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Innovative fluorescent magnetic albumin microbead-assisted cell labeling and intracellular imaging of glioblastoma cells

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ABSTRACT

Superparamagnetic nanoparticle-based polymer microbeads utilized as carriers are attractive materials widely applied in the biomedical field. However, the deficiency of toxicity, biocompatibility, and biodegradability for polymer materials often limits the application of these microbeads. In the present study, magnetic albumin microbeads (MAMbs), i.e., human serum albumin-coated γ-Fe_2O_3 nanoparticles, are synthesized to label human U251 glioblastoma multiforme cells. The effects of MAMbs on the biological behavior of U251 glioblastoma cells, including their proliferation, cell viability, cytoskeletal structure, cell cycle, and apoptosis rate, are investigated. Moreover, fluorescein isothiocyanate (FITC)-MAMbs are fabricated by reaction with fluorescent dye FITC used for intracellular imaging of U251 glioblastoma cells. MAMbs possess undetectable cytotoxicity and excellent biocompatibility with U251 glioblastoma cells, as demonstrated by the biological behavior and morphology of U251 cells exposed to MAMbs. Furthermore, the constructed fluorescent MAMbs allow effective intracellular imaging, as illustrated by fluorescence microscopic analysis. The fabricated fluorescent MAMbs have promising perspectives in biomedical research, especially in cell-targeted labeling and intracellular fluorescence magnetic dual-mode imaging in cancer-targeted diagnosis and therapy.

1. Introduction

Superparamagnetic iron oxide (SPIO) nanoparticles, particularly magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), have emerged as promising tools for various biological and medical applications, such as non-invasive behavior tracking and magnetic resonance imaging (Jung et al., 2011; Chauhan et al., 2013), cell labeling and separation (C. Wang et al., 2012 ;X. Wang et al., 2012; Xu et al., 2011; Sung et al., 2013), tissue engineering (Ramaswamy et al., 2009), DNA purification (Basu et al., 2013), biological sensing (Zhang et al., 2013), immunoassays (Wang et al., 2011; Gan et al., 2011), drug-targeted delivery, and cancer-targeted therapy (Kumar et al., 2010; Hadjipanayis et al., 2010), primarily because of their nanosized diameter and outstanding superparamagnetic properties. However, naked SPIO nanoparticles without surface coating or modification are erratic and can readily aggregate and precipitate in aqueous solutions and blood plasma and thereby seriously hinder their application either in vitro or in vivo (Tong et al., 2011). To endow SPIO nanoparticles with such characteristics, including better water solubility, stability, and low cytotoxicity, researchers have fabricated nanoparticles with coating layers, such as polymers (Petri-Fink et al., 2008; Kievit et al., 2009), dendrimers (Liu et al., 2011), polypeptides (Yang et al., 2011), and polysaccharides (Iconaru et al., 2012).

Biomedical research and clinical analyses have widely used superparamagnetic polymer microbead-aided cell labeling, which offers advantages over previous carriers (Evans et al., 2011; Maeng et al., 2010). The highly available surface-to-volume ratio of the microbeads provides many binding sites for cells, genes/drugs, and other conjugates and thus facilitates conjugate binding (C. Wang et al., 2012; X. Wang et al., 2012; Butoescu et al., 2009). Additionally, the intrinsic superparamagnetism of the microbeads allows conjugates to be targeted by delivery to target sites and released easily from the microbeads with little or no residual magnetization once the field is removed (Wahajuddin and Arora, 2012). All these merits indicate a promising future for superparamagnetic polymer microbeads in the biomedical field. However, experimental analysis shows that shortcomings, such as those in toxicity, biocompatibility, and biodegradability, often limit the further development of polymer microbeads for applications in biomedical research, especially for cell-based cancer diagnosis and treatment (Mahmoudi et al., 2009; Singh et al., 2010). Therefore, challenges remain in finding a non-toxic,

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highly biocompatible, and biodegradable method for cell labeling and imaging.

Extensive efforts have been devoted to designing nanocarriers with excellent biocompatibility. Different strategies are used to improve the biocompatibility of nanoparticles. These strategies include coating with micelles (Talelli et al., 2009), liposomes (Mahmoudi et al., 2011), homopolymers such as polyethylene glycol (Sun et al., 2008; Ujiie et al., 2011), dextran (Arbab et al., 2005), and carboxymethyl dextran (Creixell et al., 2011; Creixell et al., 2010). These strategies allow nanoparticles to be suspended in water, saline solution, and culture medium and thus make them suitable for *in vitro* and *in vivo* experiments, although most fabricated nanoparticles exhibit cytotoxicity to differing extent (Hong et al., 2011).

Moreover, one critical challenge in early cancer diagnosis and treatment using nanotechnology is the development of multifunctional nanoparticles that simultaneously serve as sensitive and localized tumor treatments. The development of multifunctional nanocarriers has attracted increasing attention because of their advantageous properties (Kaida et al., 2010; Fan et al., 2012). Fluorescent magnetic multifunctional nanocarriers that are a combination of fluorescent dye and magnetic nanoparticles have attracted great attention (Song et al., 2011; Cho et al., 2010). These fluorescent magnetic nanoparticles possess two attractive features, namely, fluorescence and superparamagnetism, which allow their intracellular movements to be controlled by magnetic force and monitored by a fluorescence microscopic system simultaneously. These features lead to effective ways to probe specific functions of bioactive molecules in localized domains or compartments of living cells without disturbing other parts of the cell. For example, fluorescent magnetic nanoparticles deliver activators or inhibitors to targeted tissue or cell domains under a magnetic field. A fluorescence microscope can then detect the positions of the particles. Toward this goal, we report the demonstration of the intracellular imaging of fluorescent FITC-modified magnetic albumin microbeads.

Here we describe a reliable and reproducible strategy, in which fluorescent magnetic albumin microbeads (FMAMbs) showing good biocompatibility and non-toxicity were fabricated for efficient cell labeling and intracellular visualization of human U251 glioblastoma multiforme cells, the most common and lethal brain malignant tumor in the central nervous system in adults (Yin et al., 2009; Ricard et al., 2012). In the present study, γ-Fe_2O_3 nanoparticles were first synthesized and then surface-coated with human serum albumin (HSA) by an improved heat-stabilization process. Combined HSA/γ-Fe_2O_3 microbeads were then incubated with human U251 glioblastoma cells at different concentrations (i.e., 25, 50, 100, 250, and 500 μg/mL) to investigate the effects of magnetic albumin microbeads on the biological behavior of glioblastoma cells. Finally, the FITC-modified FMAMbs were manufactured with the combined HSA/γ-Fe_2O_3 microbeads and fluorescent dye FITC and can be efficiently applied for the cell labeling and intracellular imaging of human U251 glioblastoma cells.

2. Experimental

2.1. Reagents and materials

The superparamagnetic γ-Fe_2O_3 nanoparticles utilized in this study were prepared from magnetite (Fe_3O_4) according to methods proposed elsewhere (Qu et al., 1999; Sun et al., 2004). Human glioblastoma cell line U251 was obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cell culture medium and fetal bovine serum (FBS) were purchased from Gibco Invitrogen Corporation (CA, USA). HSA, 3-(4,5-dimethylthiazol-2-diphenyl-tetrazolium) bromide (MTT), potassium ferrocyanide (Perls reagent), fluorescein diacetate (FDA), propidium iodide (PI), RNase, dimethyl sulfoxide (DMSO), Triton X-100 solution, and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Neutral red was obtained from Beyotime Biotech (Jiangsu, China). Fluorescent dye FITC and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Molecular Probes, Inc. (Eugene, OR, USA). Annexin V-FITC Apoptosis Detection Kit was obtained from Keygen Biotech (Nanjing, China). Rhodamine phalloidin was obtained from Cytoskeleton, Inc. (Denver, CO, USA). Other reagents and chemicals were purchased from local commercial suppliers and were of analytical reagent grade, unless otherwise stated. De-ionized water (Milli-Q, Millipore, Bedford, MA) was used to prepare aqueous solutions.

2.2. Assay principle for cell labeling and intracellular imaging with FMAMbs

The general principle of FITC-MAMb-aided cell labeling and intracellular imaging for U251 glioblastoma multiforme cells (Fig. 1) starts with the preparation of magnetic HSA/γ-Fe_2O_3 microbeads by combining magnetic γ-Fe_2O_3 nanoparticles and HSA. The MAMbs were then surface-modified by FITC through amino group reaction with HSA. Subsequently, the FITC-modified FMAMbs were internalized into human U251 glioblastoma multiforme cells for intracellular imaging and assayed with an inverted fluorescence microscope (Eclipse TE 2000-U, Nikon, Kyoto, Japan) equipped with a high-resolution CCD camera (CV-S3200, JAI Co., Japan).

2.3. Preparation of FITC-modified MAMbs

As the magnetic nanocarriers used in the present study, the MAMbs were synthesized by an improved heat-stabilization process, which involved a two-step reaction, i.e., synthesis of core superparamagnetic γ-Fe_2O_3 nanoparticles and preparation of HSA/γ-Fe_2O_3 microbeads. The preparation of magnetic HSA/γ-Fe_2O_3 microbeads followed previously reported protocol with minor modifications (Wang et al., 2009). Much information on the preparation and characterization of magnetic HSA/γ-Fe_2O_3 microbeads is provided in Supplementary information (SI 1).

HSA/γ-Fe_2O_3 microbeads were conjugated with FITC through the reaction of the amine groups of the microbeads with FITC. First, 50 mg of HSA/γ-Fe_2O_3 microbeads were resuspended in 5 mL of borate buffered saline (pH 8.4) and mixed with a solution of 100 μg/mL FITC solution. Subsequently, the suspension was incubated with slow stirring at room temperature for 2 h in the dark. The FITC-modified HSA/γ-Fe_2O_3 microbeads were then washed three times under magnetic field with phosphate buffered saline (PBS, pH 7.4). Finally, the FITC-modified FMAMbs were suspended in an RPMI-1640 medium and stored at 4 °C until use. The FMAMbs were observed with an inverted fluorescence microscope (Eclipse TE 2000-U, Nikon, Kyoto, Japan) equipped with a high-resolution CCD camera (CV-S3200, JAI Co., Japan).

2.4. Cell culture

The human U251 glioblastoma multiforme cells (obtained from the Shanghai Cell Bank) were routinely cultured in an RPMI-1640 medium supplemented with 8% (v/v) heat-inactivated FBS (Gibco), 1% L-glutamine, 1% penicillin (100 U/mL), and 1% streptomycin (100 μg/mL) in a humidified incubator at 37 °C in the presence of 5% CO_2. The cells were regularly monitored with an inverted light microscope, and the culture medium was changed every 2 d. The cells were normally passaged at a 1:3 ratio every 3 d to maintain the exponential growth phase.