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# One-pot preparation of hydrophilic manganese oxide nanoparticles as $T_1$ nano-contrast agent for molecular magnetic resonance imaging of renal carcinoma in vitro and in vivo

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ABSTRACT

Magnetic resonance imaging (MRI) contrast agents have become a necessary part for clinical practice to improve the sensitivity for the diagnosis of small lesions and injuries. Among them, manganese oxide nanoparticle (MnO NPs)-based MRI contrast agent attracts more and more attention because of their better performance in the detection of brain disease and positive enhancement in $T_1$-weighted image. However, the relatively low $r_1$ relaxivity and complex synthetic route hampered their wider applications. In this work, we proposed a one-pot approach to prepare hydrophilic MnO NPs via a polyol-like method with poly (ethylene glycol) (PEG) as both a solvent and surfactant. The obtained PEG-MnO NPs displayed a high $T_1$ relaxivity and a low $r_2/r_1$ ratio (12.942 $s^{-1}$ $mM^{-1}$ and 4.66) at 3.0 T, which was three times that of the clinical used contrast agent, Magnevist (Gd-DTPA). Additionally, when exposed to the simulated body fluid (SBF), acidic environment or glutathione, PEG-MnO NPs kept stable, favoring their further biological applications. Then, to explore their use for the molecular magnetic resonance imaging of 786-0 renal carcinoma, amino group modified AS1411 aptamer as the targeting molecule was introduced to conjugate with PEG-MnO NPs via covalent coupling reaction. The fabricated nanoprobe, AS1411-PEG-MnO, could clearly visualize 786-0 renal carcinoma cells with MRI in vitro. Furthermore, compared with PEG-MnO NPs, AS1411-PEG-MnO nanoprobe presented a prolonged retention in 786-0 renal carcinoma tumor in vivo. The intravenously injected nanoprobes were eventually excreted from the body through the renal clearance route. These results indicated the potential promising of PEG-MnO NPs as an alternative contrast agent in MRI scanning.

## 1. Introduction

Magnetic resonance imaging (MRI), as a noninvasive methodology, has been widely used in clinic diagnosis (Davies et al., 2013). To improve the sensitivity for the detection of small lesions or injuries, the employment of contrast agents (CAs) is necessary. According to the different enhancement mechanism, MRI CAs are commonly divided into two groups: $T_1$ CAs and $T_2$ CAs. For $T_1$ CAs, with $Gd^{3+}$ and $Mn^{2+}$ as representative, they can efficiently accelerate the $T_1$ relaxation process to produce a brighter $T_1$-weighted image (Barandov et al., 2016; Qin et al., 2013; Yang and Chuang, 2012; Shin et al., 2009). For $T_2$ CAs, with supermagnetic iron oxide ($Fe_3O_4$) as pioneer, they are magnetized first under an external magnetic field. The produced magnetic field by $T_2$ CAs causes local magnetic perturbations and accelarates the dephasing process of the proton nuclear spins to present a darker image. However, in clinical diagnosis, $T_1$ CAs are preferred because of the bright signal in $T_1$-weighted MRI images (McDonagh et al., 2016). Magnevist (Gd-DTPA) as $T_1$ MRI CA was approved by the FDA and European agencies in the late 1980s in clinical practice, accounting for 40–50% among MRI scanning cases (Bellin, 2006). Although widely used, Gd-chelates which were prepared to reduce the toxicity of $Gd^{3+}$ displayed relatively low relaxivity, short blood circulation time and non-specific bio-distribution (Luo et al., 2009; Fortin et al., 2007). To address this issue, $T_1$ nano-contrast agents are emerging as an alternative to traditional Gd-chelates. Although still in infant, their excellent properties have stimulated researchers to study more in this area. Nano-contrast agents were reported to possess higher water proton relaxivities, which was ascribed to the densely populated metal

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ions in a nanoparticle (Zhen and Xie, 2012). Easy conjugation with targeting molecules and longer blood circulation time further favored their biological applications. Gadolinium oxide nanoparticles ($Gd_2O_3$ NPs) with different coating agents such as polyethylene glycol (PEG) (Li et al., 2014; Faucher et al., 2012), D-glucuronic acid (Park et al., 2009a, 2009b), and bovine serum albumin (BSA) (Sun et al., 2013) have been successfully prepared and used as $T_1$ CAs for tumor MRI. Except $Gd_2O_3$ NPs, manganese oxide (MnO) nanoparticles are another kind of $T_1$ nano-contrast agent, which were first reported by Hyeon's group for the selective imaging of breast cancer cells in mouse brains (Na et al., 2007). Different from the 7 unpaired electrons for $Gd^{3+}$, there are only 5 unpaired electrons for $Mn^{2+}$, resulting in a lower relaxivity of MnO NPs than that of $Gd_2O_3$ NPs. Despite this limited $T_1$ contrast enhancement ability, MnO NPs as $T_1$ CA still attract researchers' attention because of their better biocompatibility. The potential danger of nephrogenic system fibrosis (NSF) brought by Gd-based $T_1$ CAs promoted researchers to look for suitable candidates (Zhen and Xie, 2012; Chen et al., 2015). Manganese is considered as a safer metal than Gd, because it is not only an endogenous metal, but also an essential mineral in all biological systems (Briley-Saebo et al., 2012). Thus, how to develop simple approach for the synthesis of MnO NPs with improved $T_1$ relaxivity is the major challenge for their wider biological applications in MRI.

Water-dispersed MnO NPs were commonly obtained by a two-step method. MnO-oleate complex were first fabricated by thermal decomposition of metal precursors in organic media. Then, ligands such as human serum albumin (HSA) (Huang et al., 2010), polyethylene glycol-phospholipid (Na et al., 2007), dimercaptosuccinic acid, N-(trimethoxysilylpropyl) ethylene diamine triacetic acid (TETT) silane (Chen et al., 2014a, 2014b), were introduced to convert MnO NPs into hydrophilic ones. Hydrophobic MnO NPs were also coated with a silica nanoshell (Hsu et al., 2016) or amphiphilic polymer (Abbasi et al., 2015) to make them hydrophilic. However, such two-step method has to suffer from the complex and time-consuming process. The preparation of hydrophilic MnO NPs as $T_1$ MRI CA via a one-pot approach possesses great promising. Lee et al. proposed a one-pot synthesis of hydrophilic MnO nanoparticles in a polar organic solvent, triethylene glycol (TREG), with D-glucuronic acid as surface coating agent. The obtained D-glucuronic acid-coated MnO NPs displayed a longitudinal water proton relaxivity of $r_1 = 7.02\ s^{-1}mM^{-1}$ at 1.5 T (Baek et al., 2010). Wen et al. also prepared hydrophilic and paramagnetic MnO NPs in TREG solvent by a one-pot microwave-assisted synthesis, but with poly(vinyl pyrrolidone)(PVP) as surface coating agent. The $r_1$ value was determined to be $0.81\ s^{-1}mM^{-1}$ at 0.47 T (Hu et al., 2013; Lu et al., 2013). Compared with $Gd_2O_3$ NPs, a great effort still should be made to improve the $T_1$ relaxivity of MnO NPs.

In this work, we proposed a facile one-pot approach for the preparation of hydrophilic MnO NPs with poly (ethylene glycol) bis (carboxymethyl) ether 600 as solvent and surface coating agent (Scheme 1). The obtained PEG-MnO NPs displayed a high $T_1$ relaxivity, $12.942\ s^{-1}mM^{-1}$ ($r_2/r_1$ ratio of 4.66) at 3.0 T with a blood circulation half-life of 59.76 min. Furthermore, the terminated carboxyl group of PEG molecules favored their further conjugation with amine group functionalized AS1411 aptamer to fabricate a targeting nanoprobe, AS1411-MnO, for the specific in vitro and in vivo MRI of tumor. The excellent in vitro and in vivo MRI performance of PEG-MnO NPs indicated their potential promising as $T_1$ MRI nano-contrast agent.

## 2. Materials and methods

### 2.1. Materials

Manganese nitrate, sodium chloride, and disodium hydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Poly (ethylene glycol) bis-(carboxymethyl) ether 600, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. Reagents for cell cultures including HDMEM, fetal calf serum, trypsin and RPMI 1640 were purchased from Gibco ThermoFisher Scientific Co., Ltd. (Shanghai, China). DNA oligos and the reagents for gel electrophoresis such as ammonium persulfate (APS), 40% acrylamide mix solution, 1,2-bis(dimethylamino)ethane (TEMED), DNA ladder and ethidium bromide (EB) were all obtained from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). All chemicals involved in this work were analytical-grade. All aqueous solutions were prepared with ultrapure water (≥18 MΩ, Milli-Q, Millipore). The DNA sequence was listed as follows.

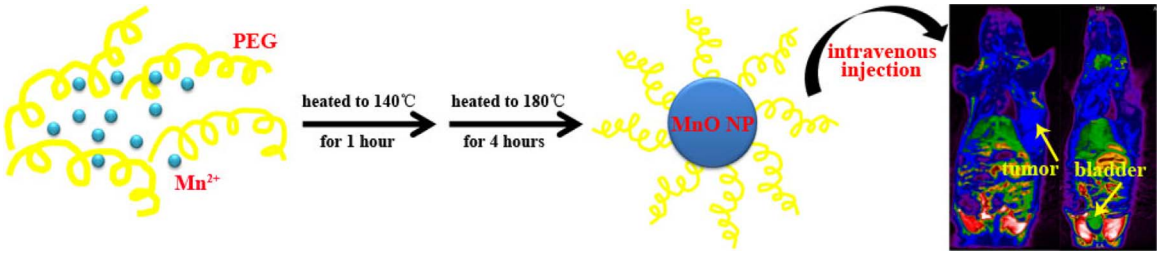
$NH_2$-AS1411: 5′-$NH_2$-C6-GGTGGTGGTGGTTGTGGTGGTGGTGG-3′

### 2.2. Apparatus and characterization

For the size and morphology characterizations of PEG-MnO NPs, transmission electron microscopic (TEM) (FEI Tecai G2F20, USA), high resolution transmission electron microscopic (HRTEM) (JEOL JEM 200CX, Japan) and dynamic light scattering (DLS) (NiComp380ZLS, USA) were employed. FT-IR spectra, UV–vis spectra and X-ray photoelectron spectroscopy (XPS) were obtained from the infrared absorption spectroscopy (Bruker, Germany), Nanodrop 2000 (Thermo Scientific, USA) and Thermo escalab 250XI (Thermo, USA), respectively. Gel imaging was carried out with gel Dox™ EZ Imager (BIO-RAD,USA). The manganese content was determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Optima 5300DV, PerkinElmer, USA). The absorbances for MTT assay were determined by a microplate reader (Multiskon MK3, USA) at 490 nm. The bright-field microscopic images were obtained from Olympus IX83 (Olympus, Japan). MRI scannings were performed on 3.0 T human magnetic resonance scanner (GE Signa, USA).

### 2.3. Cells and cell culture conditions

The 786–0 renal carcinoma cells and normal human umbilical vein endothelial cells, EA·hy926 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). They were cultured in a humidified incubator (Thermo, USA) at 37 °C under 5% $CO_2$ atmosphere with 10% FBS containing RPMI 1640 medium for 786–0 cells and 10% FBS-containing DMEM medium (Gibco, Grand Island, NY) for EA·hy926 cells, which were all supplemented with penicillin (100 μg/mL), and streptomycin (100 μg/mL).




**Scheme 1.** Scheme illustration of the one-pot preparation of hydrophilic PEG-MnO nanoparticles for magnetic resonance imaging of renal carcinoma in vivo.