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Rotavirus capsid surface protein VP4-coated Fe_3O_4 nanoparticles as a theranostic platform for cellular imaging and drug delivery

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ABSTRACT

The development of a theranostic nanoplatform based on rotavirus structural protein VP4-coated Fe_3O_4 nanoparticles (NPs) for dual modality magnetic resonance/fluorescence cellular imaging and drug delivery is reported. VP4 protein was obtained from *Escherichia coli* approach, and then chemically conjugated to Fe_3O_4 NPs premodified with meso-2,3-dimercaptosuccinnic acid (DMSA) in the presence of 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC). Next, the VP4-coated Fe_3O_4 NPs were loaded with doxorubicin (DOX), a typical anticancer drug, via formation of amide bond through the EDC approach. Prussian blue staining analysis reveals that the VP4-coated Fe_3O_4 NPs can be internalized efficiently by MA104 and HepG2 cells, thereby significantly improving cellular MRI sensitivity, compared with dextran- and BSA-coated Fe_3O_4 NPs. In addition, DOX loaded on the VP4-coated Fe_3O_4 NPs exhibits significant cytotoxicity to the cancer cells (HepG2). The current work provides a general approach toward the rational design and synthesis of a versatile theranostic nanoplatform based on functional protein-coated magnetic NPs with good biocompatibility, biodegradability, and capability of simultaneously performing multimodality imaging and therapy for optimal clinical outcomes.

1. Introduction

Theranostics, the combination of diagnostics and therapy into an all-in-one system, has attracted increasing research interests in biomedicine over the past few years [1–5]. Nanomaterials have shown promising results as potential theranostic agents. Among various theranostic nanomaterials, superparamagnetic Fe_3O_4 nanoparticles (NPs) have emerged as one of most appealing candidates due to several reasons [4,6,7]. First, Fe_3O_4 NPs have been widely used as T_2 contrast agent for magnetic resonance imaging (MRI) in clinical practice [3,8,9]. Second, these magnetic NPs have been extensively studied for their therapeutic applications such as hyperthermia and targeted drug/gene delivery [10–12]. To achieve the aforementioned biomedical applications, surface modification of the as-prepared Fe_3O_4 NPs by polymers, proteins, or other molecules is usually performed to offer these magnetic NPs physiological stability, biocompatibility, bio/magnetic targeting, imaging, and therapeutic functionalities [13–16]. For instance, the Fe_3O_4 NPs coated with a thin layer of dextran, are commercially used MRI contrast agent known as Feridex [15]. There are some reports on surface coating of Fe_3O_4 NPs with bovine serum albumin (BSA) for specific targeting of cancer cells [14] and with human serum albumin (HSA) for improving MRI contrast signal of cells [17]. Although much progress has been made in surface engineering of the magnetic Fe_3O_4 NPs with desired functionalities for specific applications, some important problems remain unsolved, which include: (1) due to cell membrane shielding effect, Fe_3O_4 NPs are distributed in the extracellular gap, and therefore unable to enter into cells for efficient drug release and/or cell labeling; (2) Fe_3O_4 NPs are taken up by cells via endocytosis, and generally remain confined to the endocytic vesicles, and the acidic environment of lysosomes may destroy Fe_3O_4 NPs [18]; (3) particles with size of 10–150 nm binding to proteins, or specific receptors may limit delivery of drug and imaging across the tight junction of epidermal cells, which exist in the blood brain barrier (BBB), vessel

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wall and so on [19]. All these drawbacks limit efficient cellular uptake of the magnetic NPs for optimal imaging and therapy.

Herein, we present a rational design and synthesis of a theranostic nanoplatform based on rotavirus capsid surface protein VP4-coated Fe_3O_4 NPs, which is illustrated in Fig. 1. Rotavirus is the major etiology agents of acute gastroenteritis in human beings, mammals and birds [20,21]. VP4, the outermost spike protein projected from the viral capsid, participates in receptor binding and cell penetration utilizing different integrins as cell receptors, and therefore plays important roles in virus cellular infection [21–23]. The coating of VP4 onto the surface of Fe_3O_4 NPs, we expect, will impart the magnetic NPs biocompatibility and physiological stability, significantly improve cellular uptake of the magnetic NPs due to VP4's excellent capability of membrane rupture, and, more importantly, effectively internalize NPs into cells without using the endosomal pathway [24], opening the space sealed by the tight junction of epidermal cells and modulating epithelial permeability [23]. As a consequence, coating of VP4 protein onto the magnetic NPs, to a large extent, enhances drug delivery efficiency and cellular imaging sensitivity. In addition, VP4 bears rich amine and carboxylic acid groups, which facilitate loading versatile imaging modalities and therapeutic functionalities onto the Fe_3O_4 NPs to form an all-in-one theranostic nanomaterial.

2. Materials and methods

2.1. Materials

Iron (III) acetylacetonate ($Fe(acac)_3$), meso-2,3-dimercaptosuccinnic acid (DMSA), dimethylsulfoxide (DMSO), 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. RPMI 1640 culture medium and fetal bovine serum (FBS) were purchased from Invitrogen. WST-1 was purchased from Biyuntian Biotechnology Institute. The guinea pig anti-VP4 antibody was obtained from Professor Yuanding Chen, Institute of Medical Biology, Chinese Academy of Medical Sciences. FITC-labeled goat anti-guinea pig IgG was purchased from Santa Crus. Dextran-coated Fe_3O_4 NPs (FeraSpin™ R) was purchased from Miltenyi Biotec. All other reagents were of analytical grade and used as received. Ultrapure water (18.2 MΩ cm^{-1}) was used throughout the experiments.

2.2. Instrumentation

The VP4 inclusion body was characterized by a Veeco Dimension 3100 atomic force microscope (AFM). The size and morphology of Fe_3O_4 NPs with different coatings were characterized by Tecnai G2 F20 S-Twin transmission electron microscopy (TEM). The protein-coated Fe_3O_4 NPs were stained by 2% tungstophosphoric acid for TEM measurement. Fluorescence spectra were obtained on a Hitachi F-4600 fluorescence spectrometer. The iron concentration was determined using atomic absorption spectrometry (SpectrAA-Duo 220 FS, Varian). WST assay was performed with a Biotek Elx 800 Microplate Reader. Cell lines were cultured in a water-jacketed CO_2 incubator (Thermo 3111). The MRI was carried out on 11.7 T Bruker micro 2.5 micro-MRI system with a conventional spin-echo acquisition. Fluorescence microscopy images were captured by a laser confocal microscope (Nikon A1). Prussian Blue Staining images were captured by a upright metallurgical microscope (Leica DM4000M).

2.3. Expression of VP4 in Escherichia coli

The VP4 gene (GenBank accession number: AY787644) was cloned from a human RV strain TB-Chen. The plasmid of pET-VP4 which carried the VP4 open reading frame (ORF) sequence was transferred into *E. coli* BL21 (DE3). The transformed cells were then incubated in Luria-Bertani (LB) liquid growth medium (containing 100 μg mL^{-1} ampicillin) at 37 °C overnight. When the optical density at 600 nm ($OD_{600\ nm}$) reached 1.5, bacterial cells were collected by centrifugation at 4000*g*. Next, the cells were suspended in lysis buffer (200 mM NaCl, 50 mM Tris–HCl, 5% Glycerol, 5% Triton X-100, 2 mM EDTA, pH 7.5) and lysed by sonication, followed by centrifugation at 12,000*g* for 30 min. The pellets were rinsed twice with urea (3 M in 10 mM Tris–HCl and 0.5 M NaCl) solution, and centrifuged at 12,000*g* for 30 min, and then characterized by AFM. Finally, the pellets (VP4 inclusion body fraction) were dissolved in 8 M urea solution (10 mM Tris–HCl, pH 8.0) and detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10%).

2.4. Renaturation of VP4

The target VP4 band was cut from the gel after staining with 150 mM KCl solution, the band was then put into the dialysis tube (MWCO = 8–14 kDa) containing electrophoresis buffer and run at 120 V for 5 h at 4 °C. After that, the VP4 protein was collected into a fresh dialysis tube and concentrated by polyethylene glycol (PEG, MW 20,000). SDS was removed from the obtained protein using the protocol described by Wessel and Flügge [25], then the SDS-free VP4 was dissolved in 8 M urea solution. Next, the VP4 was renatured by dialyzing in urea solution (2 M in 50 mM Tris–HCl) at 4 °C for 2 h, and then stepwise dialyzed in a gradient concentration of 1 M, 0.5 M, 0 M urea solution at 4 °C for 2 h. The refolded protein was dialyzed against PBS to remove the Tris–HCl. Finally, the supernatant was collected and stored at −20 °C.

2.5. Preparation of DMSA-coated Fe_3O_4 NPs

Fe_3O_4 NPs with average size of 8 nm were synthesized by thermal decomposition of $Fe(acac)_3$ according to the literature [26]. In brief, $Fe(acac)_3$ (3 mmol) was injected into a mixture containing 20 mL octylamine and 10 mL phenyl ether at 110 °C for 1 h and then, heated to reflux temperature (300 °C) for another 1 h, 7–8 nm Fe_3O_4 NPs was then obtained. Thus-prepared Fe_3O_4 NPs were coated with DMSA following a reported approach [27]. Briefly, 20 mg of the Fe_3O_4 NPs were dissolved in 2 mL toluene, then DMSA (20 mg) and DMSO (2 mL) was added to the solution and stirred at 25 °C for 12 h. The precipitant was then washed with ethyl

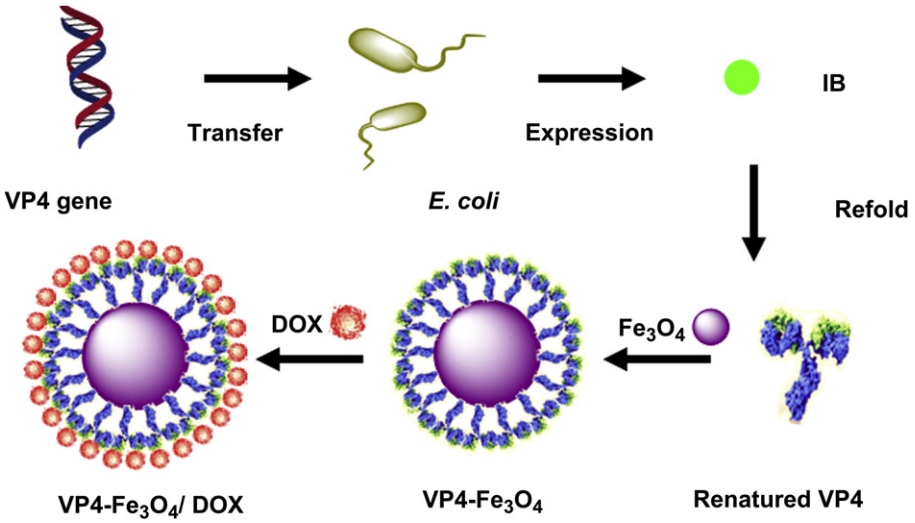

Fig. 1. Schematic diagram showing preparation of rotavirus capsid surface protein VP4-coated Fe_3O_4 NPs for dual modality magnetic resonance/fluorescence imaging and drug delivery.