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Magnetic nanoparticles (MNPs) covalently coated by PEO–PPO–PEO block copolymer for drug delivery

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ABSTRACT

A stable drug carrier has been prepared by covalently coating magnetic nanoparticles (MNPs) with PEO–PPO–PEO block copolymer Pluronic P85. The particles were characterized by TEM, XRD, DLS, VSM, FTIR, and TGA. A typical product has a 15 nm magnetite core and a 100 nm hydrodynamic diameter with a narrow size distribution and is superparamagnetic with large saturation magnetization (57.102 emu/g) at room temperature. The covalently-coated Pluronic-MNPs (MagPluronics) were proven to be stable in different conditions, such as aqueous solution, 0.2 M PBS solution, and pH 13.5 solution, which would be significant for biological applications. Furthermore, MagPluronics also possess temperature-responsive property acquired from the Pluronic copolymer layer on their surface, which can cause conformational change of Pluronics and improve load and delivery efficiency of the particles. The temperature-controlled loading and releasing of hydrophobic model drug curcumin were tested with these particles. A loading efficiency of 81.3% and a sustained release of more than 4 days were achieved in simulated human body condition. It indicates that the covalently-coated MagPluronics are stable carriers with good drug-loading capacity and controlled-release property.

1. Introduction

Magnetite nanoparticles (MNPs) have been widely applied in biomedical fields due to low toxicity and good compatibility [1–4]. It is one of FDA-approved materials used in vivo and has been utilized in magnetic separation of biological entities, hyperthermia treatment, magnetic resonance imaging (MRI), and drug delivery [2]. MNPs with high magnetization values and ultrafine particle sizes can be manipulated by external magnetic field (MF) by which human issues can be penetrated, indicating the possibility of applying them for targetable drug delivery [5,6].

To develop a more suitable drug delivery system composed of magnetite nanoparticles, some work needs to be done to diminish particle agglomeration, reduce cytotoxicity, and improve compatibility [6–10]. Many materials have been considered for functionalization of MNPs to achieve these goals, including polymers, biomolecules, surfactants, and inorganic materials [4,11]. Pluronic copolymer, among them, is especially promising for the modification. Pluronic is a kind of amphiphilic surfactant that has high molecular weight and presents excellent compatibility [12]. It is one of the very few synthetic polymers approved by US FDA for use as food additive and pharmaceutical ingredient [13]. Pluronic-involved formation of MNPs can yield particles with better aqueous-dispersity and less toxicity [14–16]. Furthermore, Pluronic can act as more than modification material by incorporating into membranes and translocating into cells, changing various cellular functions such as mitochondrial respiration, ATP synthesis, activity of drug efflux transporters, apoptotic signal transduction, and gene expression [12]. Recent studies have also confirmed several biological response modifying activities of Pluronics, such as causing drastic sensitization of multi-drug resistance (MDR) tumors to various anticancer agents, enhancing drug transport across the blood brain and intestinal barriers [17] and preventing adsorption of proteins to avoid uptake of nanoparticles by reticuloendothelial system (RES) [18].

In previous researches, various attempts have been made to modify the surface of magnetite nanoparticles with Pluronic copolymers. Ting-Yu Liu et al. synthesized MNPs embedded in

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Pluronics by co-precipitation process which yields smaller particles [13]; Qin et al. made MNPs with hydrophobic surface and then coated them with Pluronics via phase transfer [19]; Jain et al. encapsulated MNPs in Pluronics with oleic acid as "vector molecules," by coating second layer of Pluronics onto first layer of oleic acid via hydrophobic interaction [18,20]; Chen et al. developed MNPs with Pluronics bound to particle surface via electrostatic interaction [21]; Park et al. prepared MNPs modified by thiolated Pluronic molecules [22]. Despite many improvements about MNPs these researches have made, there are still problems need to be solved. The temperature-responsive property of co-precipitated carriers is limited because of incomplete coverage of Pluronics on particle surface; Pluronic layer, in most cases, is modified onto the particle by non-covalent interactions such as electrostatic interaction and hydrophobic interaction and is therefore easy to detach from the particle surface in harsh conditions in vivo, resulting in short circulation time and instant drug release of the carriers [23]; Synthesis of thiolated Pluronics involves several procedures with complex principles and is consequently difficult to carry out. These drawbacks of Pluronic-coating methods restrict the efficacy of MNPs-mediated drug delivery and have been concerns among some biologists and pharmacologists [4,11].

Our goal here is to develop a way to fabricate a drug delivery system composed of magnetite nanoparticles, which are covalently bound with Pluronic copolymers, resulting in smaller particle size, better dispersion, improved stability, higher drug-loading capacity, and sustained release. In this investigation, the physical, chemical, and physiological properties of the Pluronic-modified MNPs (MagPluronics) were characterized in detail by methods including FTIR, UV–Vis, XRD, DLS, TGA, VSM, and TEM. Curcumin was used as a model drug in our tests. After the loading of curcumin, the drug carriers were tested for drug-loading efficiency and sustained drug-release, which are important profiles for successful and efficient drug delivery.

Curcumin is a natural polyphenol found in turmeric, which has long been part of the daily diet in Asian countries, and has not been reported to cause any toxicity. The past decades have witnessed a resurgent interest in the pharmacological effects of curcumin for biomedical applications, including cancer, neurological diseases, cardiovascular diseases, and various other inflammatory diseases [24]. Unfortunately, the poor solubility and stability of curcumin in aqueous solution have greatly limited its bioavailability and clinical use [25,26]. By encapsulating the molecules of hydrophobic drug like curcumin into an aqueous nanoparticle system we made from MagPluronics, there could be a better chance that the solubility and bioavailability of such drugs can be increased enough to be supplied with their full potential.

2. Material and methods

2.1. Materials

Ethyl ether, hexane, methylbenzene, *n*-butanol, anhydrous sodium sulfate (Na_2SO_4), and ethanol were purchased from Beijing Chemical Reagents Company. Succinic anhydride, N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl) were purchased from Sigma–Aldrich. Fetal calf serum and RPMI1640 medium were purchased from Gibco. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer P85 (EO_{26}–PO_{39}–EO_{26}, MW 4600) was kindly donated by BASF. Curcumin was kindly donated by the general hospital of Chinese People's Liberation Army. Amine-groups modified (AGM) magnetic nanoparticles were obtained from Beijing GiGNano Biointerface Company. The average size of the particles is 15 nm, with saturation magnetization >70 emu/g and amino group content of about 0.2 mmol/g.

2.2. Synthesis route

2.2.1. End-group modification of Pluronic P85

The end-group modification of Pluronic P85 was carried out using a modified protocol of Custers et al. [27]. Briefly, 50 g Pluronic P85 surfactant was purified by dissolving the polymer in 200 g diethyl ether. The mixture was filtered by suction filtration, and the residue was thoroughly dried in a vacuum drying chamber. 40 g purified P85 was then dissolved in 160 ml toluene, followed by the addition of 5.04 g succinic anhydride. The reaction was performed with gentle agitation for 2.5 h at 90 °C and another 2.5 h at 100 °C, respectively. After the reaction, the solvent was evaporated in a rotatory evaporator, and the product was dissolved in water (20 wt.%). Then the product was extracted with *n*-butanol and dried overnight with a layer of anhydrous sodium sulfate. Finally, *n*-butanol was evaporated in a rotatory evaporator, and the product was redissolved in 200 g diethyl ether again, followed by suction filtration and drying in vacuum condition at room temperature. The final product was obtained after further purification by dissolving it in water and then putting the solution in dialysis membrane (MWCO = 3500) for 3 days (water was changed every 6 h). 1 wt.% solution of the product was titrated with 0.5 M NaOH for subsequent characterization, and the result showed that the product has about 5.0×10^{-4}mol acid groups per gram surfactant. The fact that P85 is polydisperse makes it difficult to give an absolute number for the conversion, despite the purification steps.

2.2.2. Synthesis of MagPluronics

For the purpose of binding the MNPs, which have amino groups on their surface, to modified Pluronic P85 molecules with carboxyl end groups, EDC was introduced as the cross-linker. EDC is one important member of carbodiimide family. In synthetic organic chemistry, compounds containing the carbodiimide functionality are dehydration agents and are often used to activate carboxyl acids toward amide or ester formation [28]. EDC is a very efficient cross-linking agent for polymers containing carboxyl groups in water, and EDC·HCl used in our experiments is water-soluble, eco-friendly, and non-cytotoxic [29].

400 mg Modified Pluronic P85 and 900 mg EDC·HCl, together with 525 mg NHS, were dissolved in 50 ml water and stirred for 6 h [30,31], followed by addition of an aqueous dispersion of AGM nanoparticles (500 mg particles in 100 ml water). The pH of the mixture was adjusted to 5.8, and the triangular flask containing the mixture was put in an oven oscillator (150 rpm) for 72 h at room temperature. The final product was washed five times with distilled water, centrifugated, and stored for subsequent use. For the physical characterization of Fourier transform infrared (FTIR) spectrum, X-ray diffraction (XRD) pattern, and thermo-gravimetric analysis (TGA), different kinds of nanoparticles were lyophilized to obtain dry samples.

2.3. Characterization of MagPluronics

2.3.1. Hydrodynamic particle size and zeta potential

The hydrodynamic diameter and size distribution of MagPluronics, and zeta potential of MNPs, MagPluronics and curcumin-loaded MagPluronics were determined using Delsa™ Nano Zetasizer (Beckman Coulter Inc., Brea, California) based on dynamic light scattering (DLS) principle. For each of these measurements, 4 ml of 500 μg/ml nanoparticle suspension was processed with ultra-sonication for 3 min, put in the sample cell and measured for 3 min at 25 °C. An average diameter and distribution of particles size were based on the average of three readings (each reading = 70 runs). The zeta potential of various nanoparticles was reported from three runs of each sample.