1b. The size of magnetite nanoparticles is a crucial factor in its magnetic properties. If the size is small enough, such nanostructures have superparamagnetic properties. The saturation magnetization value (Ms) of Fe₃O₄NPs and Fe₃O₄@HP were 68.9 emu g⁻¹ and 24.92 emu g⁻¹, respectively. These results demonstrate that both structures are superparamagnetic, which allow their rapid and easy separation from the reaction mixture. Furthermore, lower Ms of the coated Fe₃O₄ is the result of the non-magnetic layer coated on Fe₃O₄ NPs. As a result, after coating, Fe₃O₄@HP NPs are more suitable for magnetic separation for DDS.

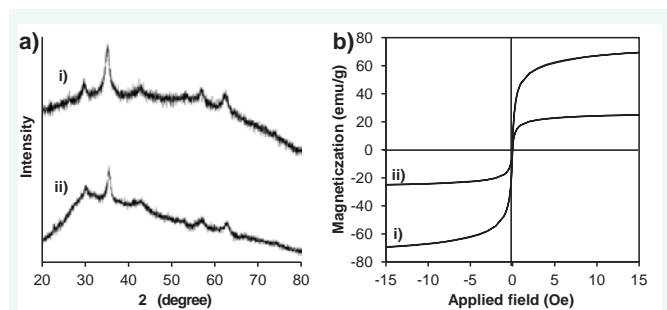


Figure 1 (a) XRD pattern and (b) hysteresis loops of (i) Fe₃O₄ and (ii) Fe₃O₄@HP NPs.

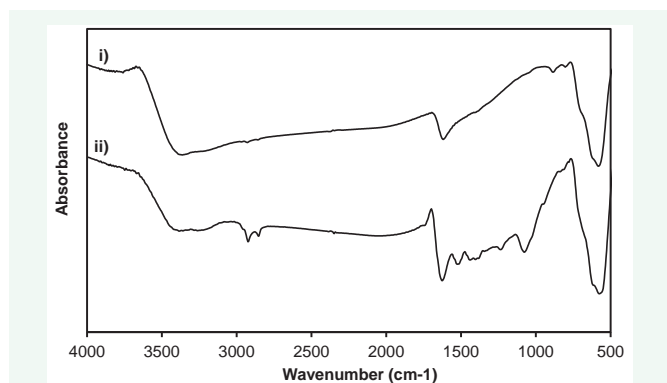


Figure 2 FT-IR spectra of Fe₃O₄ (i) and Fe₃O₄@HP (ii).

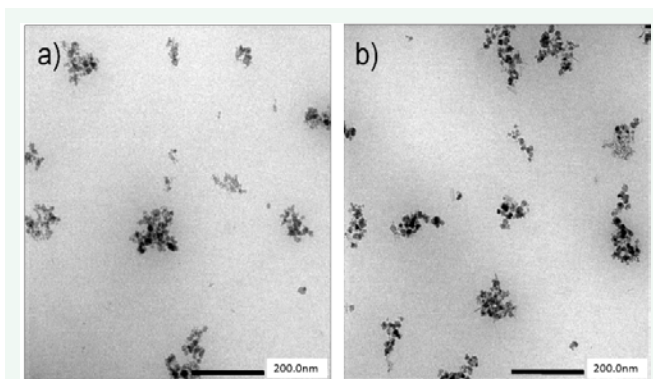


Figure 3 TEM images and particle size distribution of Fe₃O₄ (a) Fe₃O₄@HP (b).

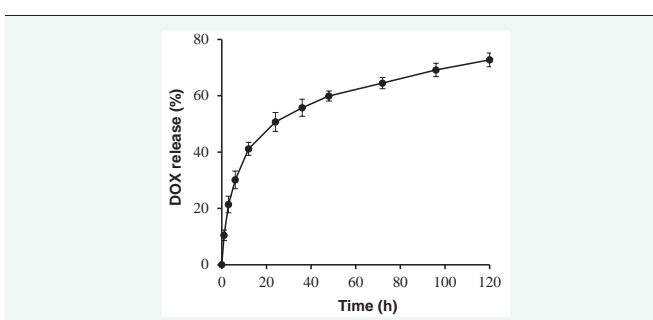


Figure 4 In vitro release profiles of DOX from Fe₃O₄@HP MNPs.

Fourier-transform infrared (FT-IR) spectra of (i) Fe₃O₄ and (ii) Fe₃O₄@HP NPs are shown in Figure 2. The characteristic peaks of Fe₃O₄ at 571 cm⁻¹ and 578 cm⁻¹ could be obtained in Figure 2i and 2ii. Moreover, the presences of Fe₃O₄ particles were identified by the O-H stretching vibration at 3416 cm⁻¹ and 3420 cm⁻¹, which were detected in both Figure 2i and ii. As compared with the spectrum of Fe₃O₄, there was a strong shift between Fe-O stretching (570-578 cm⁻¹) of Fe₃O₄ because of the existence of HP. The band appearing at 1400 cm⁻¹ is related to the C-O of alcoholic groups of Hep. Additionally, the peaks at around 2925 cm⁻¹ and at 3430 cm⁻¹ corresponds to the C-H stretching band and the hydroxyl groups of HP, which was overlapped by the O-H groups stretching vibration of Fe₃O₄NPs, respectively. These results demonstrate that HP was synthesized and attached onto the surface of Fe₃O₄NPs.

Figure 3 exhibits morphologies and particle sizes of Fe₃O₄ and Fe₃O₄@HP NPs. As shown in Figure 3, Fe₃O₄@HP NPs still maintain the morphological property of Fe₃O₄ particles except for larger particle size. It is stated that HP polymeric matrix was covered on the surface of Fe₃O₄ particles (Figure 3b). The structure of Fe₃O₄@HP NPswas looser and its size were bigger. The average particle size of Fe₃O₄ and Fe₃O₄@HP NPs were found to be 12 nm and 19 nm in diameter, respectively.

Magnetic nanoparticles have mostly used to control drug release with an external magnetic field. The corporation of magnetic particles vibrate under a magnetic force and the vibration accelerates drug release from the nanoparticles. Figure 4 shows the release profile of DOX from Fe₃O₄@HP NPs in PBS

pH 7.4 at 37°C, the nanoparticles exhibit sustained-release profile up to 120 h. The drug release ability of Hep-DOX conjugate based nanoparticle without external magnetic field in the same PBS buffer had been studied in Wenchuan's research [15]. The release of DOX from nanoparticle was only 20% at pH 7.4 after 56 h incubation. Compared when the present of Fe₃O₄ which created the magnetic field, the release ability is significant increase. The magnetic field showed the high potential in controlling drug release after incubation with PBS at 37°C for 48 h, the cumulative release amount of DOX from Fe₃O₄@HP was 59.9%. Due to the high interaction between DOX and HP, the release of loaded drug from the coated MNPs can therefore be taken a longer time for DOX to diffuse through the nanoparticles into the aqueous medium. Taken together, Fe₃O₄@HP NPs may serve as a promising candidate for controlled drug delivery system.

CONCLUSION

In this study, HP have been conjugated and successfully coated on Fe₃O₄ NPs with 19 nm in size and high saturation magnetization. The DOX-loaded magnetic nanoparticles showed a steady and sustained release profile in vitro up to 120 h. These results suggest that the DOX-loaded Fe₃O₄@HP NPs may serve as a promising platform of MNPs with dual therapeutic effects (hyperthermia combined with chemotherapy) for cancer therapy.

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