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Self-assembled magnetic fluorescent polymeric micelles for magnetic resonance and optical imaging

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ABSTRACT

Stable and cytocompatible hybrid PEGylated micelles with multimodal imaging capabilities are described. The F_3O_4-encapsulated polymeric micelles composed of cores containing magnetic nanoparticles and polyethylene glycol (PEG) shells are synthesized by self-assembly of amphiphilic poly(-HFMA-co-VBK)-g-PEG copolymers and oleic acid stabilized Fe_3O_4 nanoparticles. The Fe_3O_4 magnetic nanoparticles in the core produce T_2-weighted MR imaging functionalities, whereas the small fluorescent monomer carbazole in the polymer shell introduces good fluorescent properties. The multifunctional micelles exhibit excellent paramagnetic properties with the maximum saturation magnetization of 9.61 emu/g and transverse relaxivity rate of 157.44 m_M^{-1} S^{-1}. *In vivo* magnetic resonance imaging (MRI) studies reveal enhanced contrast between the liver and spleen. Fluorescence spectra show characteristic emission peaks from carbazole at 350 nm and 365 nm and vivid blue fluorescence can be observed by 2-photon confocal scanning laser microscopy (CLSM). *In vivo* optical imaging demonstrates the unique fluorescent characteristics of the Fe_3O_4-encapsulated polymeric micelles in the liver and spleen and the excellent multifunctional properties suggest potential clinical use as nanocarriers in magnetic resonance imaging and optical imaging.

1. Introduction

The emergence of nanotechnology and biotechnology has created an exciting and interdisciplinary area of nanobiotechnology [1–3]. Multifunctional nanocarriers possess favorable properties integrated into a single nanosystem spurring new applications such as multimodal imaging and simultaneous diagnosis and therapy [4–6]. As diagnostic techniques, they have been explored in magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), optical imaging, and so on [7,8]. However, most of them have drawbacks such as low sensitivity, weak penetrability, and insufficient spatial or temporal resolution [9–11] and there have been attempts to combine two or more imaging modalities to improve the performance [12,13], for instance, PET/CT [14], MRI/CT [15], MRI/PET [16], and MRI/optical imaging [17–21]. One of the popular approaches is to combine the MRI and optical imaging modalities [22]. MR imaging offers high spatial resolution and the capacity to simultaneously obtain physiological and anatomical information in living organisms based on the interactions between protons and molecules in the surrounding tissues, whereas optical imaging allows rapid screening [23,24].

Magnetic resonance imaging (MRI) is one of the most useful diagnostic techniques providing noninvasive and real-time detection of diseases and super-paramagnetic nanoparticles are excellent contrast agents capable of noninvasive monitoring of pathological changes on both the molecular and cellular levels *in vivo* [25,26]. However, magnetic nanoparticles are commonly stabilized with oleic acid and their biological applications are hampered because of the poor dispersion properties in blood [27,28]. Several strategies such as ligand exchange [29] and self-assembly [30,31] have been proposed to enhance their water

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solubility. In particular, amphiphilic polymers produced by controlled radical polymerization for magnetic nanoparticle encapsulation exhibit good colloidal stability compared to small molecule surfactants [32,33]. Kim et al. reported amphiphilic poly(styrene$_{250}$-block-acrylic acid$_{13}$) (PS_{250}-b-PAA_{13}) copolyolefin with magnetic nanoparticles to enclose the particles in the copolymer micelles. They demonstrated that the surrounding polymer could be cross-linked to fix the nanostructures topologically and these structures were stable to subsequent synthetic transformation of surface functional groups [34]. Ai et al. showed that oleic acid and oleylamine modified magnetic particles could be encapsulated inside the hydrophobic core of poly(-caprolactone)-b-poly(ethylene glycol)(PCL-b-PEG) micelles by ring-opening polymerization [35].

Fluorescent nanomaterials comprising π-conjugated polymers have attracted much interest lately due to their small size as well as high fluorescence and photochemical stability which render them appealing as bioprobes in labeling and imaging [36]. For example, carbazole and their derivatives have a large conjugated system, photoconductive feature, and strong intramolecular electron transfer and are excellent optical materials [37]. Li prepared water-soluble trifunctional nanoparticles with thermoresponsive, magnetic, and fluorescent hybrid *via* surface-initiated reversible addition–fragmentation chain transfer (RAFT) polymerization. They used the fluorescent RAFT agent with carbazole as the chain transfer agent, which could enhance the negative contrast in MRI [38].

In our previous work, we demonstrated the preparation of magnetite (Fe_3O_4)-loaded polymer micelles by self-assembly of fluorine-containing amphiphilic poly(HFMA-g-PEGMA) copolymers with oleic acid modified Fe_3O_4 nanoparticles in an aqueous medium [39]. However, the single imaging modality could not convey all the necessary information about the biological structure and functions of an organ. Herein, a dual-modality imaging probe is designed and fabricated and *in vivo* optical imaging and MRI experiments demonstrate their suitability as T_2-weighted negative MRI contrast agents and fluorescent probes in liver and spleen imaging.

2. Materials and methods

2.1. Materials

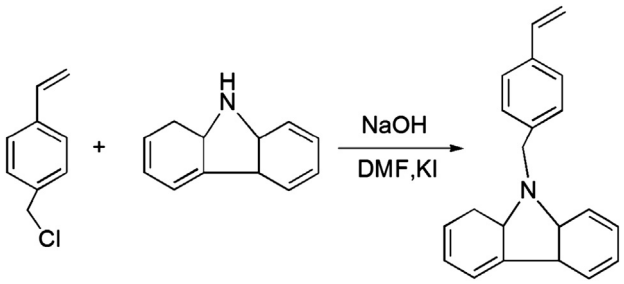
2,2,3,4,4,4-Hexafluorobutyl methacrylate (HFMA) purchased from Xeogia Fluorine–Silicon Chemical Company (Harbin, China, Chemical Purity) was distilled at reduced pressure before use. Methoxy poly(ethylene glycol) monomethacrylate (PEGMA) (average M_n 950 g/mol) was obtained from Aldrich and used without further purification and 2,2′-azobisisobutyronitrile (AIBN, analytical grade) was purified by recrystallization in ethanol. 4-Vinylbenzyl chloride (90%) and carbazole (≥95%) were supplied by Aldrich Chemical Company. Potassium iodide (KI), oleic acid (OA, analytical reagent), sodium hydroxide (NaOH), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ammonium hydroxide ($NH_3{\cdot}H_2O$, 25–28%), ethanol, hexane, N,N-dimethylformamide (DMF, analytical reagent), and tetrahydrofuran (THF, analytical reagent) were purchased from Sinopharm Chemical Reagent Co., Ltd., China and 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were bought from Sigma–Aldrich.

2.2. Synthesis of 9-(4-vinylbenzyl)-9H-carbazole (VBK)

The fluorescent monomer VBK was synthesized according to that described in the literature with some modifications [40] as shown in Scheme 1. Succinctly speaking, 2.11 g of carbazole and 0.63 g of NaOH were dissolved in 30 mL of DMF together with 0.05 g of KI as the catalyst under vigorous magnetic stirring for 3 h. 2.34 g of 4-vinylbenzyl chloride was dripped slowly into the mixture at room temperature (RT) and after stirring for 20 h at room temperature, the mixture was poured into a large amount of deionized water. The crude product was precipitated and collected by filtration and while crystals were produced by recrystallization in acetone.

2.3. Preparation of iron oxide nanoparticles

Mono-dispersed super-paramagnetic iron oxide nanoparticles were prepared by chemical co-precipitation, followed by modification with oleic acid [41]. 26.115 g of $FeCl_3{\cdot}6H_2O$ and 13.31 g of $FeCl_2{\cdot}4H_2O$ were mixed in 160 mL of deoxygenated water in a 500 mL three-necked flask. The solution was stirred under nitrogen for 0.5 h and 90 mL of $NH_3{\cdot}H_2O$ was added drop-wise to obtain a pH value of 10–11 in order to produce black precipitates and allow the growth of iron oxide nanoparticles. The solution was heated to 75 °C for 1 h and 9 g of oleic acid were added drop by drop at a constant rate and the reaction proceeded for another hour. Afterwards, the black mixture was cooled and washed by repeated dispersion and precipitation with hexane and ethanol, respectively. The final product was dispersed in hexane and the resulting ferrofluid was sealed in a glass vial for storage.



Scheme 1. Schematic illustration of the preparation fluorescent monomer VBK.

2.4. Synthesis of amphiphilic poly(HFMA-co-VBK)-g-PEG copolymers

The amphiphilic poly(HFMA-co-VBK)-g-PEG copolymers were synthesized by free radical polymerization as described in our previous paper [42]. Briefly, 1.005 g of PEGMA, 0.815 g of HFMA, and 0.102 g of VBK were dissolved in 12 mL of THF. After adding 0.068 g of AIBN as the radical initiator, the mixture was transferred to a 50 mL round-bottom flask with a magnetic stirrer and degassed by bubbling with N_2 several times in an ice bath. Polymerization proceeded at 75 °C for 24 h and the amphiphilic poly(HFMA-co-VBK)-g-PEG copolymers were collected by precipitation in hexane thrice. The purified products were dried under vacuum at 35 °C for later use.

2.5. Preparation of Fe_3O_4-encapsulated polymeric micelles

The amphiphilic poly(HFMA-co-VBK)-g-PEG copolymers were dissolved in 25 mL of distilled water and mixed with the oleic acid stabilized Fe_3O_4 nanoparticles (0.210 g) in hexane. The two-phase suspension was vigorously sonicated at 70 °C for 20 min under nitrogen bubbling to evaporate the hexane and produce the self-assembled micelles. The colloids underwent centrifugation and the collected Fe_3O_4-encapsulated polymeric micelles were washed three times with distilled water to remove the unbound polymer.

2.6. Effects of HFMA/(PEGMA + VBK) ratio on Fe_3O_4 loading efficiency

To optimize the Fe_3O_4 loading efficiency, a series of poly(HFMA-co-VBK)-g-PEG samples with variable HFMA/(PEGMA + VBK) mass ratios from 0.258/(1.004 + 0.101) to 1.516/(1.004 + 0.101) were synthesized. The oleic acid stabilized Fe_3O_4 nanoparticles were added to the amphiphilic copolymers at the same ratio described in Section 2.5. Here, the abbreviation scheme PEG–VBK–F_x is used, where *X* represents the HFMA wt% in the amphiphilic copolymer. For example, PEG–VBK–$F_{42.4}$ is the amphiphilic copolymer with 42.4 wt% of HFMA in the backbones. The samples were lyophilized and the Fe_3O_4 loading efficiency was evaluated by atomic absorption spectrophotometry (AA-680).

2.7. In vitro cytotoxicity

The cytotoxicity of the Fe_3O_4-encapsulated polymeric micelles was assessed by the MTT assay. HeLa cells were placed on the wells (10^4 cells per well) of 96-well plates, grown for 24 h, washed with PBS (pH = 7.4), and incubated with different concentrations of Fe_3O_4-encapsulated polymeric micelles (dose diluted by complete medium, 0–1000 μg/mL) for another 24 h. Afterwards, the supernatants were removed and cells were washed with PBS (pH = 7.4) three times. 40 μL of the MTT solution (2.5 mg mL^{-1}) were added to each well and after incubation for 4 h, the culture medium was discarded. Each well received 100 μL of DMSO and vigorous pipetting for 3–5 min to dissolve the precipitates. The absorption peak at 570 nm was measured on a microplate reader and data from six parallel wells were averaged to obtain the result for each sample. The relative cell viability was calculated by the following equation: Relative cell viability (%) = ($OD_{treated}/OD_{control}$) × 100%, where $OD_{treated}$ was obtained in the presence of the Fe_3O_4-encapsulated polymeric micelles and $OD_{control}$ was obtained without the Fe_3O_4-encapsulated polymeric micelles and defined as 100% viability.