Temperature-Responsive Magnetite/PEO−PPO−PEO Block Copolymer Nanoparticles for Controlled Drug Targeting Delivery

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Received July 10, 2007. In Final Form: September 26, 2007

In this study, temperature-responsive magnetite/polymer nanoparticles were developed from iron oxide nanoparticles and poly(ethyleneimine)-modified poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO−PPO−PEO) block copolymer. The particles were characterized by TEM, XRD, DLS, VSM, FTIR, and TGA. A typical product has an ∼20 nm magnetite core and an ∼40 nm hydrodynamic diameter with a narrow size distribution and is superparamagnetic with large saturation magnetization (51.34 emu/g) at room temperature. The most attractive feature of the nanoparticles is their temperature-responsive volume-transition property. DLS results indicated that their average hydrodynamic diameter underwent a sharp decrease from 45 to 25 nm while evaluating the temperature from 20 to 35 °C. The temperature-dependent evolution of the C−O stretching band in the FTIR spectra of the aqueous nanoparticles solution revealed that thermo-induced self-assembly of the immobilized block copolymers occurred on the magnetite solid surfaces, which is accompanied by a conformational change from a fully extended state to a highly coiled state of the copolymer. Consequently, the copolymer shell could act as a temperature-controlled "gate" for the transit of guest substance. The uptake and release of both hydrophobic and hydrophilic model drugs were well controlled by switching the transient opening and closing of the polymer shell at different temperatures. A sustained release of about 3 days was achieved in simulated human body conditions. In primary mouse experiments, drug-entrapped magnetic nanoparticles showed good biocompatibility and effective therapy for spinal cord damage. Such intelligent magnetic nanoparticles are attractive candidates for widespread biomedical applications, particularly in controlled drug-targeting delivery.

1. Introduction

Stimuli-responsive nanomaterials offer exciting new opportunities with respect to numerous applications,[1] particularly in biomedical fields such as controlled drug-delivery systems.[2,3] Applying an external stimulus (e.g., temperature, pH, ionic strength, etc.) to such an intelligent system could trigger the release of active agents, which is very helpful in controlling the required concentration or release pattern of drugs.[4−6] If these stimuli-responsive nanomaterials could be introduced with magnetic properties, then they would be target-guided to their desired locations by an external magnetic field; therefore, drug transportation efficiency could be improved significantly, and toxic effects could be reduced as well.[7−12] Such multi-responsive nanomaterials might be ideal candidates for controlled drug delivery. For example, Liu et al. reported magnetic-sensitive poly(vinyl alcohol) hydrogels for controlled drug release by switching magnetic fields on and off.[13] Lecommandous and co-workers developed pH-responsive magnetic nanoparticles from micelles and vesicles of amphiphilic polybutadiene-*block*-poly-(glutamic acid) block copolymer. Affected by a pH change, their hydrodynamic diameter underwent a variation as large as 50% as a result of a helix−coil transition of the PGA block.[14] Among them, temperature-responsive magnetic nanoparticles attracted more attention because the temperature change could be generated by magnetic nanoparticles in an alternating magnetic field (e.g., hyperthermia using magnetic nanoparticles). That is to say, a remote-triggered drug release might be simply achieved without further addition of a stimulus.[15] To date, there are only limited reports on temperature-responsive magnetic nanoparticles, and in most cases, the themosensitive poly(*N*-isopropylacrylamide) (PIPAAm) polymer or its copolymers were employed.[15−23] On the basis of the temperature-sensitive coil-to-globule transition

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10.1021/la702049d CCC: $37.00
Published on Web 11/08/2007

behavior of PIPAAm at the lower critical solution temperature (LCST), the organic part of these magnetic polymeric particles[15−21] or hydrogels[22,23] often demonstrated a hydrophilic−hydrophobic change, which resulted in the on−off dissociation of drug molecules as a function of temperature.

Poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO−PPO−PEO, Pluronic) block copolymer, which consists of hydrophilic PEO segments and hydrophobic PPO segments, is another important class of temperature-responsive copolymers.[24,25] Induced by a temperature change, the block copolymers would self-assemble to form micelles or vesicles at around body temperature.[26] Recent studies have proven several unique advantages of these biocompatible mesostructures as drug-delivery carriers in vivo.[27,28] For instance, Pluronic could interact with multidrug-resistant cells, which results in the sensitization of these cells with respect to the loaded anticancer agents.[27] Furthermore, Pluronic copolymers have been shown to cross many impermeable barriers such as the blood−brain barrier, which provides for enhanced transport of pharmaceutical agents.[28] Pluronic copolymer is also an excellent candidate to tailor the surface properties of inorganic nanoparticles. The PEO segment is very effective in preventing the adsorption of proteins, avoiding the uptake of nanoparitlces by the reticuloendothelial system, and thus the blood circulation time of nanoparticles would be prolonged.[29−31] Besides, the self-association of Pluronic copolymers could be well controlled by adjusting the polymer composition or adding several additives.[32−41] These properties provide the opportunity to modify inorganic nanoparticles with defined property.[42] For example, we have demonstrated the controllable synthesis of gold nanoparticles by modulating Pluronic micelles.[43] If the Pluronic copolymers could be incorporated with the magnetite nanoparticles, then their temperature-responsive properties might be utilized for modulating the drug release rate.

Herein, our aim was to develop novel temperature-responsive magnetic nanoparticles from magnetite nanoparticles and PEO−PPO−PEO block copolymer. Because of the self-assembly of Pluronic copolymers on the magnetite solid surface, the copolymer shell could act as a temperature-controlled "gate" for the upload and release of guest species. Such stimuli-responsive nanomaterials have the following advantages with respect to drug delivery: (a) thermo-controlled release of both hydrophobic and hydrophilic drugs; (b) magnetic targeting by an external magnetic field; and (c) good biocompatibility and long-term aqueous stability.

2. Experimental Section

Materials. Iron(II) chloride ($FeCl_2$•$4H_2O$), iron(III) chloride ($FeCl_3$•$6H_2O$), concentrated ammonium hydroxide (25%), and anhydrous acetonitrile were purchased from Beijing Chemical Reagents Company. Poly(ethyleneimine) (PEI, 2 kDa), 1,1′-carbonyldiimidazole, ibuprofen, and Eosin Y were purchased from Sigma-Aldrich. Poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO−PPO−PEO) block copolymer P123 (EO_{19}-$PO_{69}EO_{19}$, MW 5750) were kindly donated by BASF. Monosialotetrahexosylganglioside (GM-1) aqueous samples (5 wt %, 1.6 kDa) were purchased from Trb Pharma Argentina.

Modification of Pluronic with PEI. Pluronic-*g*-PEI (2 kDa) was synthesized using a modified protocol of Kabnov et al.[44] Briefly, 5 mmol of Pluronic P123 copolymers was first dried by co-evaporation with anhydrous acetonitrile, and then the free terminal hydroxyl groups (10 mmol) of the copolymer were activated by 1,1′-carbonyldiimidazole (3 mmol) in anhydrous acetonitrile. The reaction mixture was agitated for 3 h at 40 °C. The solution was dialyzed three times against 50% ethanol using a Membra-Cel MD34-3.5 membrane (Biocity), and the solvent was then removed in vacuo. After that, 5 mmol of activated Pluronic copolymers was reacted with 25 mmol of PEI (2kDa) in 300 mL of 0.2 M carbonate buffer at pH 8.0. After 24 h, the reaction mixture was again dialyzed three times and then lyophilized.

Conjugation of Pluronic-*g*-PEI to Anionic Magnetite Particles. One hundred millileters of an aqueous solution of iron(II) chloride and iron(III) chloride ($[Fe_{total}] = 0.13$ M with [Fe(II)]/[Fe(III)] = 0.5) containing 0.26 mmol of citrate acid trisodium salt (2% mol/mol citrate/metallic species) was stirred for 30 min under a nitrogen gas atmosphere at 80 °C. Then 20 mL of concentrated ammonium hydroxide (25%) was added to the mixture. After the solution turned black, 50 mL of the prepared Pluronic-*g*-PEI aqueous solution (40 mg/mL) was poured in, and the reactions continued for 2 h. Afterward, the precipitate was separated by magnet and washed three times with distilled water. The resulting nanoparticles were suspended in water for storage.

Characterization. *FTIR Measurement.* FTIR spectra of P123, P123-*g*-PEI, bare Fe_3O_4 nanoparticles, and P123-*g*-PEI conjugated Fe_3O_4 nanoparticles (MagPluronic) were recorded on a Bruker Vecter 22 FTIR spectrometer. Lyophilized nanoparticles was dispersed in KBr and then pelletized before measurements. Temperature-dependent FTIR spectra of a MagPluronic aqueous sample were recorded with a thermocouple inserted into a stainless steel block that hosted a sample cell. The equilibration time for each temperature was 2 min.

TEM Measurement. MagPluronic nanoparticles were characterized by a transmission electron microscope operated at 100 keV (TEM, FEI TECNAI 20). Samples were prepared by placing a drop of MagPluronic aqueous solution onto a Formvar-covered copper grid.

XRD Measurement. The X-ray diffraction (XRD) analysis of lyophilized samples of MagPluronic was carried out with an X'Pert PRO MPD (PANalytical). The X-ray diffraction patterns were taken in the 2θ range from 20° to 70° using Cu Kα radiation, with the instrument operating at 200 mA and 40 kV).

DLS Measurement. The hydrodynamic diameters of MagPluronic nanoparticles were monitored by the dynamic light scattering (DLS) method at a 90° detection angle in the temperature range of 6 to

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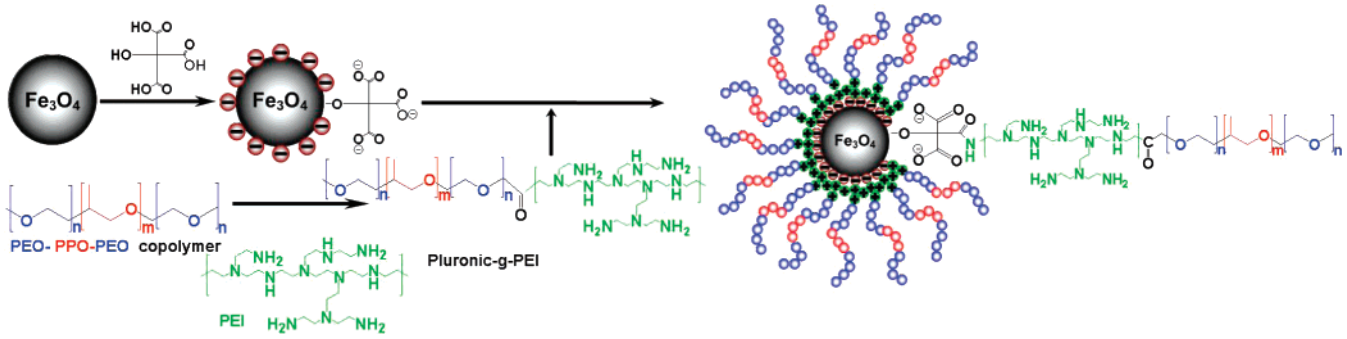

Figure 1. Schematic illustration of the fabrication strategy for MagPluronic nanoparticles

50 °C (Brookhaven Instruments, 90Plus, laser wavelength 659.0 nm). The equilibrium time for each temperature is 20 min. The intensity-averaged diameters were used for data analysis and comparison. The zeta potential of the nanoparticles was determined using the Zetasizer-3000HS analyzer (Malvern Instruments).

TGA Measurement. The number of Pluronic copolymers incorporated into magnetite particles was determined by thermogravimetric analysis (TGA, Netzsch STA 449C). Samples were heated from 30 to 800 °C at a heating rate of 10 °C/min in air.

Measurements of the magnetization of magnetic nanoparticles were carried out with a vibrating sample magnetometer at room temperature (VSM, model 155). The absorbance of the drug was measured on a Lambda Bio 40 UV−vis spectrometer (Perkin-Elmer).

Drug Loading and Release in Vitro. To determine the adsorption isotherms of hydrophilic Eosin Y on the carriers, 1 mL aqueous solutions of MagPluronic (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/mL) were added to 10 mL of Eosin Y aqueous solutions (10 mg/mL). The suspension was stirred for 2 h in a thermostated shaking bath (150 rpm) that was maintained at 0, 20, and 37 °C. The mixture was purified by magnetic separation and centrifugation, and the obtained supernatant was assayed for the loading amount of Eosin Y by measuring the absorbance at 517 nm. Similarly, 1 mL ethanol solutions of MagPluronic (20, 40, 60, 80, 100, 120, 140, and 160 mg/mL) were added to 10 mL of ibuprofen ethanol solutions (1 mg/mL). The suspension in a sealed vial was stirred for 3 days in a thermostated shaking bath (150 rpm) that was maintained at 0, 20, and 37 °C. The mixture was purified by magnetic separation and centrifugation, and the obtained supernatant was assayed for the loading amount of ibuprofen by measuring the absorbance at 272 nm. After the incubation of Eosin Y or ibuprofen with MagPluronic at 0 °C, the drug-loaded carriers were separated from solution by a magnet, quickly washed with water (for Eosin Y) or ethanol (for ibuprofen) two times, and then redispersed into 100 mL of preheated (37 °C) or precooled (0 °C) or 20 °C phosphate-buffered saline (PBS, pH 7.4). The solutions were shaken at 150 rpm at the desired temperature. At a given time, the sample was separated, and a 1 mL aliquot of solution was withdrawn for analysis and then replaced with the same volume of fresh PBS. The release profile at each temperature was measured three times, and then averages and the standard error were calculated.

Animals. Twenty adult female Sprague−Dawley rats weighing 250−300 g were kept in individual stainless steel metabolic cages and maintained in a temperature-controlled room with a 12 h light/dark cycle, with tap water and standard rat chow provided ad libitum throughout the study. All protocols were approved by the Animal Care and Use Committee of Capital University of Medical Sciences, China, and we strictly followed NIH guidelines for the care and use of laboratory animals. All rats underwent complete spinal cord transection at the T9−T10 level, and a lesion cavity approximately 2 mm in length formed between the ends of spinal cord. After that, they were randomly divided into four groups: control group I without any treatment, control group II treated with MagPluronic nanoparticles (10 mg), control group III treated with 50% PEI (2 kDa) aqueous solutions, and an experimental group treated with 10 mg GM-1 loaded MagPluronic nanoparticles (0.14 mg GM-1/mg MagPluronic) and fibrin glue introduced into the lesion cavity. Four weeks later, the rats were sacrificed and analyzed with an immunohistochemistry method (rabbit polyclonal antineurofilament antibody, NF-200). The specimens were processed for electron and light microscopy (Leica-DMLA).

3. Results and Discussion

Fabrication Strategy. Generally, the direct immobilization of a polymer with few reactive functional groups such as Pluronic copolymer to magnetite nanoparticles could not be easily achieved. The concept of the proposed temperature-sensitive magnetic nanoparticles composed of a magnetite nanoparticle core and an amphiphilic PEO−PPO−PEO block copolymer shell (MagPluronic) is schematically illustrated in Figure 1. Here, Pluronic P123 $(EO)_{19}(PO)_{69}(EO)_{19}$ was selected as one example (the cmc value at 25 °C is 0.03 wt %, and the cmt value of a 5.0 wt % aqueous solution is about 24.5 °C.[24]) but was not limited to illustrate the fabrication of MagPluronic carriers and their applications. Monodisperse superparamagnetic iron oxide nanoparticles were first synthesized by chemical coprecipitation, and hydroxyl groups of their surfaces were exchanged with citrate ligands to provide excess carboxylate groups and make the surface anionic.[45] The terminal hydroxyl groups of P123 were covalently grafted to the amino groups of poly(ethyleneimine) (PEI) polymer, which is a randomly branched cationic polyelectrolyte with every third amino nitrogen atom being protonated.[44] Then, the PEI-modified P123 polymers were conjugated to the anionic surface of magnetite nanoparticles through strong ionic interactions. The excess amino groups of PEI are confined at the inner layer and provide functional groups for further interaction with guest molecules such as drugs. Consequently, therapeutic molecules would be encapsulated inside and protected by the outer layer of the Pluronic copolymer.

Figure 2 schematically shows how the proposed MagPluronic works for targeted drug delivery and controllable release. A distinctive feature of Pluronic block copolymers is their temperature-responsive micellization over a broad temperature range depending on the composition and molecular weight when they freely dissolve in aqueous solution.[24−26] Although the copolymers are now embedded, it is conceivable that a micellization-like self-assembly would occur on magnetite surfaces. At low temperature, the copolymer chains are fully extended by interacting with water, and thus the polymer shell is open. Therefore, it is much easier for guest molecules to enter into the vector, and the uploaded amount could be enhanced. Increasing temperature above the critical micellization temperature (cmt) will induce the copolymer dehydration and stronger interactions between the polymer blocks themselves (e.g., PPO/PPO, PEO/PEO).[32−41] The polymer shell is contracted, closing and forming compact barriers that would effectively inhibit the diffusion of

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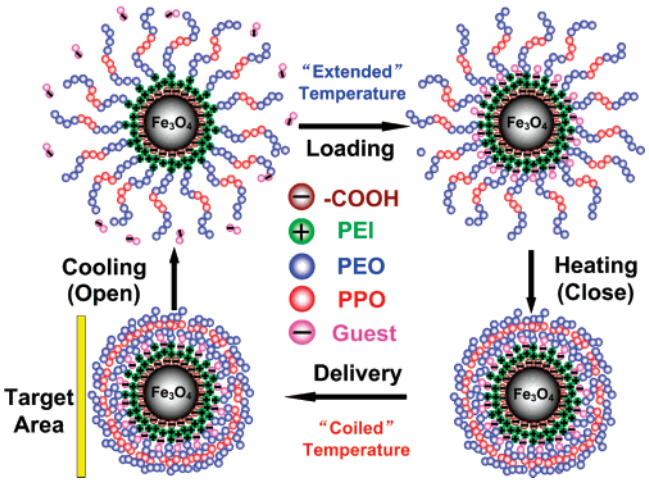

Figure 2. Schematic representation of temperature-responsive MagPluronic nanoparticles working as a targeted drug-delivery system with controlled payload and release. The polymer shell composed of the PEO−PPO−PEO block copolymer acts as temperature-responsive gate. A temperature change around the cmt would trigger the opening (extended polymer conformation) and closing (coiled polymer conformation) of the polymeric shell, which favors or prevents the transit of guest substances, respectively.

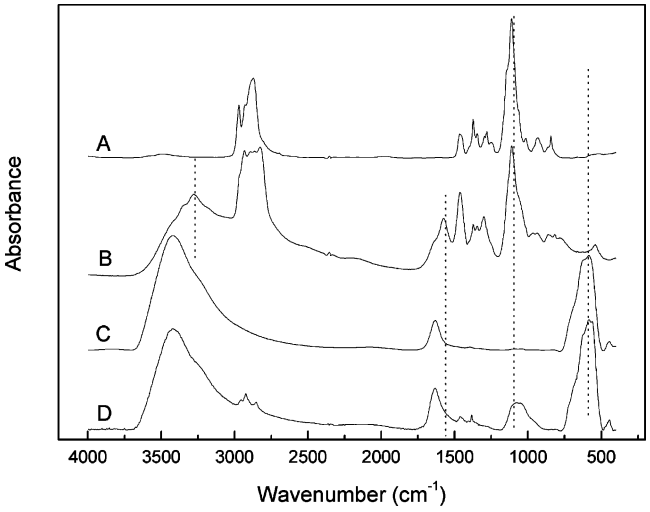

Figure 3. FTIR spectra of (A) pure Pluronic P123, (B) P123-*g*-PEI, (C) bare Fe_3O_4 nanoparticles, and (D) Fe_3O_4 nanoparticles conjugated with P123-*g*-PEI (MagPluronic nanoparticles).

loaded molecules out of the polymer matrix. Because of the magnetic sensitivity introduced by iron oxide, the magnetic nanoparticles could be directed to the desired locations by an external magnetic field. After that, the subsequent cooling would open the polymeric shell again, and then the cargo could be downloaded. Moreover, the temperature transition region could also be tailored by using different types of Pluronic copolymers because the cmt value varies over a wide range depending on the composition of the polymers, such as the PPO/PEO ratio, and the molecular weight.[24−26] The evaluation of the success of each step and the characterization are described in the following text.

Characterization of MagPluronic Nanoparticles. The modification of Pluronic and incorporation into magnetite particles were confirmed by Fourier transform infrared (FTIR) spectroscopy. Figure 3 shows the FTIR spectra of pure Pluronic P123 copolymer (A), PEI-modified P123 (B), bare magnetite nanoparticles synthesized in the presence of pure P123 (C), and MagPluronic nanoparticles (D). The broad peak between 1200

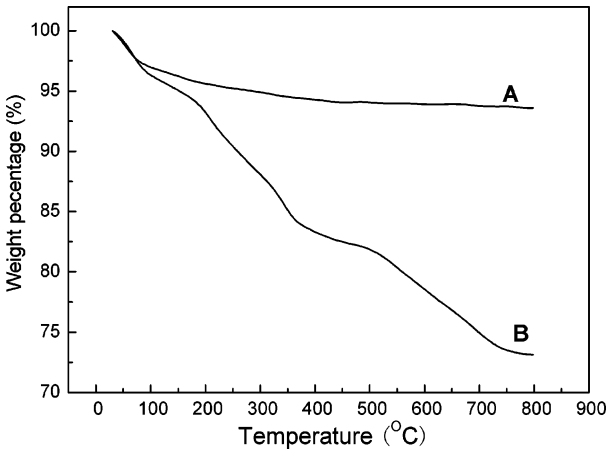

Figure 4. TG analysis of (A) bare Fe_3O_4 nanoparticles and (B) Fe_3O_4 nanoparticles conjugated with P123-*g*-PEI (MagPluronic nanoparticles).

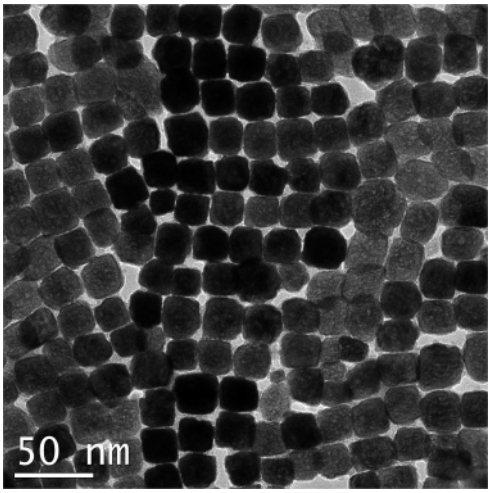

Figure 5. TEM image of MagPluronic nanoparticles.

and 1000 cm^{-1} is due to the C−O stretching vibration, which is a feature of the Pluronic copolymer. The bands around 3200 cm^{-1} are assigned to the stretching vibration of N−H bonds of PEI, and the bands at 1650 and 1581 cm^{-1} result from the amine band that covalently links PEI and P123 (Figure 3B). The peak around 600 cm^{-1} corresponds to iron oxide particles. It is obvious that P123 was successfully conjugated onto the surface of magnetite nanoparticles by PEI linkage (Figure 3D), but there was no bonding of P123 to the particle surface when the copolymer was not modified (Figure 3C). The conclusions were further validated by thermogravimeric analysis (TGA). As shown in Figure 4, MagPluronic shows a mass loss of about 28 wt % after heating to 700 °C, at which temperature the P123 copolymers decomposed (Figure 4B). In contrast, only about a 5 wt % mass loss was observed for the sample prepared in the presence of pure P123 (Figure 4A). That is to say, the content of PEI-*g*-P123 in MagPluronic is about 23 wt % with respect to the total formulation weight.

A typical product of MagPluronic has an ∼20 nm spherical magnetite core and an ∼40 nm hydrodynamic diameter with a narrow size distribution in water at room temperature, as measured by TEM (Figure 5) and dynamic light scattering (DLS), respectively. The prepared MagPluronic nanoparticles showed good aqueous stability and monodispersity even after 3 months of storage. X-ray powder diffraction (XRD) spectra of MagPluronic nanoparticles exhibits reflex peaks that are in agreement with the known reflex positions of magnetite (Figure 6). Debye−

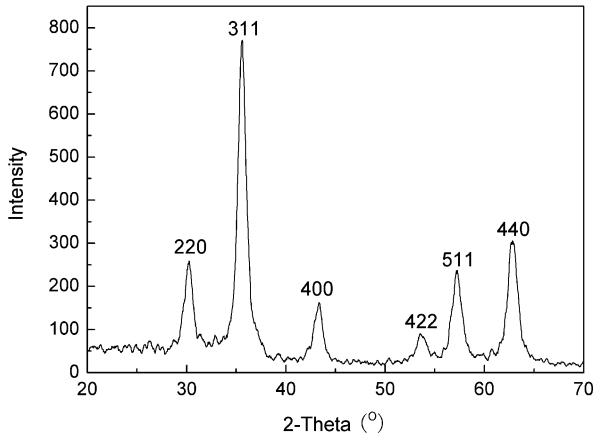

Figure 6. XRD powder pattern of MagPluronic nanoparticles.

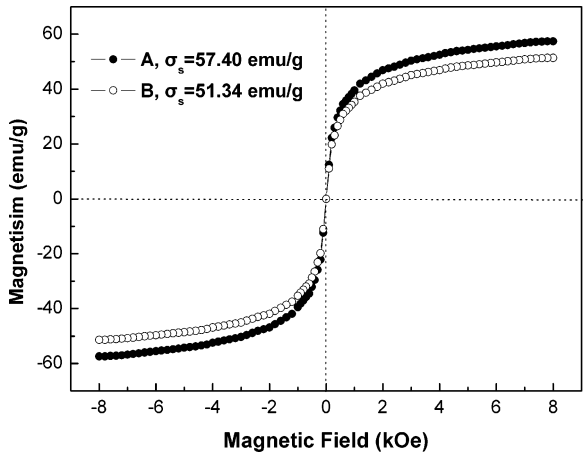

Figure 7. Room-temperature magnetization curves of (A) bare Fe_3O_4 nanoparticles and (B) Fe_3O_4 nanoparticles conjugated with P123-*g*-PEI (MagPluronic). No obvious loss in saturation magnetization was observed after the Pluronic coating was applied.

Scherrer calculations[46] predict an average diameter of 17.6 nm for the magnetite core, which is close to the value obtained from TEM. The magnetic properties of MagPluronic nanoparticles were investigated with a vibrating sample magnetometer (VSM). The saturation magnetization value of MagPluronic is 51.34 emu/g at 25 °C (Figure 7B). No obvious loss of saturation magnetization was observed after Pluronic conjugation compared with that of bare magnetite nanoparticles (Figure 7A). Neither magnetic remanence nor coercivity was observed, which indicates that MagPluronic nanoparticles are superparamagnetic. Superparamagnetic particles no longer show magnetic interactions after elimination of the magnetic field, and therefore aggregation between the particles could be reduced.[8] The zeta potential of citrate-treated pristine magnetite nanoparticles is −39 mV and changed to +36 mV after coating PEI-*g*-P123 as a result of the overcharge of PEI. The positive charge of MagPluronic nanoparticles was completely neutralized after Eosin Y, an anionic hydrophilic dye, (−3 mV), or ibuprofen, an anionic hydrophobic model drug (−4 mV) was encapsulated. The evolution of zeta potentials again demonstrated the layer-by-layer synthesis of MagPluronic nanoparticles and the subsequent uptake of anionic guest molecules.

Temperature Responsivity of MagPluronic Nanoparticles. As shown in Figure 8, the P123 copolymer-conjugated magnetite nanoparticles (2 wt % aqueous solution) exhibit temperature-responsive volume transition behavior in water over the range of 6−50 °C. DLS results indicate that the average hydrodynamic diameter of the MagPluronic particles underwent a sharp decrease from 45 to 25 nm while the temperature was evaluated from 20 to 35 °C. This phenomenon may be attributed to the thermo-induced self-assembly of the immobilized PEO−PPO−PEO block copolymers on the magnetite solid surfaces. Vibrational spectroscopy, especially FTIR spectroscopy, is a very powerful technique for investigating the self-association of amphiphilic polymers and the conformation state of polymer chains during that process.[47] Figure 9 shows the temperature-dependent evolution of the C−O stretching band in the FTIR spectra of the aqueous MagPluronic solution. With temperature increasing, the band shifted to a higher wavenumber from ∼1085 to ∼1100 cm^{-1}, which indicates a dehydration process of the ether backbone of P123 that is due to the broken hydrogen bonds between ether oxygen atoms and water hydrogen atoms.[32,33] The removal of water molecules from the backbone of P123 copolymers would result in the effective clustering of both PPO and PEO blocks. As a result, the polymeric shell of MagPluronic became much more compact at higher temperature and was accompanied by a conformational change from fully extended to highly coiled

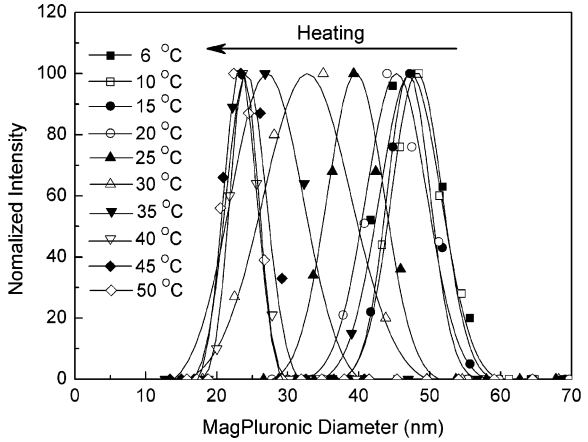

Figure 8. DLS profiles of aqueous MagPluronic nanoparticle solution over the temperature range of 6−50 °C. The hydrodynamic diameters of MagPluronic decreased from 45 to 25 nm while the temperature was evaluated from 20 to 35 °C.

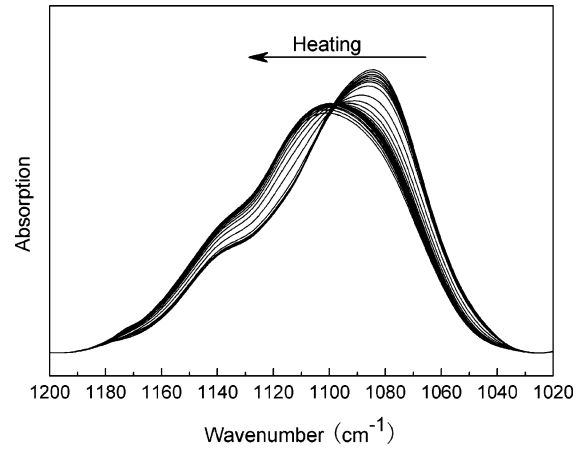

Figure 9. Temperature-dependent evolution of the C−O stretching vibrational band in the FTIR spectra of aqueous MagPluronic solution over the temperature range of 0−50 °C. With increasing temperature, the band shifted to a higher wavenumber, from ∼1085 to ∼1100 cm^{-1}

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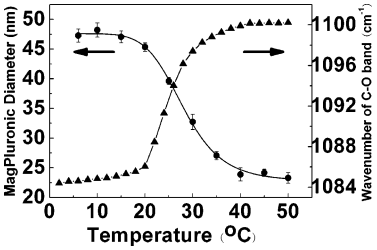

Figure 10. Temperature-responsive transition of the hydrodynamic diameter (left) and C−O stretching band (right) of MagPluronic nanoparticles. Both transformation regions matched exactly.

according to our previous studies on the micellization of Pluronic copolymer.[32−40] The temperature transformation region of the C−O band was exactly matched with that of the hydrodynamic diameter (Figure 10). As a result, we conclude that a temperature change would trigger the opening (extended state) and closing (coiled state) of the polymeric shell during the temperature-responsive volume transition of MagPluroic particles.

It is noteworthy that the temperature transformation region of P123-modified magnetic nanoparticles is around body temperature, which makes them especially suitable for human biomedical applications such as controlled drug delivery. As soon as they were injected into the body, increased temperature (37 °C) would induce the polymeric shell to close, which might result in sustained release of the enclosed drug. A cold-shock treatment below 20 °C might lead to pulsatile release as well. Such cold-shock treatments were reported to be effective at triggering the temperature responsivity of Pluronic, where Park and co-workers succeeded in utilizing cross-linked Pluronic nanocapsules to break up the intracellular endosomal compartment.[48,49]

Controlled Payload and the Release of Drug Molecules. For proof-of-concept experiments, ibuprofen (IB) and Eosin Y (EY) were selected as hydrophobic and hydrophilic model molecules, respectively. The amine groups inside MagPluronic nanoparticles interact strongly with the carboxyl group of IB or EY. To examine whether the temperature-responsive closing and opening of the copolymer shell could be utilized for controlled drug delivery, the payload and release of the two model molecules were performed at 20 and 37 °C, respectively, where the temperature is just below or above the transformation region of the particle volume and copolymer chain conformation (20 to 35 °C). The payload and release at 0 and 20 °C were also compared to test the effects of drug solubility when the copolymer shell is opening. Figure 11a,b shows the adsorption isotherms of IB and EY at 0, 20, and 37 °C. The adsorption of both molecules follows Langmuir patterns at each temperature. The maximum saturated adsorptions at 0 and 20 °C are almost the same, about 0.09 g of IB/g of MagPluronic and 0.22 g of EY/g of MagPluronic, respectively. In contrast, they are 0.038 g of IB/g of MagPluronic and 0.12 g of EY/g of MagPluronic at 37 °C. About a 2-fold payload enhancement was achieved at "extended" temperature (0 or 20 °C) compared with that at "coiled" temperature (37 °C) for both molecules. The release tests in vitro were performed in a phosphate-buffered saline (PBS, pH 7.4) solution on a rocking platform (150 rpm) at the desired temperature. Figure 11c,d indicates the cumulative release profiles of IB and EY from MagPluronic particles at 0, 20, and 37 °C. At extended temperature (0 or 20 °C), almost all adsorbed molecules dissociated within 6 h. In contrast, at coiled temperature (37 °C), only about 20% of the drug molecules were released in 6 h, and more than 95% were released in 3 days. Although the solubility of Eosin Y or ibuprofen almost doubles when the temperature increases from 0 to 20 °C,[50] there is no significant difference in the adsorption curves at 0 and 20 °C because the nanoparticles remained saturated when adsorption was performed at each temperature. The release of Eosin Y or ibuprofen speeded up slightly more at 20 °C than at 0 °C because of the increase in solubility; however, it slowed down tremendously when the temperature was 37 °C. Consequently, compared with the change in drug solubility, the temperature triggered the opening or closing of the polymer shell, which plays a significant role in controlling drug loading and release. Besides, a continual drug release of about 3 days could be achieved in the simulated human body environment, which is particularly required in tumor therapy but is still a challenge for most reported magnetite/polymer nanocomposites (with the bound drug rapidly dissociating within 2 h in most cases).[9,51−53]

The magnetically guided efficiency of MagPluronic nanoparticles was tested by placing a magnet near a glass bottle as shown in Figure 12. The original solution of EY was red (left). EY-loaded MagPluronic was attracted to the magnet within 10 s, and the color of the solution disappeared immediately (right), which indicates an effective magnetic site direction of drug-loaded MagPluronic particles.

In Vivo Mouse Experiments of MagPluronic Particles. The in vivo-targeted drug-delivery efficacy of MagPluronic particles was evaluated by injecting monosialotetrahexosylganglioside (GM-1)-loaded MagPluronic into damaged spinal cords of living rats. GM-1 has been shown to reestablish the functional recovery of central nervous system structures that have suffered damage.[54,55] In past decades, great effort has been made to explore more effective administration routes of GM-1 with respect to spinal cord injury. Generally, GM-1 aqueous solutions were injected intravenously or into the neuron tissues around the lesion cavity; in this way, the efficacy of GM-1 was low, and a large dose was required (50−100 mg/kg).[56−58] The pharmaceutical efficiency of GM-1 might be greatly improved if it could directly act on the spinal cord lesion cavity for a long time. However, it is still a challenge because GM-1 is very soluble in the aqueous environment inside the lesion cavities and quickly flows away with fluid exchange. One solution is to encapsulate GM-1 in some carriers so that GM-1 can be released continually inside the cavity, and the temperature-responsive magnetic nanoparticles seem to be an excellent candidate.

Fifteen adult female Sprague−Dawley rats (250−300 g) underwent complete spinal cord transection at the T9−T10 level, and a lesion cavity approximately 2 mm in length formed between the ends of spinal cord. After that, they were randomly divided into three groups: control group I without any treatment, control group II treated with MagPluronic nanoparticles, and an experimental group treated with 10 mg of GM-1-loaded MagPluronic nanoparticles (0.14 mg of GM-1/mg of MagPluronic). Four weeks later, the rats were sacrificed and were analyzed using the immunohistochemistry method. The specimens were processed for electron and light microscopy. Figure 13 gives the histological cross sections of control case I (A), control case II

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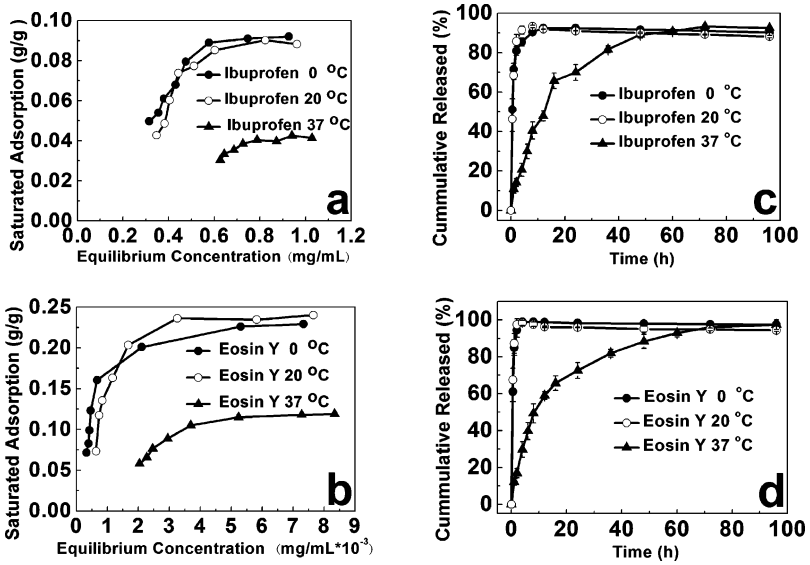

Figure 11. Adsorption isotherms and release profiles of ibuprofen (a, c) and Eosin Y (b, d) at 0, 20, and 37 °C.

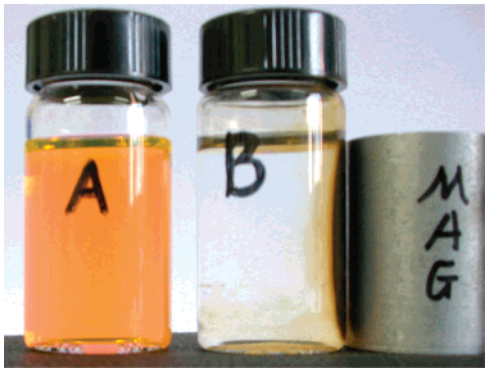

Figure 12. Original aqueous solution of Eosin Y (left) and magnetic capture of MagPluronic loaded with Eosin Y (right).

(B), and the experimental case (C). The spinal cord transaction resulted in a lesion cavity approximately 2 mm in length (A). The lesion cavity still exists after treatment with MagPluronic nanoparticles, and no evidence of the recovery of the lesions was observed (B). In the case of treatment with GM-1-loaded MagPluronic nanoparticles, significant recovery of the spinal cord was observed, which is indicated by the black circle between the two ends of the broken spinal cord (C). Details of all sections are shown in Figure 14. A wealth of nerve fibers regenerated toward the lesion area where GM-1-loaded MagPluronic is located (C). On the contrary, nonregeneration of the nerve fibers was observed in control cases I (A) and II (B). In our experiments, the injected amount of GM-1 is calculated to be about 6 mg/kg, which is much lower than the traditional dose but still resulted in good recovery of the spinal cord injury in the mouse. Therefore, we can conclude that MagPluronic nanoparticles could transport therapeutic molecules to desired locations efficiently.

It is worth mentioning that PEI is known to be toxic because of its high cationic charge. However, in contrast to this, PEI is extensively discussed in the development of efficient nonviral gene carriers, where it could encapsulate genetic material by complexation.[44] Previous studies have proven that many factors affect the cytotoxicity of PEI (e.g., the molecular weight and degree of branching). Generally, low-molecular-weight linear PEI polymer leads to lower toxicity. The toxicity could be reduced or even avoided if PEI was modified with biocompatible polymers (e.g., PEG).[59−61] To test the toxicity of MagPluronic nanoparticles, in addition to the above three groups, another group treated with

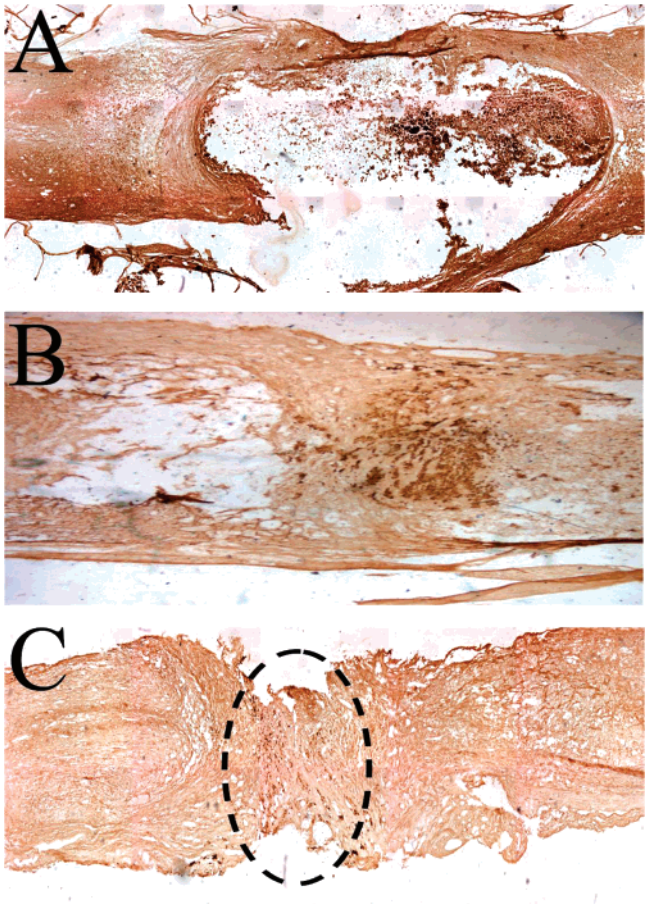

Figure 13. Overview of the histological cross sections of control case I without any treatment (A), control case II treated with MagPluronic nanoparticles (B), and the experimental case treated with GM-1-loaded MagPluronic nanoparticles (C).

PEI (2 kDa) was monitored as control III. All of the rats lived well for at least 4 weeks after injection, and no significant differences were reported with respect to mortality, body weight,

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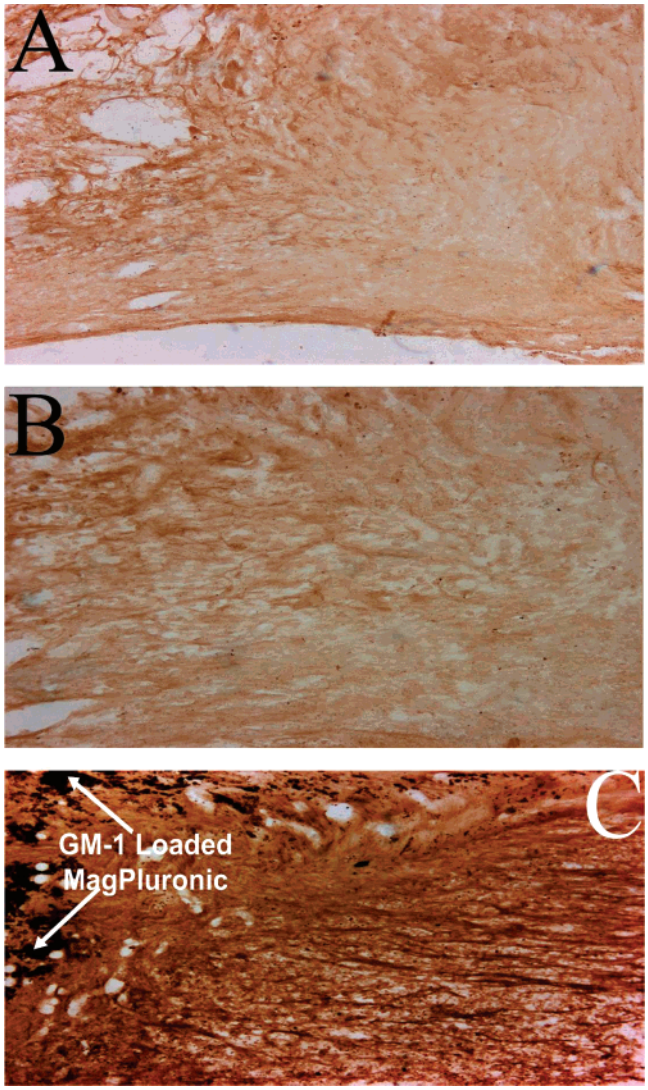

Figure 14. Region details of histological cross sections of control case I without any treatment (A), control case II treated with MagPluronic nanoparticles (B), and the experimental case treated with GM-1-loaded MagPluronic nanoparticles (C).

and feed consumption among the four groups. It is conceivable that both PEI (2 kDa) and MagPluronic nanoparticles are nontoxic because PEI of the lowest molecular weight was used in this study and was modified with biocompatible Pluronic copolymer. However, the drug-loading capacity of MagPluronic nanoparticles was very limited when PEI (2 kDa) was used. To improve the loading capacity, PEI with a higher molecular weight or degree of branching will be of great help because more positive charge could be provided. Then the toxicity of MagPluronic nanoparticles has to be further investigated when different PEI polymers are employed in the future.

4. Conclusions

We have successfully developed a novel kind of temperature-responsive magnetic nanoparticle from iron oxide nanoparticles and poly(ethyleneimine)-modified poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) block copolymer. The nanoparticles have shown good aqueous stability, monodispersivity, and superparamagnetism. The most attractive feature is their temperature-responsive volume-transition property. DLS results indicated that the average hydrodynamic diameter of the magnetic nanoparticles underwent a sharp decrease from 45 to 25 nm around 25 °C. As monitored by temperature-dependent FTIR spectra of an aqueous nanoparticle solution, the reason was attributed to the thermo-induced self-assembly of the immobilized block copolymers occurring on the magnetite solid surfaces, which was accompanied by a conformational change from the fully extended state to the highly coiled state of the copolymer. The uptake and release of both hydrophilic Eosin Y and hydrophobic ibuprofen were well controlled by tuning the opening and closing of the copolymer shell at different temperatures. The sustained release of both molecules for about 3 days was also achieved in a simulated human body environment. GM-1-loaded magnetic nanoparticles showed good biocompatibility and proved to be effective therapeutic effects for spinal cord damage in mouse models. As a result, we can envision that aqueous stability, biocompatibility, and magnetic and temperature-responsive properties of the magnetite/PEO−PPO−PEO block copolymer nanoparticles would endow them with promising potential for widespread utility in biomedicine and biotechnology applications.

Acknowledgment. This work was financially supported by the National Natural Science Foundation of China (nos. 20221603, 20676137, and 20490200) the and National High Technology Research and Development Program of China (863 Program) (no. 20060102Z2049).

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