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A novel drug vehicle capable of ultrasound-triggered release with MRI functions

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ABSTRACT

A novel remotely triggered drug vehicle, magnetic hydroxyapatite (HA)-coated liposome (i.e. HA-coated liposome decorated with superparamagnetic iron oxide (SPIO) nanodots; HA/SPIO-coated liposome), was developed to exhibit ultrasound-triggered release behavior, magnetic resonance imaging (MRI) contrast and ultrasound-induced MRI contrast change. In this study, the effects of the HA/SPIO coating layer on the background leakage, response to ultrasound and MR signal were investigated. The background leakage of the liposome was significantly reduced due to HA/SPIO coating of the liposome. This coating layer also enhanced the sensitivity of the drug vehicle to ultrasound under sonication conditions at high frequencies (1 and 3 MHz) and low power densities (0.2–0.4 W cm^{-2}). Moreover, the ultrasound-triggered vehicle exhibited a concentration-dependent T_2 (spin–spin relaxation time) contrast in MR images due to their decoration with SPIO nanodots. In addition, r_2 and r_2^* (transverse relaxivity) values increased with increasing amounts of SPIO decoration, suggesting that the MR images of HA/SPIO-coated liposomes could be probed by the T_2 signal. Most importantly, the $r_2^* - r_2$ value of HA/SPIO-coated liposomes decreased after sonication, which was more prominent for the sample with lower SPIO amounts. This suggests that this novel drug vehicle can be used not only as an MR image-guided drug vehicle capable of ultrasound-triggered release, but also as an MR reporter to probe the status of vehicles after ultrasonic triggering.

1. Introduction

In recent years, ultrasound (US)-triggered drug vehicles have received considerable attention due to the effectiveness of ultrasound as a diagnostic tool. US is a technique with low electronic interference that allows for easy focus whilst penetrating into deep soft tissue in a non-invasive manner. It is commercially available, and has an established safety record compared to temperature, light and electric and magnetic stimuli [1–5]. Moreover, US can enhance the intercellular uptake of drugs not only by breaking the tight junction between cells, but also by acoustically enhancing the permeability of the cell membrane to induce endocytosis of drug-loaded micelles [6–9]. Therefore, an ultrasonically triggered vehicle can potentially be developed into a promising remotely triggered drug delivery system, which can be used in clinical applications.

An US image-guided drug delivery system is one in which ultrasonically triggered vehicles accumulated at a specific site can be simultaneously probed and triggered by US, and in recent years, there has been growing interest in it for the clinical treatment of many diseases [10–13]. However, whether sufficient drug vehicles travel to the triggering site (i.e. US focus area) must be determined by probing a series of US images, and one limitation of this technique is that the drug carriers might be triggered earlier and/or at the wrong sites during this serial probing with US. Therefore, magnetic resonance imaging (MRI) might be a better imaging tool than US imaging for probing US-triggered drug vehicles since MRI is a powerful non-invasive technique for probing real-time images of living bodies with high resolution at the cellular and molecular level [14–16]. On this basis, it is necessary to develop a drug vehicle with MRI contrast and ultrasonically triggered release functions, with the expectation that images of vehicles can be probed by MR, and the release of the drug can then be subsequently triggered via US.

In general, drug vehicles exhibiting MR image contrast could be achieved via encapsulating differing amounts of paramagnetic and/or superparamagnetic nanoparticles into polymeric vehicles. Jain et al. developed oleic acid-coated iron oxide nanoparticles to encapsulate doxorubicin and demonstrate MRI contrast [16]. In addition, a magnetic nanovehicle consisting of a magnetite core and a starch shell has been reported to monitor targeting delivery for brain tumors [15]. In these studies, drug released from the polymeric shell was not remotely triggered by US. Hence, in our previous study, a multi-functional drug vehicle exhibiting MRI contrast and ultrasonically triggered behaviors was prepared by encapsulating superparamagnetic nanoparticles inside hydroxyapatite (HA)-coated liposomes [17]. However, encapsulating nanoparticles

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in the vehicle might decrease the loading volume of the bioactive agent. This was overcome by the novel vehicle developed in the present study. The superparamagnetic iron oxide (SPIO) nanodots were semi-embedded onto the surface of the HA-coated liposome to form a core–shell structure decorated with SPIO nanodots (i.e. HA/SPIO-coated liposome) that will have the ability to exhibit MRI contrast and ultrasonically triggered behaviors. However, it remains unclear whether the SPIO embedded onto the HA coating layer alters the US-triggered release behavior, MRI T_2 contrast and US-induced contrast change of MR images. Few studies have been performed on this issue, which deserves systematic investigation.

The objective of this study was to prepare the HA/SPIO-coated liposome and investigate the influences of SPIO amount and US parameters (i.e. frequency and power density) on background leakage and ultrasonically triggered behaviors of HA/SPIO-coated liposomes. In addition, a preliminary investigation on the MRI contrast of the HA/SPIO-coated liposomes in relation to the SPIO amount and US triggering was also carried out. An understanding of the ultrasonically active behaviors of HA/SPIO-coated liposomes could provide fundamental and valuable information that can be useful in the design and fabrication of MRI-guided drug carriers capable of remotely triggered release.

2. Materials and methods

L-α-Lecithin (minimum 60% TLC), xylenol orange sodium salt, phosphoric acid (99%), calcium acetate hydrate, ferrous chloride, aqueous ammonia and methyl alcohol (anhydrous, 99%) were purchased from Sigma–Aldrich. Xylenol orange sodium salt was employed as the model drug for the present study because it demonstrated good stability after the long-term release test and US-induced release test.

2.1. Preparation of drug-loaded liposome, HA-coated liposome and HA/SPIO-coated liposome

Drug-loaded liposome (which will be referred to as "liposome" henceforth) was prepared using the following procedure. L-α-Lecithin was dissolved in 1 ml of methyl alcohol at 54 °C. The methyl alcohol was then evaporated using a vacuum evaporator, and a thin film of lipid was coated onto the wall of a 20 ml glass round flask. The obtained thin lipid film was loaded with a neutrally charged model drug (xylenol) after rehydrating the film with 10 ml of a xylenol/DI solution (1000 ppm). The resulting solution was vortexed and sonicated by probe sonication for 2 min to obtain drug-loaded liposomes. The excess unloaded drug in the liposome suspension was removed by gel-filtration chromatography.

The HA-coated liposome loaded with drug (called HA-liposome) was prepared by the co-precipitation method [17,18]. In brief, the above-mentioned drug-loaded liposomes were filtered using size-exclusion chromatography to remove unloaded drug. The drug-loaded liposome suspension collected from the filtration column was then immediately mixed with calcium acetate solution (2.5×10^{-2} M, 10 ml). Subsequently, a phosphoric acid solution (1.5×10^{-2} M, 10 ml) was added dropwise to the suspension, and the pH value was maintained at 11 by adding ammonia solution (25 vol.%). The thickness of HA was tuned using a reaction time of 2 h. The resulting suspension containing the drug-loaded vehicles was mildly centrifuged at 4000 rpm, and then filtered by a gel column to remove the free HA and leaked drug for further characterization and release tests. The names of the drug vehicle samples and their features are summarized in Table 1.

The HA/SPIO-coated liposomes loaded with drug were prepared as follows [19]: 3 ml of aqueous ammonia (25 vol.%) was slowly added to the above-mentioned drug-loaded liposome suspension; then 400 μl from each, 2.9×10^{-2} M calcium acetate aqueous solution, 2.3×10^{-2} M ferrous chloride aqueous solution, and 1.7×10^{-2} M phosphate solution, was added dropwise; the mixture was then stirred for 10 min for uniform distribution of the content; the above steps were repeated five times. The molar ratios XCa/P (1.67) were kept constant, so as to cover the surface of liposomes with a layer of SPIO-decorated hydroxyapatite shell. After centrifuging the resulting suspension, the precipitate was dispersed in water.

In addition, drug vehicles decorated with various amounts of SPIO nanodots were obtained by controlling the concentration of the iron precursor. Vehicles with a smaller number of SPIO nanodots (called HA-liposome-S1) were obtained under the above parameters. Vehicles with a larger number of SPIO nanodots (called HA-liposome-S10) were obtained by increasing the concentration of the above iron precursor by 10 times (i.e. 400 μl of 2.3×10^{-1} M ferrous chloride solution).

2.2. MRI characterization

MRI experiments were performed with a 3.0-T clinical scanner (Siemens). The effective transverse relaxation time (T_2^*) incorporates the natural transverse relaxation time (T_2) and the effect of magnetic inhomogeneities (T_2') produced by superparamagnetic particles (i.e. $1/T_2^* = 1/T_2 + 1/T_2'$). The transverse relaxation rate (R_2; $R_2 = 1/T_2$) was determined by probing transverse images using a two-dimensional (2-D) spin-echo MR sequence with 30 echoes and an echo time of 22.5 ms. The imaging parameters used were: a repetition time of 2000 ms, a field of view of 320 mm, a slice thickness of 3 mm, a 192 × 192 imaging matrix and a flip angle of 180°. The effective transverse relaxation rate (R_2^*; $R_2^* = 1/T_2^*$) was determined by probing transverse images using a 2-D gradient-echo MR sequence with 12 echoes and an echo time of 4 ms. The imaging parameters used were: a repetition time of 300 ms, a field of view of 320 mm, a slice thickness of 2 mm, a 192 × 192 imaging matrix and a flip angle of 30°. Finally, the slopes of the curves plotting the R_2 and R_2^* relaxation rates as a function of vehicle concentration were calculated and defined as relaxivity values r_2 and r_2^*, respectively [20].

2.3. Material characterization

The magnetic properties of HA/SPIO-coated liposome were characterized via superconduction quantum interference device (SQUID) at a temperature of 300 K. Microstructural observations were performed using a Philips Tecnai 20 transmission electron microscope (TEM) operated at 200 keV. The size distributions of drug vehicles were measured via a particle size analyzer (Malvern, ZS90) using the dynamic light scattering (DLS) method.

2.4. Determination of background leakage

The background leakage of drug-loaded vehicles was measured using a side-by-side diffusion cell. While unloaded drug was removed via gel chromatography, the suspension containing the drug-loaded vehicles was immediately added to the donor cell. A dialysis membrane was fixed between the donor and receptor cells. Phosphate buffered saline (PBS) solution was used as the release medium (pH 7.4 ± 0.02, 37 ± 0.2 °C). The drug released in the donor cell could permeate through the dialysis membrane to the receptor cell, where the concentration of the permeated drug was measured using UV–visible spectroscopy (Agilent 8453) at an absorption peak of 431 nm for each juncture. The vehicles were kept in the donor cell to avoid inaccuracies in determining concentration caused by optical interference from nanosized vehicles in the receptor cell. The drug release profile was recorded by plotting the (M_t/M)–t