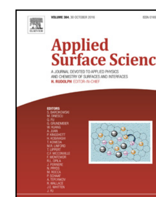
Applied Surface Science 389 (2016) 560–566

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc



Perspective Article

Synthesis and characterization of superparamagnetic iron oxide nanoparticles as calcium-responsive MRI contrast agents

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ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 20 July 2016
Accepted 28 July 2016
Available online 30 July 2016

Keywords:
Superparamagnetic iron oxide nanoparticles
EGTA
Contrast agents
Calcium
MRI

ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPIONs) as T2 contrast agents have great potential to sense calcium ion (Ca^{2+}) using magnetic resonance imaging (MRI). Here we prepared calcium-responsive SPIONs for MRI, formed by combining poly(ethylene glycol) (PEG) and polyethylenimine (PEI) coated iron oxide nanoparticle (PEI/PEG-SPIONs) contrast agents with the straightforward calcium-sensing compound EGTA (ethylene glycol tetraacetic acid). EGTA was conjugated onto PEI/PEG-SPIONs using EDC/sulfo-NHS method. EGTA-SPIONs were characterized using TEM, XPS, DSL, TGA and SQUIID. DSL results show that the SPIONs aggregate in the presence of Ca^{2+}. MRI analyses indicate that the water proton T2 relaxation rates in HEPES suspensions of the EGTA-SPIONs significantly increase with the calcium concentration because the SPIONs aggregate in the presence of Ca^{2+}. The T2 values decreased 25% when Ca^{2+} concentration decreased from 1.2 to 0.8 mM. The aggregation of EGTA-SPIONs could be reversed by EDTA. EGTA-SPIONs have potential as smart contrast agents for Ca^{2+}-sensitive MRI.

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1. Introduction

Calcium ions (Ca^{2+}) are involved in many crucial biological processes and pathologies. Ca^{2+} as a second messenger in the brain participates in a variety of physiological activity regulation of cells and tissues, including metabolism, muscle contraction, secretion and cell division [1,2]. The concentration of Ca^{2+} within cells falls within the range of 20–100 nM, whereas in extracellular fluids the concentration is of the order of 1.2 mM. Changes in extracellular Ca^{2+} concentrations have been documented in neuronal firing as well as in diseases such as ischemia, hypoglycemia and seizures [3]. To observe changes in Ca^{2+} concentrations noninvasively is very helpful for biomedical purposes, especially in neuroimaging [4]. Currently, Ca^{2+} imaging (generally intracellular calcium) is mostly based on optical methods using different fluorophores that suffer from low tissue penetration and restricted fields of view, and therefore cannot be used for Ca^{2+} imaging of deep tissues and organs [3–5]. Magnetic resonance imaging (MRI) has become a powerful tool in the imaging of deep tissues. Attempts have been made to prepare smart contrast agents for Ca^{2+}-sensitive MRI. Most of the positive MRI contrast agents for Ca^{2+} are based on functionalized gadolinium (Gd^{3+}) complexes, in which a change in the longitudinal relaxation time (T1) of water protons is observed upon binding to Ca^{2+} [6–9]. Further work toward the practical application was done to evaluate the influence of these Ca^{2+}-sensitive agents on the standard cellular physiological processes, normal neural signaling and consequently Ca^{2+} fluctuations [9]. Yet these gadolinium-containing T1 contrast agents also have some disadvantages, such as raising toxicity and low relaxivity. The levels of signal changes produced by these contrast agents are just comparable to the blood oxygenation level-dependent (BOLD)-based functional magnetic resonance imaging (fMRI). Chemical exchange saturation transfer (CEST) is a widely used MRI contrast mechanism to sense the presence of Ca^{2+} through their substrate binding kinetics [10,11]. However, the delivery of relatively high amount of smart contrast agents and the weak MRI signal changes observed remain the major limitations of these methodologies [9]. Superparamagnetic iron oxide nanoparticles (SPIONs) have been intensively studied for their biomedical applications as T2 contrast agents in MRI [12,13]. Compared with other nanoparticles, SPIONs exhibit high magnetic responsivity which can reduce the amount of the contrast agents needed for calcium-responsive MRI, low cytotoxicity, higher biocompatibility and chemical stability [14]. Furthermore, nontoxic polymer materials can be easily coated onto magnetic nanoparticles for surface modification with amino or carboxyl

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http://dx.doi.org/10.1016/j.apsusc.2016.07.160
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groups, which can be further conjugated with other functional biomolecules [4,15]. Ethylene glycol tetraacetic acid (EGTA) as a Ca^{2+}-specific chelating agent is a promising probe for Ca^{2+} [16]. In this work, a calcium-responsive system based on SPIONs and EGTA was synthesized and characterized. PEI/PEG-SPIONs conjugated with EGTA aggregated in the presence of Ca^{2+}. The aggregation in turn affected the transverse relaxation of the water protons in the vicinity, which caused dramatic T2 contrast changes, showing the calcium-responsive property of EGTA-SPIONs.

2. Experimental

2.1. Chemicals and materials

Iron(III) acetylacetonate ($Fe(acac)_3$, 98%) was provided by Tokyo Chemical Industry Co. Ltd., Tokyo, Japan. Ethylene glycol tetraacetic acid (EGTA, 99%) and ethylene diamine tetraacetic acid (EDTA, 99%) were from Amresco LLC, America. Calcium chloride anhydrous ($CaCl_2$, 96%), toluene (99%) and acetone (99%) were purchased from Xilong Chemistry Co. Ltd., Shantou, China. Polyethylenimine (PEI, 99%, MW 1800) and polyethylene glycol (PEG, 99%, MW 1000) were provided by Aladdin Industrial Corporation, Shanghai, China. 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC, 97%), and *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS, 98%) were purchased from Adamas Reagent Co. Ltd., Shanghai, China. Unless otherwise specified, all reagents and chemicals were analytical grade. Milli-Q water (18 MΩ) was prepared using a Milli-Q Synthesis System (Millipore, Bedford, MA, USA). All the chemicals were used without further purification.

2.2. Surface conjugation of PEI/PEG-SPIONs with EGTA by EDC/sulfo-NHS

PEI/PEG-SPIONs were synthesized as previously reported [13,17–19] by the thermal decomposition of $Fe(acac)_3$ in PEG added with PEI.

0.25 mmol EGTA was added to 10 mL deionized water, the pH of the solution was adjusted to 6.3 by addition of 0.1 M NaOH, then 0.25 mmol EDC and 0.75 mmol sulfo-NHS were added into the sample. After 6 h incubation at 60 rpm, 25 °C, then 0.25 mmol PEI/PEG-SPIONs in 10 mL water solution was added in, and the pH was adjusted to 7.4 by addition of 0.01 M NaOH, the solution was removed to a shaker and incubated for another 30 min at 60 rpm, 25 °C, then was put into the refrigerator at 4 °C to rest for overnight. The nanoparticles were collected with the help of a magnet and were dispersed again in deionized water to obtain the EGTA-SPIONs solution.

2.3. Chelating of Ca^{2+} with EGTA-SPIONs

0.2 mM (iron content) EGTA-SPIONs was added into $CaCl_2$ water solution with a final 20 mL volume at Ca^{2+} concentrations of 0, 0.8, and 1.2 mM. The mixture was then placed on a shaker and incubated for 30 min at 60 rpm, 25 °C to obtain the samples of Ca^{2+}@EGTA-SPIONs.

2.4. Characterization methods

The size and morphology of the SPIONs were determined using transmission electron microscopy (TEM, JEM 2100F). SPIONs dispersed in deionized water were drop-cast onto copper grids coated with a carbon film, and the grids were air-dried at room temperature. The hydrodynamic sizes and zeta potential of SPIONs in aqueous media were measured by a nanoparticle zeta potential analyzer (Nano ZS90, Malvern). Magnetic properties of the nanoparticles were measured by the superconducting quantum interference device (SQUID, Quantum Design, MPMS XL). Thermogravimetric analyses (TGA) were performed under nitrogen at a heating rate of 10 °C/min from room temperature up to 900 °C using a TGA Q500 (TA Instruments) analyzer. The iron concentration of the SPIONs solution was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 8000, Perkin Elmer). The chemical compositions of the surfaces of the SPIONs were determined by X-ray photoelectron spectroscopy (XPS) on ESCALAB 250Xi (Thermo Electron Corporation, USA).

2.5. MRI

Ca^{2+}@EGTA-SPIONs solutions were put in a group of six Eppendorf tubes and fixed with a form plate, then placed in an Agilent 7 T/160/AS small-animal MRI scanner with 95/63 volume coil. A fast spin echo multi-slice (FSEMS) sequence was used to collect T2 weighted images as scout view. A multi-echo multi-slice (MEMS) sequence was used to acquire T2 weighted images at several different echo times, and the parameters were set as follows: repetition time (*TR*): 2000 ms, echo time (*TE*) ranged from 10 to 100 ms, 10 ms increments, slice thickness: 3 mm, data matrices: 128 × 96, and acquisition time: 12 min and 48 s per scan. Relaxation rates were calculated by exponential fitting to the image data. All data analysis was performed with VnmrJ 4.0.

3. Results and discussion

3.1. Morphology characterization

The representative TEM images with the particle size distributions of the PEI/PEG-SPIONs, EGTA-SPIONs and Ca^{2+}@EGTA-SPIONs

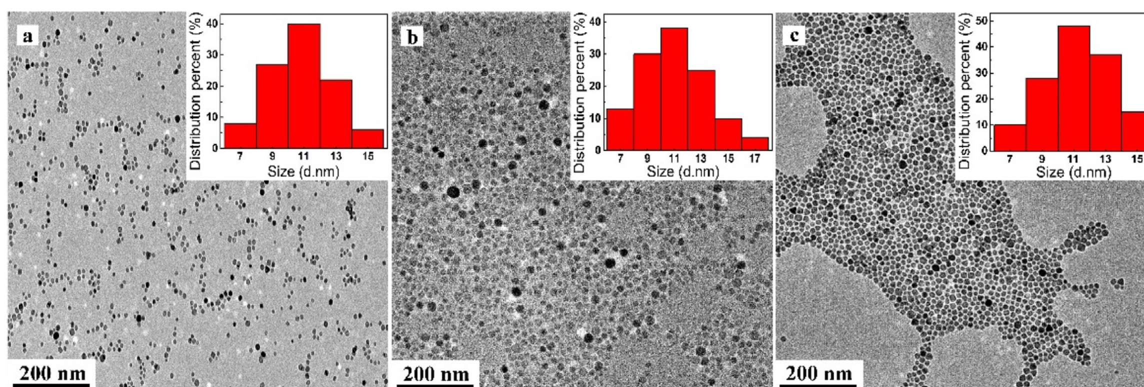

Fig. 1. TEM images with the size distributions of the PEI/PEG-SPIONs (a), EGTA-SPIONs (b) and Ca^{2+}@EGTA-SPIONs (c).

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