Journal of Colloid and Interface Science 492 (2017) 127–135

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

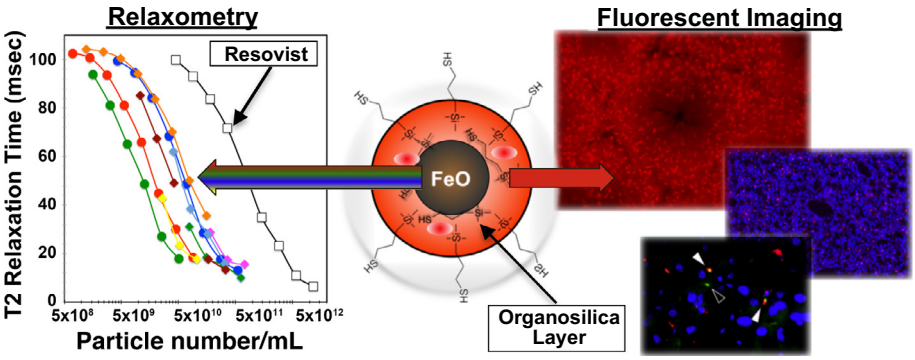
Regular Article

Relaxometric property of organosilica nanoparticles internally functionalized with iron oxide and fluorescent dye for multimodal imaging

Michihiro Nakamura [a,*], Koichiro Hayashi [b], Hitoshi Kubo [c], Takafumi Kanadani [a,d], Masafumi Harada [e], Toshinobu Yogo [b]

[a] Department of Organ Anatomy, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[b] Division of Materials Research, Institute of Materials and Systems for Sustainability, Fro-cho, Chikusa-ku, Nagoya 464-8603, Japan
[c] Advanced Clinical Research Center, Fukushima Medical University, 1 Hikariga-oka, Fukushima 960-1295, Japan
[d] Student Lab, Tokushima University Faculty Medicine, 3-18-15 Kuramoto, Tokushima 770-8503, Japan
[e] Department of Radiology, Tokushima University Graduate School of Medical Sciences, 3-18-15 Kuramoto, Tokushima 770-8503, Japan

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 September 2016
Revised 31 December 2016
Accepted 3 January 2017
Available online 5 January 2017

Keywords:
Multimodal imaging
Organosilica nanoparticles
Fluorescent imaging
Magnetic resonance imaging (MRI)
Mesoscopic imaging
Macrophages

ABSTRACT

Multimodal imaging using novel multifunctional nanoparticles provides a new approach for the biomedical field. Thiol-organosilica nanoparticles containing iron oxide magnetic nanoparticles (MNPs) as the core and rhodamine B in the thiol-organosilica layer (thiol OS-MNP/Rho) were synthesized in a one-pot process. The thiol OS-MNP/Rho showed enhanced magnetic resonance imaging (MRI) contrast and high fluorescence intensity. The relaxometry of thiol OS-MNP/Rho revealed a novel coating effect of the organosilica layer to the MNPs. The organosilica layer shortened the T2 relaxation time but not the T1 relaxation time of the MNPs. We injected thiol-OS-MNP/Rho into normal mice intravenously. Injected mice revealed an alteration of the liver contrast in the MRI and a fluorescent pattern based on the liver histological structure at the level between macroscopic and microscopic fluorescent imaging (mesoscopic FI). In addition, the labeled macrophages were observed at the single cell level histologically. We demonstrated a new approach to evaluate the liver at the macroscopic, microscopic level as well as the mesoscopic level using multimodal imaging.

* Corresponding author.
E-mail addresses: nakam@yamaguchi-u.ac.jp (M. Nakamura), hayashi@imass.nagoya-u.ac.jp (K. Hayashi), kubo-h@fmu.ac.jp (H. Kubo), taka_t_m_u@yahoo.co.jp (T. Kanadani), masafumi@tokushima-u.ac.jp (M. Harada), yogo@esi.nagoya-u.ac.jp (T. Yogo).

http://dx.doi.org/10.1016/j.jcis.2017.01.004
0021-9797/

1. Introduction

Multimodal imaging combines two or more imaging modalities into one system and is an important approach for advanced imaging studies [1–7]. Many types of nanoparticles (NPs) have been investigated and applied for various types of imaging. Magnetic nanoparticles (MNPs) have been used as T2-weighted MRI contrast agents [8–11]. MNPs show high efficacy in enhancing images and enabling the detection of focal lesions in living subjects through a noninvasive approach. For FI, semiconductor nanocrystals, often referred to as quantum dots (QDs) [12], fluorescent silica NPs [13] have been used as fluorescent probes in cell labeling, tracking, and molecular imaging. These fluorescent nanoparticles (FNPs) have advantages, such as higher fluorescent intensity and photostability compared with fluorescent molecules. In addition, NPs have a specific biodistribution compared with small molecules. NPs are uptaken by macrophages due to their size and are distributed in the reticular endothelial system. Imaging of macrophages using their naturally high endocytosis activity toward NPs is called macrophage imaging. Macrophage imaging is expected to be used in the clinical evaluation of patients [14]. In the liver, NPs are also rapidly taken up by specialized macrophages, Kupffer cells. MNPs increase the contrast between healthy tissue and tumor tissue lacking Kupffer cells. MNPs are applied for clinical diagnosis using MRI. The usefulness of liver macrophage imaging in MRI using MNPs is recognized. However, neither FI using FNPs nor multimodal imaging using nanoparticles for liver macrophage imaging has been investigated.

Recently, novel types of silica NPs were prepared from a single organosilicate and were referred to as organosilica NPs [15–26]. These organosilica NPs are both structurally and functionally different from typical (inorgano)silica particles prepared from tetraethoxyorthosilicate (TEOS) because the organosilica particles contain both interior and exterior functionalities. Unlike QDs nor MNPs, silica nanoparticles do not possess an inherent function, such as a signal that can be exploited for sensitive imaging applications. However, silica nanoparticles can be effectively functionalized with molecules. One type of organosilica particles, thiol-organosilica particles, possesses surface thiol residues, allowing for surface functionalization [15,17,19,20,26]. Furthermore, these organosilica particles can be internally functionalized with fluorescent dyes and other nanomaterials [15–26]. These characteristics of organosilica particles allow for functional fusion of the advantages of MNPs and FNPs. In this paper, we report the first one-pot synthesis of thiol-organosilica NPs containing MNPs as the core and rhodamine B in the thiol-organosilica layer (thiol-OS-MNP/Rho). The relaxometry of thiol-OS-MNP/Rho revealed a novel coating effect of the organosilica layer to the MNPs. We also demonstrated the mesoscopic FI at the level between macroscopic and microscopic FI using thiol-OS-MNP/Rho.

2. Experimental

2.1. Materials

3-Mercaptopropyltrimethoxysilane (MPMS), rhodamine B, and 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Sigma-Aldrich Chemical co, (St. Louis, MO). MNPs EMG606 and EMG707 were from Ferrotec Corp. (Tokyo, Japan). Ferucarbotran (Resovist) was from Schering (Berlin, Germany). Ethyl alcohol and 28% NH_4OH were purchased from Wako Fine Chemicals Inc. (Osaka, Japan). Anti-mouse CD68 antibody (rat IgG2a, clone FA-11) was obtained from AbD Serotec (Oxford, UK), and anti-rat IgG FITC antibody from eBioscience (San Diego, CA).

2.2. Preparation of thiol-OS-MNP/Rho

Various concentration of MPMS (1.6–50.0 mM), 0.0625% EMG707, and 27% NH_4OH, were mixed and then incubated at 100 °C for 10 h to grow the organosilica layer. Thiol-OS-MNP/Rho composed of MNPs and rhodamine B were prepared by the addition of rhodamine B (0.03–2.0 mM) to the reaction mixture described above. After incubation, the reaction mixture was subjected to centrifugation to remove remaining reagents, and the pellet was sonicated. The particles were washed extensively with distilled water.

2.3. Characterization of thiol-OS-MNP/Rho

The size and shape of the particles were observed electron microscopy. The particles were fixed on a 400-mesh copper grid coated with nitrocellulose, and transmission electron microscopy (TEM) images were obtained with a H7650 electron microscope (Hitachi, Tokyo, Japan). At least one hundred of the diameters of particle were measured to calculate average diameter and coefficient of variations (CVs). Fluorescence spectra of 1 mg/mL solutions of the particles and 5 μg/mL rhodamine B were obtained with a Nanodrop ND3300 Fluorospectrometer (Wilmington, DE). Fourier transform infrared (FT-IR) spectra were recorded within the 4000–500 cm^{-1} range using a Thermo Scientific Nicolet iS10 FT-IR spectrometer (Madison, WI) with diffuse reflectance method and operating in the transmittance mode.

2.4. Field-dependent magnetization measurements

The field-dependent magnetizations of NPs were measured with a vibrating sample magnetometer (VSM) (Type 5, Toei Kogyo, Tokyo, Japan) at room temperature and a superconducting quantum interference device (Model MPMS-7, Quantum Design, San Diego, CA) from 10 K to 300 K.

2.5. Relaxation times measurement

Relaxation times (T1 and T2) of NPs were measured with a 3-T Signa Excite HDxt (GE Healthcare, Milwaukee, WI, USA) with 8ch head coil. Phantom materials containing of various concentrations of Resovist (0.06, 0.12, 0.24, 0.49, 0.98, 1.95, 3.90, 7.81, 15.62, and 31.25 g/mL), EMG707, or thiol-OS-MNP/Rho and organosilica nanoparticle without MNP (7.81, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 g/mL) with 1.5% agar were prepared. T1-weighted images were obtained with a number of fast inversion recovery sequences with different inversion times (TI) of 100, 200, 400, 800, 1000, 1500, 2000, and 3000 ms, a repetition time (TR) of 4000 ms, echo time (TE) of 12.8 ms, 7 slices with 4 mm thickness and 1 mm spacing, FOV of 180 × 126 mm^2, matrix of 128 × 128, and 1NEX. T2-weighted images were obtained with a spin echo sequence having fixed TR of 3500 ms and echo time values of 8.5, 17.1, 25.6, 34.2, 42.7, 51.3, 59.8, and 68.4 ms. All analyses and calculations of relaxation times were performed with ImageJ (NIH, Bethesda, MD) and Microsoft Excel 2010. Signal intensity was measured using ROI function and calculation of relaxation times was done using the method described in the literature [27].

2.6. MRI of mice injected thiol-OS-MNP/Rho

Male C57 BL/6 J mice (10 weeks) were injected intravenously with 0.1 mL of a solution containing 10 mg/mL thiol-OS-MNP/Rho or Resovist, respectively. After 1 day, these animals were examined with MRI. MRI images were obtained using a 3-T Signa Excite HDxt with 8ch head coil. MRI images were reconstructed using OsiriX software (OsiriX Foundation, Geneva, Switzerland).