Biomaterials 30 (2009) 3882–3890

Contents lists available at ScienceDirect

Biomaterials

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

Superparamagnetic iron oxide nanoparticle-embedded encapsulated microbubbles as dual contrast agents of magnetic resonance and ultrasound imaging

Fang Yang [a], Yixin Li [a], Zhongping Chen [a], Yu Zhang [a], Junru Wu [b], Ning Gu [a,*]

[a] Jiangsu Laboratory for Biomaterials and Devices, State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China
[b] Department of Physics, University of Vermont, Burlington, VT 05405, USA

ARTICLE INFO

Article history:
Received 1 February 2009
Accepted 29 March 2009
Available online 22 April 2009

Keywords:
Superparamagnetic iron oxide nanoparticles
Encapsulated microbubbles
MRI (magnetic resonance imaging)
Ultrasound imaging
Contrast agents

ABSTRACT

An encapsulated microbubble (EMB) of a novel construct is proposed to enhance the magnetic resonance imaging contrast by introducing superparamagnetic iron oxide (SPIO) nanoparticles (mean diameter is 12 nm) into the polymer shell of the microbubble. Such microbubble vesicle has nitrogen gas in the core and its mean diameter is 3.98 μm. An *in vitro* MR susceptibility experiment using a phantom consisting EMBs has shown that the relationship between the transverse relaxation rate R_2 and the Fe_3O_4 nanoparticle concentration in the shell (the volume fraction of EMBs is kept constant) can be fitted to a linear function and an exponentially growth function is observed between R_2 and the SPIO-inclusion microbubble concentration. The *in vivo* MRI experiments also show that the SPIO-inclusion microbubbles have longer contrast-enhancement duration time in rat liver than non-SPIO-inclusion microbubbles. An *in vitro* ultrasound imaging experiment of SPIO-inclusion microbubbles also shows that they can enhance the ultrasound contrast significantly. Additionally, the interaction between the SPIO-inclusion microbubbles and cells indicates that such microbubble construct can retain the acoustic property under the ultrasound exposure by controlling the SPIO concentration in the shell. Therefore, the proposed SPIO nanoparticle-embedded EMBs potentially can become effective MR susceptibility contrast agents while also can be good US contrast agents.

1. Introduction

When a small magnetic object is introduced into a medium exposed to a uniform static external magnetic field along z direction, B_z, the locally arisen perturbation to the magnetic field is related to the object's geometry, dimension and magnetic susceptibility [1]. For a spherical particle located at the origin of the spherical coordinates, the magnetic-field perturbation δB_z at the position (r, θ) is described by Eq. (1) in SI units [1,2]:

$$\delta B_z(r,\theta) = (1/3)B_z\Delta\chi(R/r)^3\left(3\cos^2\theta - 1\right) \quad (1)$$

where R is the radius of the particle, $\Delta\chi$ is the magnetic susceptibility difference between the object and the medium. The perturbation is short-ranged as $1/r^3$ and positive when $0^\circ \le \theta \le 54.7^\circ$ and $125.3^\circ \le \theta \le 180^\circ$ (it reaches the maximum at $\theta = 0^\circ$ and 180°), and is negative in other region of θ. The contrast enhancement in magnetic resonance imaging (MRI) by the presence of the magnetic particle is the result of the above-described local non-uniformity of the magnetic fields, which leads to a rapid dephasing of protons and shortens the spin–spin relaxation time (T_2) and increases the transverse relaxation rate (R_2).

Encapsulated microbubbles (EMBs) consisting of a specific gas surrounded by a polymer shell are commonly used as contrast agents to enhance contrast in ultrasound (US) imaging because their acoustic impedances are significantly different from that of the surrounding tissue or fluid [3,4]. It has been shown [5,6] that EMBs excited by the moderate ultrasound (the negative peak acoustic pressure $P < 0.25$ MPa) can transiently increase permeability of membranes of cells letting DNA, antibodies, or anticancer drugs in a solution entering the cells; this technique is called sonoporation. It has been further shown that EMBs can also be used as contrast agents in MRI [7]. The rationale is that the presence of an EMB in tissue introduces the magnetic susceptibility difference $\Delta\chi$ at the gas–tissue interface; the contrast is particularly evident when B_z is strong and the core gas is paramagnetic [8]. Ueguchi

* Corresponding author. Tel.: +86 25 83792576; fax: +86 25 83272460.
E-mail address: guning@seu.edu.cn (N. Gu).

0142-9612/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2009.03.051

et al. [9] have used EMBs in a phantom experiment and showed that they were useful as MRI contrast agents when B_z was 1.5 T or greater. Other known applications of EMBs include functional lung MR imaging [10] and intra-cardiac pressure sensing for noninvasive manometry associated with MRI [11].

For a given EMB concentration, R_2 is known to be linear to B_z [1]. Although it has been shown that EMBs can be used as MRI contrast agent in B_z as low as 1.5 T, significant contrast enhancement can only be achieved *in vivo* by using much higher B_z [9]. Various techniques have been adopted to break this limitation [7–9,12]. A practical and convenient way, in our opinion, is to embed paramagnetic iron oxide, or other magnetic materials into the shell of an EMB to increase the MR susceptibility. To minimize the possible distortion to the overall quality of the MRI image, magnetic materials introduced into the shell should be small while still obtain high susceptibility [1]. Since it is known that superparamagnetic iron oxide (SPIO) nanoparticles can enhance T_2-weighted MRI images [13–15], they can be ideal candidates to be embedded into EMBs' shell for MRI contrast agents.

Ultrasound is a real-time, non-ionizing, cost effective, and widely available imaging modality, but it has poor tissue discrimination ability [16,17]. MR imaging is also a noninvasive imaging tool with exquisite soft tissue contrast and multi-planar imaging capacities, but it cannot provide real-time images and usually it has a relatively long imaging time [18]. In many clinical applications, US and MRI are complimentary; both modalities are usually needed to discern possible pathological changes in tissue. If the dual-modality contrast agents combining the advantages of the US and MR imaging can be applied clinically, adequate and comprehensive imaging information can be obtained [19–21] by just using one kind of contrast agents. It is not only convenient to medical professionals but also reduces the health-care cost. In this work, we have prepared a special microbubble construct which is loaded with both gas and superparamagnetic iron oxide (Fe_3O_4) nanoparticles. The Fe_3O_4 SPIO particles have susceptibility as high as 70 (in comparison, tissue in general is weakly diamagnetic and its susceptibility is in the range: $-11.0\times10^{-6}<\chi_{tissue}<-7.0\times10^{-6}$) [1]. This construct using nanometer size particles can effectively emulate the desired large susceptibility difference between EMBs and their surrounding medium. Thus, such EMBs can potentially serve as effective MRI contrast agents.

First, we present briefly the preparation method as well as the main physical characterization features of the SPIO Fe_3O_4 nanoparticle-inclusion EMBs. To measure their characteristics as MR contrast agents *in vitro* and *in vivo*, a 7 T MR scanner was used with the scanner sequence of T_2 (spin–spin interaction time constant)-weighted fast spin-echo. We identified that transverse relaxation rate, R_2, for the SPIO nanoparticle-inclusion EMBs increases exponentially when the EMBs' volume fraction increases, which is different from the linear relation of the transverse relaxation rate, R_2, *vs* the EMB volume fraction for non-SPIO-inclusion EMBs. The possible different physical mechanisms of transverse relaxivity enhancement for SPIO-inclusion EMBs and non-SPIO-inclusion EMBs were explored. Further, the longer contrast-enhancement duration time of SPIO-inclusion EMBs was found in rat liver MRI experiments, which was quantified by the signal-to-noise ratio (SNR) of the acquired MR images.

In addition to their MRI properties, the ultrasound contrast-enhancement experiment and the interaction between the cells and SPIO-inclusion EMBs under US exposure were also studied. We have shown the newly designed EMBs can oscillate vigorously under a moderate acoustic field (the negative peak acoustic pressure $P<0.25$ MPa). The Fe_3O_4 nanoparticles can be delivered *via* a transient sonoporation process into the cells through the cell membranes [22,23]. The cell experiments indicate that the EMBs with appropriate concentration of SPIO Fe_3O_4 nanoparticles encapsulated in the shell retain the highly echogenic and distensible properties, which can make the SPIO-inclusion EMBs not only serve as ultrasound contrast agents [24], but also as the effective MR imaging contrast agents.

2. Materials and methods

2.1. Fabrication of EMBs

Microbubbles with polyvinyl alcohol (PVA) outer layer and a poly(DL-lactide) (PLA) inner layer were prepared using a double emulsion solvent evaporation interfacial deposition (water-in-oil-in-water emulsion) process. It was reported that both PVA and PLA have good biocompatibility and biodegradability [25,26]. An organic solution (10.00 ml) was prepared containing PLA (0.50 g, purchased from the Shandong Key Laboratory of Medical Polymer Materials, China) and hydrophobic SPIO Fe_3O_4 nanoparticles [27] (mean diameter = 12 nm, Jiangsu Laboratory for Biomaterials and Devices) in methylene chloride at 25 °C. The amount of added SPIO nanoparticles was varied. EMBs with different SPIO-inclusion amounts were prepared. To generate first SPIO-inclusion bubble emulsion, Milli-Q water (1.00 ml) and Span 80 (0.50 ml) were added to the organic solution and continuously sonicated at 100 W with a probe while constant purging using a steady (4 ml/min) stream of N_2 gas for 5 min. The free SPIO nanoparticles were separated by using the magnetic isolation method [28]. The first bubble emulsion was then poured into a 5% PVA (w/v, from Alfa Aesar®) solution including Tween 80 (0.5 ml) and mixed mechanically for 2 h to form stable double-layered emulsion bubbles without the residual organic solvent. After reaction, the final emulsion became milk-white. The microbubble solution was then transferred to a custom made centrifuge tube to isolate different size microbubbles by using the differential centrifugation methods [29]. The collected agent was stored at 4 °C in tightly capped vials sealed with paraffin films for experimental analysis. Before the sample was used, it was diluted to the concentration of $7–8\times10^8$ microbubbles/ml in a phosphate buffer solution (PBS, pH = 7.4 ± 0.1). The EMBs without SPIO inclusion was prepared by the same way just without adding the SPIO Fe_3O_4 nanoparticles in the methylene chloride. Fig. 1 is the schematic diagram illustrating the designed SPIO-embedded EMB structure.

2.2. The characterization of EMBs

The mean diameter size and size distribution of EMBs were analyzed using a Laser Diffraction Particle Size Analyzer (Mastersizer 2000, England). The concentration of EMBs was measured by a hemocytometer. The concentration of Fe_3O_4 nanoparticles embedded in the shell of EMBs was determined by an Atomic Absorption Spectrometer (180-80 Hitachi, Japan) [30].

The morphology of nanoparticle-embedded EMBs was studied using a transmission electron microscope (H800-3 Hitachi, Japan) operating at 100–175 kV accelerating voltage. Samples were fixed in 1% OsO_4 for 1 h, dehydrated in acetone and then embedded in liquid epoxy resin. After hardening, the resin blocks were sectioned in 50 nm sections and stained in uranyl acetate and lead citrate before photographed.

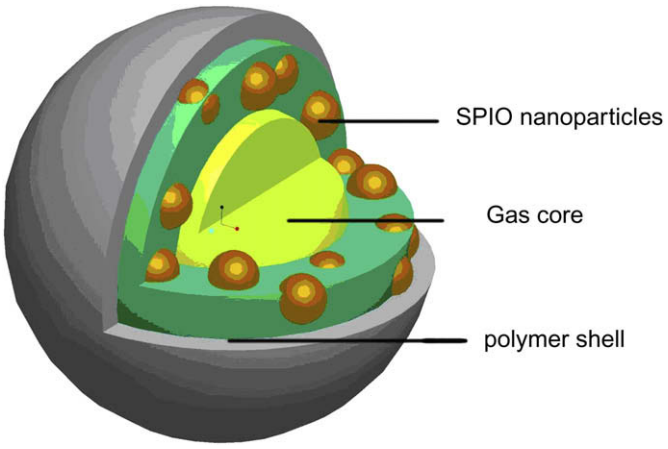

Fig. 1. The schematic diagram of the designed SPIO-inclusion EMB. The structure of an EMB can be briefly described as follows: the core gas of the first emulsion microbubble was N_2, encapsulated by a shell of poly-D,L-lactide (PLA). Hydrophobic SPIO nanoparticles were distributed in the PLA layer during the formation of the first bubble emulsion. The second shell was poly(vinyl alcohol), PVA, solution.