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Preparation of Biodegradable Magnetic Nanoparticles via a Simple Assembly Process for Controllable Drug Delivery

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***Abstract*— In this research, a functional cross-linked PEI assembled with superparamagnetic iron oxide nanoparticles (SPIONs) were prepared via a simple assembly process for delivery of doxorubicin (DOX). The functional cross-linked PEI was synthesized by Michael addition reaction, where N,N-Bis(acryloyl) cystamine served as cross-linker, PEI, and dopamine (DA) acted as comonomers. Here the cross-linked PEI is directly assembled to the surface of SPIONs by the ligand exchange reaction forming the SPIONs@PEI, and then the DOX was encapsulated into the nanoparticles forming the drug loaded biodegradable magnetic nanoparticles (SPIONs@PEI@DOX). The hydrophilic PEI moiety provides the nanoparticles colloidal stability and good-dispersity in aqueous solution. TEM images showed that the SPIONs and SPIONs@PEI 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.**

***Index Terms*— SPIONs, Biodegradable, Controllable DOX delivery.**

INTRODUCTION

In recent years, superparamagnetic iron oxide nanoparticles (SPIONs) have been extensively developed as a promising candidate for tumor-targeting drug delivery in cancer therapy[1-6]. However, poor water-solubility and severe aggregation under *in vivo* condition are still challenging and impeding the practical uses of SPIONs. Therefore, in attempt to solve the problems functional polymers were widely used as the coating materials to develop SPIONs-based stable magnetic nanoparticles in aqueous solution. Polymer coating can remarkably enhance the structural stability for drug delivery system to endure the complex biological environment[7-11], thus, functional magnetic nanoparticles by incorporation of SPIONs into polymer coating have been demonstrated a promising system to stabilize magnetic nanoparticles for *in vivo* medical uses. These functional magnetic nanoparticles were reported with excellent biodegradability, prolonged blood circulation, and good bio-stability. However, non-degradable structure shows low therapeutic 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 and minimized side effects show great potential use in cancer chemo-therapy. 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 under in vivo condition because of the weak bond between SPIONs and polymer coatings[14-15]. 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. Therefore, catechol-containing molecules have been widely used to immobilize SPIONs to avoid unexpected disassembly of polymer coated magnetic nanoparticles. However, to our best knowledge, systems based on the biodegradable magnetic nanoparticles were scarcely studied for controllable drug delivery so far.

In this study, a redox-sresponsive magnetic nanoparticle (SPIONs@PEI) with water-stability and high DOX loading capacity was prepared for drug delivery in cancer therapy. The shell of the magnetic nanoparticle was a cross-linked PEI, synthesized with N,N-bis(acrylate) cystamine (BACy), dopamine (DA), and PEI, where BACy served as the biodegradable cross-linker and DA moiety as anchor to immobilize SPIONs. Subsequently, DOX molecules were encapsulated into the SPIONs@PEI forming the drug loaded biodegradable magnetic nanoparticles (SPIONs@PEI@DOX). Based on the experimental results, this functional magnetic nanoparticle exhibited excellent stability, high drug loading, quick degradation in redox environment.

EXPERIMENTAL

Materials

DOX (Beijing HuaFeng United Technology), cystamine dihydrochloride (J&K Chemical), acryloyl chloride (J&K Chemical), methacrylic anhydride (Aladdin), dopamine hydrochloride (Aladdin), $Na_2B_4O_7$ (Fucheng Chemical Reagent), PEI (600 Da Shanghai Sangon Biotech, China), were used as received. *n*-hexane (Kelon Chemical), N,N-dimethylformamide (DMF, Tianjin Zhiyuan Chemical Reagent), ethylene acetate (EA, Jinshan Chemical Reagent),

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dimethyl sulfoxide (DMSO, Tianjin Zhiyuan Chemical Reagent), tetrahydrofuran (THF, Tianjin Zhiyuan Chemical Reagent), Methanol (Kelon Chemical), and dichloromethane (DCM, Shandong Jinling Chemical Industry) were used after purification. The other chemicals were used as received.

Synthesis of SPIONs

$FeCl_3$ (5.00 g) and $FeCl_2$ (2.00 g) were dissolved in 10 mL of distilled water. When 10 mL of ammonium hydroxide were added to this solution with vigorous agitation at 70 ^oC for 30 min, magnetite slurry was precipitated. 1 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 THF, so finally, a THF-based magnetic fluid was obtained.

Synthesis of BACy

Cystamine dihydrochloride (2.30 g) was dissolved in distilled water (18 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 (2 mL) in DCM (3 mL) and NaOH solution 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 cross-linked PEI

A typical procedure for preparing the cross-linked micelles is as following: 1.2 g PEI, 0.135 g BACy, and 0.056 g DMA were dissolved in a water/methanol solution and added to a 25 mL flask. flask was sealed and purged with nitrogen. The reaction proceeded at 45 °C for 24 h, and the product was dialyzed against water for 2 days. Finally, the solution was frozen and lyophilized.

Preparation method of SPIONs@PEI

The cross-linked PEI was coated to SPIONs surface via ligand exchange reaction. 0.5 g of cross-linked PEI were dissolved in 5 mL of DMSO and mixed with the SPIONs. The mixture was placed in a flask with vigorous sonication, sealed and purged with nitrogen. The mixture was allowed to react for 24 h at room temperature in dark. After that, the nanoparticles were separated by centrifugation, and the obtained SPIONs@PEI purified by a dialysis method to remove the unbound copolymer. Finally, the solution was frozen and lyophilized.

Preparation method of SPIONs@PEI@DOX

5 mg SPIONs@PEI and 5 mg of DOX were dissolved in 5 mL of DMSO. The mixture was placed in a flask with vigorous sonication, sealed and purged with nitrogen. The mixture was stirred for 24h at room temperature in dark. After that, the nanoparticles were separated by centrifugation, and the obtained SPIONs@PEI@DOX purified by a dialysis method. Finally, the solution was frozen and lyophilized.

RESULTS AND DISCUSSION

Preparation and characterization of the cross-linked PEI

In this work, the cross-linked PEI was synthesized by Michael addition reaction, where DMSO as solvent. The cross-linked PEI contains there basic units: BACy served as the biodegradable cross-linker for redox-sensitivity, DA as metal binding unit, PEI as hydrophilic corona to ensure colloidal stability. Subsequently, SPIONs were immobilized into the cross-linked PEIPAMAM to prepare the SPIONs@PEI by a simple ligand exchange reaction. Finally, the DOX molecules were encapsulated into the SPIONs@PEI forming a functional magnetic nanoparticles SPIONs@PEI@DOX. It could be anticipated that controllable drug release from the SPIONs@PEI@DOX under GSH reduction environment could be chieved in this nano-composites. The chemical structures of the synthesized PAMAM crosslinked copolymer were characterized by ^1H NMR. The representative ^1H NMR spectrum of cross-linked PEI in D_2O was depicted in Fig. 1. The resonance signals at around δ = 2.9~3.1 (a), 3.4~3.5 (c), and 3.6~3.7 (a) were belong to the methylene group (b) of the PEI, methylene group (c) of DMA units, and methylene group (a) in BACy, respectively. Based on the experimental results, the cross-lined PEI was successfully synthesized.

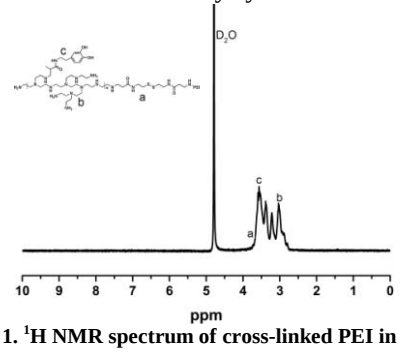

Fig. 1. ^1H NMR spectrum of cross-linked PEI in D_2O.

The morphology of the nano-particles was investigated by TEM. As shown in Fig. 2, the SPIONs and SPIONs@PEI were well-dispersed in water indicating a good colloidal stability of the functional nano-particles in aqueous media, which could by explained by their hydrophilic property of the PEI coating. Base on the TEM photos, the SPIONs exhibited a spherical morphology with average size of about 10 nm, and the SPIONs@PEI showed a core-shell structure that the clustered SPIONs assembled inside the polymer coating MNPs-DOX with average size of about 110 nm. In conclusion, the SPIONs@PEI were successfully prepared via ligand exchange reaction with a spherical morphology, and were well-dispersed in water.

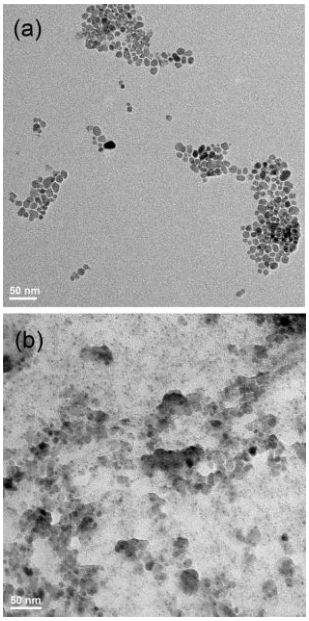

Fig. 2. TEM photos of SIPONs and SPIONs@PEI.

The hydrodynamic diameters and Zeta potentials of the obtained SPIONs, SPIONs@PEI, and SPIONs@PEI@DOX were measured by DLS. The SPIONs@PEI, and SPIONs@PEI@DOX exhibited similar size about 190 nm and 210 nm, respectively. The gradually increase in size of these nanoparticles was attributed to the encapsulation of the DOX molecules. Additionally, the Zeta potentials of the SPIONs, SPIONs@PEI, and SPIONs@PEI@DOX were of -0.71, 29.2, and 15.7 mv, respectively. It was indicated that the DOX molecules and SPIONs were successfully incorporated into the cross-linked PEI with a core/shell nano-structure. No significant changes in size were observed for weeks indicating that the SPIONs@PEI@DOX retained good stability.

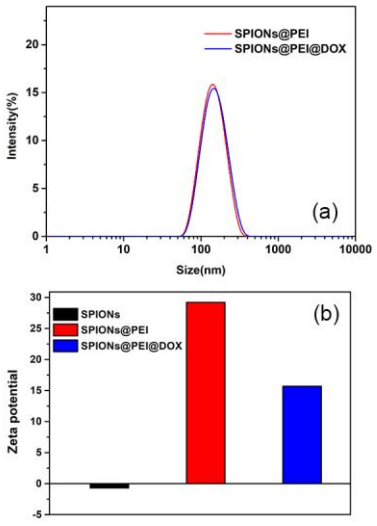

Fig. 3. The size distribution and Zeta potentials of the SIPONs SPIONs@PEI, and SPIONs@PEI@DOX.

CONCLUSION

In summary, we demonstrated a simple method to prepared redox-responsive magnetic nanoparticles for drug delivery of cancer chemotherapeutics. The as prepared SPIONs@PEI@DOX exhibited good colloidal stability with a nano-sized structure. It can be anticipated that the SPIONs@PEI@DOX system can minimize drug release in physiological environment whereas an enhanced drug delivery could be attained in the reductive intracellular environment. Although the cell assay was not carried out to evaluate the cellular uptake and cytotoxicity of the SPIONs@PEI@DOX in this study, we still convince that this biodegradable magnetic nanoparticle with excellent stability, and redox-responsibility holds great promise for controlled drug delivery in cancer therapy.

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