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Multifunctional nanoprobe for cancer cell targeting and simultaneous fluorescence/magnetic resonance imaging

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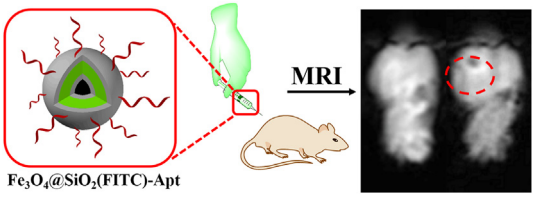
HIGHLIGHTS

- Multifunctional nanoprobe exhibits good dispersion, low cytotoxicity and excellent biocompatibility.
- The nanoprobe targets cancer cells, providing for simultaneous fluorescence and magnetic resonance imaging.
- The nanoprobe is used for real-time imaging in early liver cancer diagnosis.

GRAPHICAL ABSTRACT



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ABSTRACT

Multifunctional nanoprobes with distinctive magnetic and fluorescent properties are highly useful in accurate and early cancer diagnosis. In this study, nanoparticles of Fe_3O_4 core with fluorescent SiO_2 shell (MFS) are synthesized by a facile improved Stöber method. These nanoparticles owning a significant core-shell structure exhibit good dispersion, stable fluorescence, low cytotoxicity and excellent biocompatibility. TLS11a aptamer (Apt1), a specific membrane protein for human liver cancer cells which could be internalized into cells, is conjugated to the MFS nanoparticles through the formation of amide bond working as a target-specific moiety. The attached TLS11a aptamers on nanoparticles are very stable and can't be hydrolyzed by DNA hydrolytic enzyme *in vivo*. Both fluorescence and magnetic resonance imaging show significant uptake of aptamer conjugated nanoprobe by HepG2 cells compared to 4T1, SGC-7901 and MCF-7 cells. In addition, with the increasing concentration of the nanoprobe, T_2-weighted MRI images of the as-treated HepG2 cells are significantly negatively enhanced, indicating that a high magnetic field gradient is generated by MFS-Apt1 which has been specifically captured by HepG2 cells. The relaxivity of nanoprobe is calculated to be 11.5 $mg^{-1}s^{-1}$. The MR imaging of tumor-bearing nude mouse is also confirmed. The proposed multifunctional nanoprobe with the size of sub-100 nm has the potential to provide real-time imaging in early liver cancer cell diagnosis.

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1. Introduction

In the past few years, numerous attention has been paid to develop early cancer diagnosis using single or combined imaging modalities, such as optical, computed X-ray tomography (CT), ultrasound, positron emission tomography (PET) and magnetic resonance imaging (MRI). Among them, MRI, a sophisticated promising three-dimensional noninvasive tomographic diagnostic technique, has gained wide acceptance in diagnosis and medical research. It can penetrate deeply into tissue providing excellent soft tissue contrast with submillimeter resolution on clinical scanners and avoid using harmful ionizing radiation [1–4]. Superparamagnetic iron oxide nanoparticles, a kind of powerful multifunctional T_2 contrast agents, are commonly used as magnetic nonviral vectors for magnetic resonance imaging (MRI) [5–9]. They come into being an induced magnetic field under an additional field, and disturb the spin–spin relaxation (T_2) courses of protons in the vicinity, leading to negative enhanced (dark) MRI image [9,10]. However, their applications in MRI were largely restricted due to their poor dispersion or easy aggregation/deposition and hard surface modification [11,12]. In order to achieve the efficient imaging application, many efforts have been made to improve the stability and biocompatibility of the superparamagnetic iron oxide nanoparticles by coating a hydrophilic surface, such as silica shell [13], polymers [14,15] and biomolecules [16]. With the development of surface chemistry, silica is considered to be an exceptional candidate for encapsulating magnetic NPs owing to its good biocompatibility, outstanding physicochemical stability, and easy modification [11,17,18].

Optical imaging, on the other hand, has high sensitivity at the cellular level but could not provide spatial resolution and 3D tissue details. When the cellular-sensitive fluorescent imaging is combined with MR imaging, high resolution/sensitive imaging of both tissues and cells could be obtained. So far, many nanocomposites have been developed for simultaneous fluorescence and MR imaging. For example, Acharya group designed a novel multifunctional healthcare nanocomposite material for fluorescence and simultaneously for MRI imaging, including chitosan encapsulated iron oxide as MRI contrasting agent, CdS nanoparticles as fluorescent probe and podophyllotoxin as anticancer drug [17]. Eghbali and his coworkers reported a stable bimodal contrast agent for both MRI and fluorescence imaging, consisting of rhodamine B as fluorescent probe and APTES-modified superparamagnetic iron oxide nanoparticles as MRI contrasting agent [19]. Zhu and his colleagues designed a multifunctional peptide-fluorescent-magnetic nanocomposites, in which fluorescence dye (Cy5.5) was linked on the surface of Fe_3O_4@PEI for MRI and fluorescence imaging [20].

Aptamer is the artificial single-stranded nucleic acid sequence with extremely high specificity to certain targets that fold into secondary and tertiary structures. It is able to recognize and specifically bind to various targets ranging from small molecules, proteins to entire cells [21]. Compared with antibodies, aptamers show the advantages of lower toxicity, better thermally stability and are usually prohibited from being discovered by the immune system as foreign agents [22]. More importantly, it provides a potential approach for early diagnosis of cancers [23]. TLS11a aptamer is the first aptamer to be identified as specific for human liver cancer cell [24,25] and shows great binding affinity for corresponding hepatocellular carcinoma cell line-LH86. More importantly, TLS11a can also function as a membrane protein to protect the nanocomposites to internalize into cells. In the present study, we prepared a multifunctional fluorescent-magnetic nanocomposite modified with TLS11a aptamer (Fe_3O_4@SiO_2(FITC)-Apt1) *via* a facile method with low cytotoxicity and good biocompability, which was potentially for liver cancer targeting and imaging. Iron oxide magnetic nanoparticles were coated with a fluorescent silica shell which would allow their visualization by optical means, and then TLS11a aptamers were introduced on the surface of nanoparticles through the formation of amide bond to achieve cancer targeting capability [26–29]. HepG2, MCF-7, 4T1 and SGC-7901 cells were chosen as our model cancer cell lines for the in vitro studies. The cellular presence of the nanoprobe was confirmed by both fluorescence and MR imaging. Cancer cell targeting and MR imaging ability were further tested *in vivo*. All the data highlighted that the prepared nanoprobe had good potential for targeted diagnostic biomodal imaging.

2. Experimental section

2.1. Materials and reagents

Magnetic nanoparticles (MNPs, 10 nm, 2 mg mL^{-1}), modifying with mercaptopropionic acid on the surface, were obtained from Nanjing Nanoeast Biotech Co. LTD (Nanjing, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), tetraethoxysilane (TEOS), (3-aminopropyl)-triethoxysilane (APTES) and fluorescein isothiocyanate isomer I (FITC) were obtained from Sigma-Aldrich Inc. (USA). 4′,6′-Diamidino-2-phenylindole solution (DAPI) was purchased from TCI Moving Your Chemistry Forward (Japan). 3-(4,5-Dimethylthiazol-2-yl)-2-diphenyltetrazolium bromide (MTT) was received from KeyGen Biotech. Phosphate buffer solution (PBS) was prepared by mixing 8 g NaCl, 0.2 g KCl, 1.42 g Na_2HPO_4 and 0.27 g KH_2PO_4 in 1 L of ultrapure water. Dulbecco's modified eagle medium (DMEM) and Roswell park memorial institute (RPMI-1640) medium both containing 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin were gained from KeyGen Biotech. Human hepatoma (HepG2), human mammary cancer (MCF-7), mouse mammary tumor cell (4T1), gastric cancer (SGC-7901) cells were obtained from Department of Microbiology and Immunology, Medical School of Southeast University. All of other chemicals were analytical grade. Ultrapure water (18.2 MΩ cm) was got through Thermo purification system.

Aptamers were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high performance liquid chromatography (HPLC). TLS11a aptamer could specifically bind to the membrane surface of hepatocellular carcinoma cells. The TLS11a aptamer modified with –COOH at the end of 5′ was named to Apt1 and used for coupling with nanoparticles, while the intact complementary DNA of TLS11a aptamer was modified with a fluorescent dye of Cy3 at the 5'-end and named to Apt2. Their sequences were:

TLS11a aptamer (Apt1): 5′–COOH–ACA GCA TCC CCA TGT GAA CAA TCG CAT TGT GAT TGT TAC GGT TTC CGC CTC ATG GAC GTG CTG-3′.

Complementary to Apt1 (Apt2): 5′-Cy3-CAG CAC GTC CAT GAG GCG GAA ACC GTA ACA ATC ACA ATG CGA TTG TTC ACA TGG GGA TGC TGT-3′.

2.2. Apparatus

UV–*vis* spectra were carried on a UV-2450 spectrophotometer (SHIMADZU, Japan). The morphology and size of the nanoparticles were observed with a transmission electronmicroscope (TEM, Model S-2400 N, Hitachi, Japan) and a field emission scanning electron microscope (FESEM, Hitachi S-4800, Japan). Fluorescence spectra were carried out on a FluoroMax-4 spectrofluorometer with Xenon discharge lamp excitation (HORIBA, USA). Confocal laser microscopy images were carried out by a confocal laser scanning microscopy (CLSM, FluoView™ FV1000, Olympus, Japan). MRI was