Biomaterials 30 (2009) 2919–2928

Contents lists available at ScienceDirect

Biomaterials

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

Manganese ferrite nanoparticle micellar nanocomposites as MRI contrast agent for liver imaging

Jian Lu [a], Shuli Ma [a], Jiayu Sun [b], Chunchao Xia [b], Chen Liu [a], Zhiyong Wang [a], Xuna Zhao [c], Fabao Gao [b], Qiyong Gong [b], Bin Song [b], Xintao Shuai [d], Hua Ai [a,b,*], Zhongwei Gu [a]

[a] National Engineering Research Center for Biomaterials, Sichuan University, 29 Wangjiang Road, Chengdu 610064, China
[b] Department of Radiology, West China Hospital, Sichuan University, China
[c] Philips Healthcare, China
[d] School of Chemistry and Chemical Engineering, Sun Yat-sen University, China

ARTICLE INFO

Article history:
Received 11 November 2008
Accepted 2 February 2009
Available online 20 February 2009

Keywords:
Manganese ferrite
Block copolymer
MRI
Mouse liver
Contrast agent

ABSTRACT

Iron oxide nanoparticles are effective contrast agents for enhancement of magnetic resonance imaging at tissue, cellular or even molecular levels. In this study, manganese doped superparamagnetic iron oxide (Mn-SPIO) nanoparticles were used to form ultrasensitive MRI contrast agents for liver imaging. Hydrophobic Mn-SPIO nanoparticles are synthesized in organic phase and then transferred into water with the help of block copolymer mPEG-*b*-PCL. These Mn-SPIO nanoparticles are self-assembled into small clusters (mean diameter ≈ 80 nm) inside micelles as revealed by transmission electron microscopy. Mn-SPIO nanoparticles inside micelles decrease PCL crystallization temperatures, as verified from differential scanning calorimetry and Fourier transform infrared spectroscopy. The Mn-SPIO based nanocomposites are superparamagnetic at room temperature. At the magnetic field of 1.5 T, Mn-SPIO nanoparticle clustering micelles have a T_2 relaxivity of 270 (Mn + Fe) $mM^{-1}\,s^{-1}$, which is much higher than single Mn-SPIO nanoparticle containing lipid–PEG micelles. This clustered nanocomposite has brought significant liver contrast with signal intensity changes of −80% at 5 min after intravenous administration. The time window for enhanced-MRI can last about 36 h with obvious contrast on liver images. This sensitive MRI contrast agent may find applications in identification of small liver lesions, evaluation of the degree of liver cirrhosis, and differential diagnosis of other liver diseases.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Magnetic resonance imaging (MRI) is among the best noninvasive methodologies today in clinical medicine for assessing anatomy and function of tissues. The MRI technique offers several advantages such as excellent temporal and spatial resolution, the lack of exposure to radiation, rapid *in vivo* acquisition of images, and long effective imaging window [1–4]. However, MRI is much less sensitive than nuclear medicine or fluorescence imaging when used to monitor small tissue lesions, molecular activity, or cellular activities [3–5]. Searching for ultrasensitive contrast agents has drawn a lot of attention during the last decade.

Superparamagnetic iron oxide (SPIO) nanoparticles are strong enhancers of proton relaxation with superior MR T_2 (transverse relaxation) shortening effects, and can be used at a much lower concentration than paramagnetic agents [6,7]. With the help of SPIO contrast agents, MRI has made great progress in studying gene delivery, cell trafficking, drug delivery, tumor diagnosis and many other fields [4,5,8–14]. The most commonly used and clinically approved SPIO contrast agents are dextran- or carboxydextran-coated SPIO nanoparticles such as Feridex or Resovist. The continued search of sensitive contrast agents with different compositions led to the development of other superparamagnetic nanocrystals including FePt alloy nanocrystals [15] and metal-doped iron oxide nanoparticles [16–19]. One potential candidate discovered recently is manganese ferrite ($MnO{\cdot}Fe_2O_3$) nanocrystals, which have higher magnetization than magnetite nanoparticles and other metal-doped iron oxide nanoparticles such as $CoO{\cdot}Fe_2O_3$ and $NiO{\cdot}Fe_2O_3$. More importantly, for the same sized metal-doped iron oxide nanoparticles, Mn-doped $MnFe_2O_4$ nanoparticles have the strongest MR contrast effect in T_2-weighted images with much higher T_2 relaxivity [18,19].

* Corresponding author. National Engineering Research Center for Biomaterials, Sichuan University, 29 Wangjiang Road, Chengdu 610064, China. Tel./fax: +86 28 85413991.
E-mail address: huaai@scu.edu.cn (H. Ai).

0142-9612/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2009.02.001

High quality Mn-doped SPIO (Mn-SPIO) nanoparticles can be synthesized in organic phase at high temperatures for precise control of particle size and morphology [16,17,20,21]. These nanoparticles are well dispersed in organic solvents but not in water. Three important prerequisites need to be satisfied by the nanoparticles before any biological applications. First, they need to be well dispersed in water for good solubility; second, they need to preserve their magnetic properties; third, the coating materials need to be biocompatible for *in vivo* applications.

Polymeric micelles are potential candidates to meet the above requirement. Polymeric micelles are nanoscopic core–shell structures self-assembled by amphiphilic block copolymers in an aqueous solution [22,23]. Their various advantages such as low critical micellar concentration (CMC), reduction in toxic side effects of the drug and long blood circulation time, etc., have led to their use in hydrophobic small molecule anti-cancer drug encapsulation [22,24–26], and some of the formulations are already in clinical trials [27,28]. Recently, applications have been extended to encapsulation of functional hydrophobic nanoparticles including magnetite [12,29,30] and quantum dots [31,32] for bioimaging. The magnetic and optical properties of original particles were preserved, and these nanocomposites have shown good biocompatibility both *in vitro* and *in vivo*. Particularly, multiple magnetic nanoparticles can be encapsulated inside the hydrophobic core of one micelle, forming a closed packing structure – clustering, which resulted in much stronger T_2 effects than micelles containing a single particle at the same iron concentration [29]. This structure provides opportunities for design and development of ultrasensitive MR probes.

In this study, we choose amphiphilic diblock copolymer methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone) (mPEG-*b*-PCL) for encapsulation of multiple Mn-SPIO nanocrystals to form the clustering nanocomposites. Magnetic properties were examined through magnetization measurement and T_2 relaxivity was measured at the magnetic field of 1.5 T with a clinical MR scanner. The biocompatibility was investigated using mouse macrophage cell line Raw 264.7 and Human hepatocarcinoma cell strain HepG2 *in vitro*. Finally, contrast effect of MRI was assessed on mouse liver at 3.0 T Philips MRI scanner.

2. Materials and methods

2.1. Synthesis and characterization of Mn-SPIO nanoparticles

Iron(III) acetylacetonate [$Fe(acac)_3$], manganese acetylacetonate [$Mn(acac)_2$], 1,2-hexadecanediol (97%), benzyl ether (99%), oleic acid (90%), and oleylamine (>70%) were purchased from Aldrich Chemical Co. and used as-received. The nanoparticles were synthesized following a published procedure by Sun et al. [20]. Briefly, $Fe(acac)_3$ (1 mmol) and $Mn(acac)_2$ (0.5 mmol) were mixed with 1,2-hexadecanediol (5 mmol), oleic acid (3 mmol), and oleylamine (3 mmol) in benzyl ether (10 ml) under dry and deoxidized argon atmosphere. Then the mixture was heated to reflux (300 °C) for 1 h. After cooling to room temperature, the solution was treated with ethanol and then centrifuged to yield a dark-brown precipitate. The product was redispersed in hexane and reprecipitated with ethanol. Finally, these nanocrystals were dispersed in anhydrous hexane for storage.

Samples for transmission electron microscopy (TEM) analysis were prepared by drying a dispersion of the Mn-SPIO nanoparticles in hexane on amorphous carbon coated copper grids. Particles were imaged using an FEI Tecnai 20 microscope. Size distribution of nanoparticles dispersed in hexane was characterized by dynamic light scattering (DLS) using a Nanosizer (Zetasizer Nano ZS, Malvern, U.K.). The structure of the particles was characterized using high resolution (HRTEM) and selected area electron diffraction (SAED). The measured lattice spacing, d (Å), was compared to the standard atomic spacing of $MnFe_2O_4$ (*hkl* indexes from the standard PDF database). Molar ratio of iron and manganese of nanocrystals was determined by atomic absorption spectroscopy (AAS) (AA800, Perkin–Elmer, USA).

2.2. Synthesis and characterization of mPEG-b-PCL block copolymer

Diblock copolymer methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone) (mPEG-*b*-PCL) was synthesized by ring-opening polymerization of ε-caprolactone using monomethoxy-terminated PEG (mPEG5k, Fluka) as a macroinitiator and stannous octoate ($Sn(Oct)_2$, Aldrich) as a catalyst [25,33]. ε-Caprolactone was dried over calcium hydride (CaH_2) powder then purified by distillation under reduced pressure, while mPEG was dehydrated by azeotropic distillation with toluene then dried to constant weight in vacuum oven. $Sn(Oct)_2$ was used as-received. The whole reaction system was placed in oil bath at 105 °C for 48 h with magnetic stirring. After cooling at room temperature, the resulting block copolymers were dissolved in tetrahydrofuran (THF) and precipitated in excess amount of cold ether. Then the precipitates were dried in vacuum oven.

^1H NMR spectra were obtained with deuterium chloroform ($CDCl_3$) as solvent and TMS as internal standard, using a Bruker AM 400 apparatus (400 MHz) at 25 °C. The actual PCL content of copolymer was calculated from the integration of hydrogen shown in ^1H NMR. Average molecular weight and its distribution were determined by gel permeation chromatography (GPC) (Waters ALC/GPC 244, USA) operating with THF as eluent and calibrated with polystyrene standards.

2.3. Self-assembly and characterization of mPEG-b-PCL/Mn-SPIO nanocomposites

Mn-SPIO nanoparticles in hexane were dried under argon flow and redispersed in THF together with mPEG-*b*-PCL (polymer/Mn-SPIO mass ratio = 2:1). Then, mixed solution was slowly added into Milli-Q water (produced by Milli-Q Biocel, Milli-pore, USA) with sonication. The Mn-SPIO-loaded micelle formation is illustrated in Scheme 1. The mixture was under shaking overnight and remaining THF was removed through rotary evaporation. The size distribution and morphology of Mn-SPIO nanoparticle-loaded micelles were characterized by DLS and TEM respectively, and the morphology of Mn-SPIO-loaded micelles was characterized by AFM (MFP-3D, Asylum Research, USA) using AC mode (i.e. Tapping Mode).

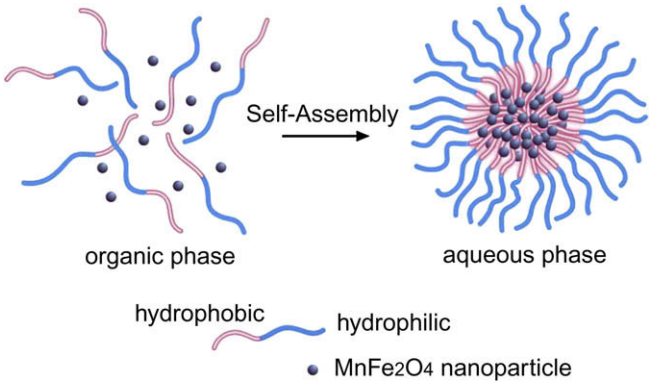
2.4. Magnetization and T_2 relaxivity studies

Elemental analyses of manganese and iron from mPEG-*b*-PCL/Mn-SPIO nanocomposite samples were performed using AAS. Magnetic studies were carried out using an MPMS7 Quantum Design SQUID magnetometer (Quantum Design, San Diego, U.S.) at 300 K. A sample in aqueous phase was lyophilized and then measured with the scope of −20 kOe to 20 kOe, 4 quadrants. T_2 relaxivities were measured at 1.5 T on a clinical MR scanner (Siemens Sonata) at room temperature as described before [29]. The T_2-weighted images were acquired with a conventional spin echo acquisition (TR = 5000 ms) with TE values ranging from 6 to 170 ms. Relaxivity values of r_2 were calculated through the curve fitting of $1/T_2$ relaxation time (s^{-1}) vs the magnetic atoms (Mn + Fe) concentration (mM).

2.5. Interaction between Mn-SPIO nanoparticles and mPEG-b-PCL copolymer

To investigate the interaction between Mn-SPIO nanoparticles and PEG-*b*-PCL in micelles, a series of mPEG-*b*-PCL/Mn-SPIO micelles were synthesized using the above method with polymer/Mn-SPIO mass ratios of 1:1, 2:1, 4:1 and 6:1. The empty mPEG-*b*-PCL micelle was used as control. After removal of THF, all samples and the control were lyophilized then investigated by differential scanning calorimetry (DSC, Q2000, equipped with Refrigerated Cooling System, TA Instruments, USA).

The procedure for the DSC test was as follows: first, samples were heated to 100 °C at a heating rate of 10 °C/min, held isothermal for 10 min, cooled as rapidly as possible to −90 °C, and held isothermal for 10 min. The samples were again heated to 100 °C at 10 °C/min and held isothermal for 10 min, and the glass transition temperature (T_g) and melting temperature (T_m) signals were collected in this stage. Finally the samples were cooled to −90 °C at a rate of 10 °C/min and the crystallization temperature (T_c) signals were collected in this stage.



Scheme 1. Schematic illustration of mPEG-*b*-PCL/Mn-SPIO micelle formation and Mn-SPIO nanocrystal clustering inside the micelle core.

Download English Version:

https://daneshyari.com/en/article/10086

Download Persian Version:

https://daneshyari.com/article/10086

Daneshyari.com