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Preparation of Fe_3O_4/poly(styrene-butyl acrylate-[2-(methacryloxy) ethyl]trimethylammonium chloride) by emulsifier-free emulsion polymerization and its interaction with DNA

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ABSTRACT

Cationic magnetic polymer particles Fe_3O_4/poly(styrene-butyl acrylate-[2-(methacryloxy)ethyl]trimethylammonium chloride), a type of potential gene carrier, were prepared by emulsifier-free emulsion polymerization with oleic acid modified magnetite Fe_3O_4, styrene, butyl acrylate and [2-(methacryloxy)ethyl]trimethylammonium chloride) (METAC). The morphology of the particles was characterized by transmission electron microscopy and the composites of particles were characterized by FT-IR spectroscopy, X-ray diffraction. These results showed that magnetic particles were well dispersed in polymers with the content of about 15%(wt/wt). The composites exhibited superparamagnetism and possessed a certain level of magnetic response. The interactions between the particles with calf-thymus DNA (ct DNA) were confirmed by zeta potential measurement, UV–vis spectroscopy and fluorescence spectroscopy. The DNA-binding capacity determined by the agarose gel electrophoresis showed good binding capacity of the emulsion to DNA. These results suggested the potential of the cationic magnetic polymer emulsion as gene target delivery carrier.

1. Introduction

Magnetic polymer microspheres consisting of magnetic core and polymer shell have received much attention due to their wide range of potential applications in the fields such as rapid protein immobilization and separation [1–3], drug carrier and delivery [4–6], magnetic resonance imaging (MRI) [7–9]. As the core of the composite microsphere, magnetic nanoparticles (MNPs) can be manipulated by an external magnetic force. In addition, magnetic core can also respond to an alternation magnetic field, leading to the temperature elevating. Above certain temperature, the cancer cells can be effectively killed, which can be applied for cancer treatment. If magnetic particles embedded in temperature sensitive polymers or liposome, the composites can combine hyperthermia therapy with drug delivery to provide a synergistic treatment strategy [10] .To perform real-time bioapplication, MNPs are often encapsulated with functional polymers containing carboxyl [3,11], hydroxide [12,13], epoxy [14,15], amino [16,17] and other functional groups [18], which are easy to covalent link with various bimolecular like enzymes, peptides and nucleic acid.

Cationic lipids and cationic polymers have recently gained increasing interest in gene therapy as a kind of non-viral transfer vectors [19,20]. Typically, interaction of cationic vectors with DNA involves both electrostatic and hydrophobic interactions. The positively charged groups of the vectors are able to combine with the negatively charged phosphates of DNA via electrostatic interaction. The hydrophobic interaction comes from the components of the DNA duplex and polymer or lipid backbone [21]. Compared to cationic lipids, cationic polymers are more attractive dues to they are stable, easy to manipulate, more economical and more tailorable for functionalization such as incorporation of targeting ligands [22]. A variety of polycations have been proposed and investigated for non-viral transfer system, such as poly-L-lysine (PLL) [23], polyethyleneimine (PEI) [24,25], chitosan [26,27], methacryl oxyethyl trimethylammonium chloride (MOTAC) [28,29]. Furthermore, a number of strategies have been explored based on polycations combined with magnetite because association of polycations with magnetic particles allows the vectors to respond to an applied magnetic force, which enables to speed up the gene transfer vectors to the target cell surface, shorten the time of gene delivery and allow the use of lower dose

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of vectors with high efficiency, thus minimize the toxic risk caused by high concentration of gene delivery vectors [30]. Most of them introduced polyethylenimine (PEI) by electrostatic adherence [31,32] or utilizing linker [33] onto magnetic particles. However, the stability of electrostatic adherence is easily destroyed when the biological solution is changed [34]. Though the high molecular weights of PEI ensure the binding capacity with DNA, they induce insufficient linkage with magnetic particles, cause extensive bridging flocculation and lead the morphology uncontrollable [18]. Encapsulation magnetic particles with monomers by in-situ polymerization may solve these drawbacks because small molecules can easily adsorb onto the surface of magnetic particles without the steric hindrance of long chains and the morphology of the polymer shell can be more controllable by changing the process of polymerization.

At present, many approaches have been employed to prepare magnetic polymer microspheres with functional monomers, such as emulsion polymerization [35,36], seed-emulsion polymerization [37], dispersion polymerization [38,39], emulsifier-free emulsion polymerization [40], miniemulsion polymerization [41]. Among these methods, emulsifier-free emulsion polymerization invokes more interest in bioapplication because this method without initially adding surfactants can produce monodisperse and "clean" particles, avoiding cytotoxic and antibacterial effects of the emulsifier. Several methodologies for the preparation of emulsifier-free magnetic polymer microsphere have been reported. Chiu et al. prepared Fe_3O_4/PMMA composite particles and studied nucleation mechanism of emulsifier-free polymerization [40]. Xie et al. investigated the emulsifier-free emulsion polymerization of styrene-butyl acrylate-methacrylic acid [poly(St-BA-MAA)] in different kinds of polar solvent [42].

However, to our knowledge, those studies just focus on emulsifier-free emulsion polymerization with negatively charged surface. Reports on cationic magnetic particles are obviously far fewer compared to those on anionic microspheres. Cationic magnetic microsphere prepared by emulsifier-free polymerization may be a new way to obtain functional composites in gene therapy as mentioned above. Herein, our work focuses on exploring the preparation of cationic magnetic polymer emulsion by emulsifier-free emulsion polymerization and the interactions with DNA in vitro. The objective is to explore the prospect of the application of polymer nanoparticles as gene carriers and drug delivery. In this study, copolymer shell were comprised of styrene, butyl acrylate and a cationic comonomer of METAC, which was selected as a DNA-binding site because it provides permanent high charged to the particles [43]. The final magnetic emulsions were characterized by X-ray diffraction (XRD), vibrating sample magnetometer (VSM), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), transmission electron microscopy (TEM) and Mastersizer particle size analyzers. The physiochemical characteristics of the emulsion/DNA complexes were analyzed by zeta potential measurements, UV–vis spectrophotometry, fluorescent spectroscopy and agarose gel electrophoresis.

2. Material and methods

2.1. Materials

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), oleic acid (OA), a 36% ammonium hydroxide solution ($NH_3 \cdot H_2O$) and ethanol, isopropanol, hexane were purchased from Sinopharm Chemical Reagent Co. Ltd, China. Styrene (St) and butyl acrylate (BA) were distilled under reduced pressure and stored at 5 °C. All the reagents were AR.

2,2-azobis-(2-amidinopropane) hydrochloride (AIBA) from Aldrich is 97% pure. An aqueous solution (75 wt%) of [2-(methacryloxy) ethyl]trimethylammonium chloride) (METAC) was purchased from Aldrich and used as received and distilled water was used throughout the study.

Calf-thymus DNA (ct DNA) was purchased from Sigma (St. Louis, MO, USA).The stock solution of calf-thymus DNA was prepared by dissolving ct DNA in doubly distilled water and stored at 0–4 °C. The concentration of working solution DNA was 64 mg/L. Plasmid DNA (2.5 kbp), loading buffer and other biochemicals were supplied by the group of Prof. Ma (Faculty of Biology Science, Hubei University).

2.2. Preparation of magnetic nanoparticles

Fe_3O_4 nanoparticles were prepared as the method of our previous work without sodium dodecylsulphate [44], which consists of Fe(III) and Fe(II) coprecipitation in alkaline solution. In detail, 13.5 g $FeCl_3 \cdot 6H_2O$ and 6 g $FeCl_2 \cdot 4H_2O$ were dissolved in 150 mL distilled water under nitrogen at room temperature, 40 mL $NH_3 \cdot H_2O$ was quickly added into the solution with vigorous stirring, the mixture rapidly forming a black precipitate. Then the mixture was heated to 60 °C for 1 h. The black precipitate was isolated from the solution by magnetic separation and washed with distilled water until the pH value reached 7.

2.3. Modification of the magnetic nanoparticles

The modification of the magnetic nanoparticles was carried out in a three-necked flask equipped with a stirring paddle, a condenser and nitrogen inlet. The wet precipitate (50 g), 200 mL distilled water were added into the reactor and stirred. When the mixture was heated to 80 °C, 3 g oleic acid was introduced to modify the particles. The system was kept at 80 °C for 1 h. Then the reaction was ended by cooling the mixture to room temperature, the oleic acid-modified magnetite fluid was separated by a magnet and washed several times with ethanol and hexane until the upper solution became transparent. Then the black slurry was dispersed in hexane.

2.4. Preparation of cationic magnetic composite Fe_3O_4/poly(St-BA-METAC) particles

The magnetic slurry (0.5 g), St (2.3 g) and BA (2.0 g) constituted the oil phase, and the METAC (0.5 g), isopropanol (20 mL) and water (80 mL) constituted the aqueous phase. Two different phases were then put into a 250 mL three-necked flask and homogenized at 50 °C with vigorous stirring for half an hour. The polymerization was carried out after 0.3 g AIBA was added into the flask and the temperature was heated to 80 °C. The air in the flask was replaced by a stream of nitrogen and the mixture was kept under nitrogen atmosphere until the polymerization sustained for 10 h. The resulting composite emulsion was purified by overnight dialysis in distilled water solution to remove isopropanol and monomers. Then the purified emulsion was added dropwise to saturation calcium chloride/methanol solution to remove unreacted species from the composites. The resultant floccus was washed with methanol and water for several times, and then dried under vacuum for 12 h at 40 °C. In the end, the powder was obtained for further characterization.

3. Characterization

The morphology and structure of the composite emulsion were determined by transmission electron microscopy (TEM, Tecnai G20,