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Preparation of Biodegradable Magnetic Nanoparticles via a Simple Assembly Process for Targeting Doxorubicin Delivery and MR Imaging

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***Abstract*— In this research, a multi-functional copolymer assembled with superparamagnetic iron oxide nanoparticles (SPIONs) were prepared via a simple assembly process for targeting delivery of doxorubicin (DOX) and magnetic resonance (MR) imaging detection. The multi-functional copolymer, polyamidoamine (PAMAM), was synthesized by Michael addition reaction, where N,N-Bis(acryloyl) cystamine served as cross-linker, DOX, dopamine (DA), folic acid–polyethylene glyco (FA-PEG) and PEG acted as comonomers. Here the PAMAM is directly assembled to the surface of SPIONs by the ligand exchange reaction with SPIONs forming the drug loaded biodegradable magnetic nanoparticles (MNPs-DOX). The hydrophilic PEG moiety provides the nanoparticles colloidal stability and good-dispersity in aqueous solution. Colloidal stability of the MNPs-DOX in aqueous media by their anti-fouling property. TEM images showed that the MNPs-DOX were well dispersed with high stability in water after the ligand exchange process. All the results showed that the prepared biodegradable magnetic nanoparticle could serve as a promising vehicle for targeting anticancer drug delivery and efficient detection through MR imaging in theranostics.**

***Index Terms*— SPIONs, Biodegradable, Targeting DOX delivery, MR Imaging.**

INTRODUCTION

In recent years, superparamagnetic iron oxide nanoparticles (SPIONs) have been extensively developed as a promising candidate for both magnetic resonance (MR) imaging and tumor-targeting targeting drug delivery in theranostics[1-6]. However, the practical uses of SPIONs have been disappointing because of poor water-solubility, acid erosion, oxidation, and severe aggregation under *in vivo* condition. Therefore, recently, to avoid these disadvantages polymers were used as the coating materials to develop SPIONs-based stable magnetic nanoparticles in aqueous solution. Polymer micelles can remarkably enhance the structural stability for drug delivery system to endure the complex biological environment[7-11], thus, micellar magnetic nanoparticles by incorporation of SPIONs into polymer coating have been demonstrated a promising method to stabilize magnetic nanoparticles for in vivo medical uses. These micellar magnetic nanoparticles were reported with excellent biodegradability, prolonged blood circulation, good biostability, and MR imaging contrast effect. However, nondegradable micellar structure shows low therapeutic and diagnostic efficiency, because the excessively stabilized nanoparticles will prevent the drug release at aimed sites, accumulate in the cells or tissues causing a long-term toxicity. Therefore, SPIONs with biodegradable polymer coating with improved drug delivery efficacy, minimized side effects, and enhanced contrast effect for MR imaging show great potential use in theranostics.

Biodegradable nanoparticles with redox-responsibility were proved to be an effective vehicle for intracellular and triggered drug release owing to 1000 times higher concentrations of glutathione (GSH, 2~10 mM,) in the various subcellular organelles in cytoplasm than in the extracellular fluids (about 2-20 μM)[12-13]. The redox-responsive nanoparticles can be biodegraded in presence of the reducing agent such as GSH, due to the chemical cleavage of disulfide bond by a thiol/disulfide exchange process. In additional, a triggered release of the anticancer drug from the carriers is enabled under the reductive environments. Therefore, to achieve an efficient drug delivery, redox-responsive nanoparticles are of great interests for drug delivery in cancer therapy.

On the other hand, SPIONs with polymer coating often show short-term stability and are easily disassociated caused by massive dilution or disruptions in the chemical environments, such as pH, temperature, and ion concentration, because of the weak bond between SPIONs and polymer coatings[14-15]. Therefore, stable magnetic nanoparticles are needed to achieve long circulation in vivo. In recent years, catechol-containing molecules are proved to have the property to adhere to almost any material of either organic or inorganic origin. 3,4-dihydroxy-L-phenylalanine (L-DOPA) found in mussel specialized adhesive proteins and its analog dopamine (DA) have the catechol functional group, which could form strong bonds on inorganic/organic materials surfaces such as coordination of metal ions, formation of □-electrons, and hydrogen bond interactions. Therefore, catechol-containing molecules have been widely used to immobilize SPIONs to avoid unexpected disassembly of polymer coated magnetic nanoparticles. For example, Chen et al. developed a magnetic nanoparticle with enhanced MR imaging and pH-sensitive drug release kinetics by a simple and controllable method in cancer theranostics. Recently, our group reported a redox-responsive cross-linked micellar magnetic nanoparticle demonstrating controllable drug release and effective MR imaging detection. However, most magnetic nanoparticles can only passively accumulate in tumors, but to enhance the uptake of drug-loaded nanoparticles into the target cells, active targeting should be attempted. To our

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best knowledge, there was no report on redox-responsive cell targeting magnetic nanoparticle in theranostic system so far.

Herein, our object is to design a redox-sresponsive magnetic nanoparticle and fabricate a biodegradable, robust, and cell targeting MR imaging contrast agent for future theranostic application. The shell of the magnetic nanoparticle was a linear multi-functional copolymer, polyamidoamine (PAMAM), synthesized with N,N-bis(acrylate) cystamine (BACy), dopamine (DA), DOX, folic acid–polyethylene glyco (FA-PEG), and PEG by Michael addition reaction, where BACy served as the disulfide bonded backbone, DA moiety as anchor to immobilize SPIONs, DOX as anti-cancer drug unit, PEG provided colloidal stability, and FA as cell targeting ligand forming the drug loaded biodegradable magnetic nanoparticles (MNPs-DOX). Based on the experimental results, this theranostic magnetic nanoparticle exhibited excellent stability, high drug loading, quick degradation in redox environment.

EXPERIMENTAL

Materials

Folic acid PEG Amine (95%, Mn=2000, Ponsure), Methoxypoly (Ethylene Glycol) Amine (95%, Mn=2000, Ponsure), cystamine dihydrochloride (96%, J&K Chemical), Acryloyl chloride (96%, Aladdin), dopamine hydrochloride (98%, Aladdin), GSH (98%, Aladdin), DOX (98%, Aladdin). Dichloromethane (DCM), tetrahydrofuran (THF), ethylene acetate (EA), Dimethylsulfoxide (DMSO), ethanol, and ether were used after purified. The other chemicals were used as received.

Synthesis of SPIONs

$FeCl_3$ (0.020 mol) and $FeSO_4$ (0.011 mol) were dissolved in 20 mL of distilled water. When 10 mL of ammonium hydroxide (25%) were added to this solution with vigorous agitation at 90 ^oC for 30 min, magnetite slurry was precipitated. 0.4 mL of oleic acid were then added. In this process, with the evaporation of ammonia gas thus changing the magnetite nanocrystals coated with hydrophobic oleic acid upon continuous heating. As a result, a distinct phase separation between the upper organic portion and the lower aqueous portion appeared. Most of the aqueous phase was removed using a pipette and the heating of the residue was continued until the remaining water had been completely evaporated. Oleic acid-coated magnetite nanocrystals were then washed with ethanol to eliminate excess oleic acid and centrifuged. Ethanol was completely removed from the resulting precipitation under reduced pressure at room temperature. The dried oleic acid-coated magnetite nanocrystals were dispersed in 10 mL of chloroform, so finally, a chloroform-based magnetic fluid was obtained.

Synthesis of BACy

Cystamine dihydrochloride (4.6 g, 0.020mol) was dissolved in distilled water (36 mL) and added to a three-necked, 500 mL flask equipped with a stirrer, a thermometer, and a dripping funnels. After the mixture was cooled to 0 ^oC in ice-water bath for 30 min, acryloyl chloride (3.6 mL, 0.044 mol) in DCM (6 mL) and NaOH (3.2 g, 0.080 mol) dissolved in water (8 mL), were added dropwise slowly into the three-necked flask, and the reaction mixture was cooled in ice-water bath and stirred at 0 ^oC for 30 min and then at room temperature for another 4 h. The organic phase was extracted with DCM, and subsequently dried over anhydrous $MgSO_4$, the solvent was removed under vacuum. The raw BACy product was purified by recrystallization from EA.

Synthesis of PAMAM crosslinked copolymer

A typical procedure for preparing the cross-linked micelles is as following: Molar ratio of comonomers was set at 50 : 19 : 16 : 12 : 3 (BACy : DA : mPEG-NH_2 : DOX : FA-PEG- NH_2), 13.0 mg BACy, 7.8 mg DA, 28.3 mg mPEG-NH_2, 9.0 mg DOX and 4.5 mg FA-PEG- NH_2 added to a 25 mL flask. 5μL triethylamine dissolved in 1mL DMSO was added to the flask, and the flask was sealed and purged with nitrogen. The reaction proceeded at 55 °C for 48 h. and the product was precipitated by the organic solvent ether and was purified by a dialysis method, and the solution was frozen and lyophilized.

Preparation method of MNPs-DOX

The PAMAM crosslinked copolymer was coated to SPIONs surface via ligand exchange reaction. 10 mg of PAMAM polymer were dissolved in 1 mL of DMSO and mixed with 4 mL of THF containing 2 mg nanoparticles. The mixture was placed in a flask with vigorous sonication, sealed and purged with nitrogen. The mixture was allowed to react for 24h at room temperature in dark. After that, the nanoparticles were separated by centrifugation, and the obtained MNPs-DOX purified by a dialysis method to remove the unbound copolymer. And then the solution was frozen and lyophilized.

RESULTS AND DISCUSSION

Preparation and characterization of the MNPs-DOX

In this work, the PAMAM was synthesized by Michael addition reaction, where DMSO as solvent. The PAMAM contains four basic units: BACy served as the disulfide bonded backbone for redox-sensitivity, DA as metal binding unit, DOX as the anti-cancer drug unit, and PEG as hydrophilic corona to ensure colloidal stability. Finally, SPIONs were immobilized into the PAMAM to prepare the theranostic MNPs-DOX by a simple ligand exchange assembly process of catechol in DA unit with the oleic acid layer on SPIONs. It could be anticipated that controllable drug release from the MNPs-DOX under GSH reduction environment and excellent contrast for MR imaging detection could be both achieved in this theranostic system.

The chemical structures of the synthesized PAMAM crosslinked copolymer were characterized by ^1H NMR. The representative ^1H NMR spectrum of PAMAM in DMSO-d_6. was depicted in Fig. 1. The resonance signals at δ = 1.21 (h), 2.82 (d), 3.02 (b), 3.25 (g), 3.52 (e), 3.94 (i), 5.61 (a), 6.64~8.31 (f), and 8.56 (c) were belong to the methylene group (a and b) of the BACy crosslinker, methylene group (j) of the BACy crosslinker, methylene group (d) and phenolic hydroxyl (c) in DA units, the glycol units in

FA-PEG-NH_2 and mPEG-NH_2 (e), methoxyl (g) of the mPEG-NH_2 units, phenyl group and imino group (f) of the FA-PEG-NH_2 units,

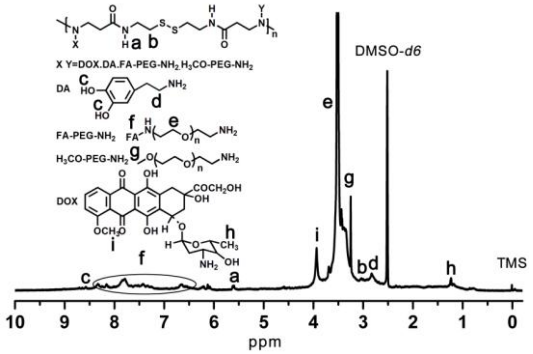

Fig. 1. ^{1}H NMR spectrum of cross-linked copolymer in a DMSO.

methyl (h) and methoxyl (i) of the DOX units, respectively. The appearance of the methoxyl unit in mPEG-NH_2, phenyl group and imino group units in FA-PEG-NH_2, methylene signal in BACy, methoxyl unit in DOX, and phenolic hydroxyl in DA unit indicated the successfully synthesis of the PAMAM cross-linked copolymer.

As shown in Fig. 2a, herein, the polymer component weight ratios were referred to the feeding and loading ratios before and after polymerization, respectively. The small difference between the feeding and loading of monomers indicated high efficient conversion during the polymerization reaction. Therefore, in our system the drug/polymer composition in the end product can be precisely controlled by altering the feeding ratio of monomers. MNPs-DOX was also characterized by UV–vis and fluorescence spectroscopy to further confirm and quantify the DOX loading. Free DOX emits red fluorescence at 590 nm and the spectrum shown in Fig. 2b confirms that MNPs-DOX conserved the fluorescent property of DOX with the absorbance and emission maxima at 480 and 590 nm. Using the absorbance measured from MNPs-DOX, we quantified that the weight of DOX accounts for 6.3% of the total polymer weight.

Colloidal stability of the MNPs-DOX in aqueous media by their anti-fouling property. TEM images showed that the MNPs-DOX were well dispersed with high stability in water after the ligand exchange process (shown in Fig. 3c). The hydrodynamic diameter of the obtained SPIONs, PAMAM, and MNPs-DOX were also characterized by DLS measurement. The gradually increase in size of these nanoparticles was observed (SPIONs~40 nm, PAMAM~80 nm, and MNPs-DOX~160 nm). It was indicated that the SPIONs were successfully incorporated into PAMAPM polymer chain forming a core/shell structure. No significant changes in size were observed for weeks, indicating that the MNPs-DOX retained good stability.

CONCLUSION

In summary, we demonstrated a simple assembly process to prepared redox-responsive magnetic nanoparticles for drug delivery of cancer chemotherapeutics and MR imaging detection. The MNPs-DOX exhibited the excellent property to minimize drug release in physiological environment whereas increase in the reductive environment. Although the cell assay was needed to further evaluate the cellular uptake and cytotoxicity of the MNPs-DOX, we are still convinced that this biodegradable magnetic nanoparticle with supraparamagnetism, excellent stability, and redox-responsibility holds great promise for controlled drug delivery and MR imaging detection in cancer theranostics.

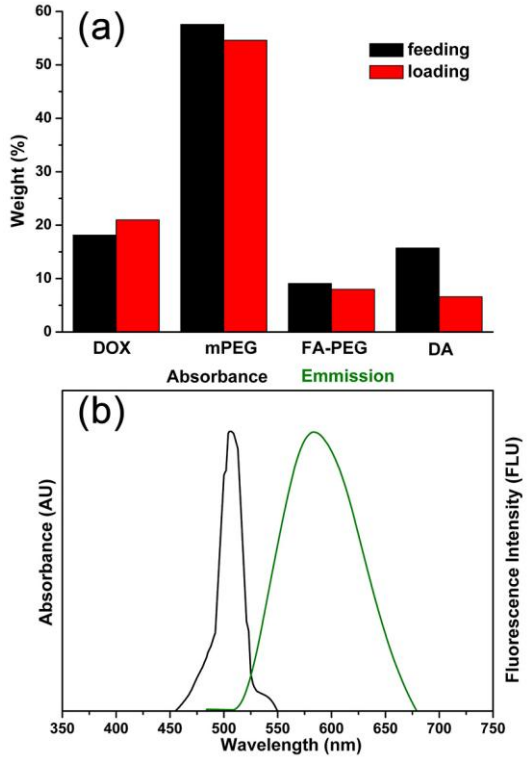

Fig. 2. (a) Comparison chart of DOX, mPEG, FA-PEG, and DA feeding and loading ratios in PAMAM. (b) Absorbance and fluorescence spectra of DOX in MNPs-DOX.

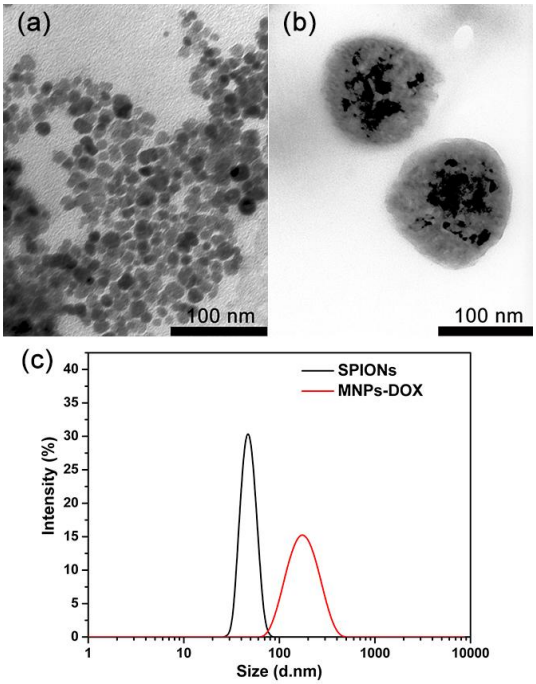

Fig. 3. TEM images of oleic acid-coated SPIONs (a), and after (c) the ligand exchange process of MNPs-DOX; (d) DLS data showing the changes of hydrodynamic size (in diameter) of SPIONs and MNPs-DOX.

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