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A novel ultrasound-triggered drug vehicle with multimodal imaging functionality

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ABSTRACT

A novel remotely triggered drug vehicle having multimodal imaging functionality was developed. It exhibits magnetic resonance (MR) imaging, ultrasound (US) imaging, encapsulation of a hydrophobic agent and US-triggered release behavior. Lipophilic superparamagnetic iron oxide (SPIO) nanoparticles were self-assembled with an amphiphilic chitosan derivative, carboxymethyl hexanoyl chitosan (CHC), to form superparamagnetic CHC/SPIO micelles and then loaded with camptothecin (a hydrophobic anti-cancer agent). The superparamagnetic micelles were then conjugated with albumin-based microbubbles (MBs) to form superparamagnetic micelle-decorated MBs (CHC/SPIO-decorated MBs). The albumin MBs and CHC/SPIO-decorated MBs both demonstrated in vitro concentration-dependent US imaging contrast. Interestingly, the in vitro US contrast was enhanced by decoration. In vivo US images showed that the B-mode contrast of the proposed vehicles could be clearly observed in the veins and arteries of Sprague–Dawley rats. Moreover, the proposed vehicle exhibited significant US-triggered release behavior under therapeutic US sonication at a frequency of 1 MHz and power density of 2.4 W cm^{-2} for 30 min. However, similar behavior was not observed under diagnostic US bombardment at a frequency of 12 MHz and mechanical index of 0.5. On the other hand, in vitro MR images of the CHC/SPIO-micelle-decorated MBs also revealed a significant concentration-dependent T_2 (spin–spin relaxation time) contrast due to their decoration with superparamagnetic micelles. Most importantly, the $r_2^* - r_2$ value of the CHC/SPIO-decorated MBs decreased after therapeutic US bombardment for 30 min. This might be considered as an index to probe destruction of the drug-loaded CHC/SPIO micelles.

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1. Introduction

In recent years the concept of the integration of diagnosis and therapy in a single box has been widely accepted and applied to the clinical treatment of many diseases [1–3]. To realize this concept many drug delivery systems (DDSs) associated with imaging and targeting functions, or image-guided DDSs, have been developed in order to detect diseases, deliver therapeutic agents, and track the distribution of drug vehicles [4–6]. Drug vehicles with imaging contrast could be probed using diagnostic imaging systems such as computed tomography (CT), magnetic resonance (MR), fluorescence, and ultrasound (US) [7–9]. Because each imaging technology has distinct advantages and limitations there has been growing interest in combining two or more complementary imaging techniques to probe an agent with multimodal imaging functionality [10,11]. A multimodal imaging contrast agent combined with drug encapsulation and triggered release functions is expected to be helpful for precise determination of the position and trigger timing of vehicles accumulated at a specific site.

Ultrasonically triggered drug vehicles have received extensive attention because US can not only trigger drug release from the vehicle but also enhance the intercellular uptake and transmembrane permeation of drugs by breaking the tight junctions between cells and perturbing the cell membrane, respectively [12–14]. In addition, US imaging is a non-invasive, ionization-free, and cost-effective diagnostic tool, so US image-guided DDSs, which simultaneously probe vehicles accumulated at a specific site by US imaging and trigger drug release by US energy, show promise for use in clinical applications. However, in some cases, when using clinical scanners to probe the objects far from the transducer or in a large organ, limited resolution restricts the application of US in molecular level imaging [15]. In contrast, MR imaging (MRI) might be an alternative tool for probing US-triggered drug vehicle because MRI can provide not only structural information at high resolution (i.e. at the cellular and molecular level) but also functional information regarding living bodies in a non-invasive manner [16]. Several newly developed drug vehicles have been proposed in order to demonstrate MR image contrast. Chertok et al. and Jain et al. proposed novel MR-guided drug vehicles to

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monitor targeted delivery [17,18]. Therefore, it would be advantageous to develop an ultrasonically triggered drug vehicle exhibiting MR/US image contrast function, with the expectation that vehicle images can be probed by MR and/or diagnostic US (frequency range 5–15 MHz, power density < 0.5 W cm^{-2}), and drug release can then be triggered by therapeutic US (frequency range 1–3 MHz, power density < 3 W cm^{-2}).

In general, nano-sized vehicles are suitable for extravascular targeted imaging and delivery due to the fact that the leaky vasculature in tumor tissue (with fenestration sizes of hundreds of nanometers, varying with the tumor type) allows nanoparticles to escape from blood capillaries and access the tumor cells surrounding them [19]. On the other hand, micron-sized vehicles are usually employed to enhance intravascular targeted imaging because the extravasation of micron-sized vehicles is restricted (i.e. the vehicles remain in the vessel lumen). Microbubbles (MBs) conjugated with vascular endothelium growth factor (VEGF) have been used to demonstrate US vascular imaging to monitor the progression of angiogenesis of metastatic cancers [15]. Therefore, multifunctional vehicles with a combination of nano-sized liposomes and micron-sized bubbles have been developed [20]. Albumin-based and lipid-based MBs have long been employed as commercial US contrast agents having good safety in both cardiac and abdominal ultrasound applications [21,22]. Liu et al. used US to trigger the destruction of albumin MBs, which can enhance gene transfection efficiency in cardiac myocytes [23]. Jiang et al. developed lipid-based MBs to encapsulate hydrophobic drugs and demonstrated US/optical imaging functions by embedding quantum dots in lipid layers [24]. However, very few studies have examined albumin-based MBs exhibiting dual modal (MR/US) imaging functionality and ultrasonically triggered behavior. These capabilities were realized by the novel vehicle proposed in the present study in which a hydrophobic antitumor agent and lipophilic superparamagnetic iron oxide (SPIO) nanoparticles were self-assembled with an amphiphilic chitosan derivative developed by our group, carboxymethyl hexanoyl chitosan (CHC), to prepare superparamagnetic micelles (CHC/SPIO micelles) [25]. These drug-loaded micelles were then conjugated with albumin-based MBs to form superparamagnetic micelle-decorated MBs (CHC/SPIO-decorated MBs). Studies of superparamagnetic micelle-decorated albumin MBs have not been reported, although they deserve systematic investigation.

The objective of this study was to prepare CHC/SPIO-decorated albumin MBs and investigate the effects of the superparamagnetic micelles on in vitro MR and US images. In addition, a preliminary investigation of in vivo MR and US images was also conducted to demonstrate the dual modal imaging capability of the CHC/SPIO-decorated MBs. Subsequently, the US-induced MR signal change in and US-induced drug release from the proposed vehicle were studied. An understanding of the ultrasonically activated behavior of the superparamagnetic micelle-decorated MBs could provide valuable fundamental information to support the design and fabrication of multimodal image-guided drug carriers capable of intravascular imaging, extravascular imaging, extravascular delivery and remotely triggered release.

2. Materials and methods

Bovine serum albumin (BSA), camptothecin (CPT), $FeCl_2$, glucose (99%), glycerol, glutaraldehyde (GA), and hexane were purchased from Sigma–Aldrich. CPT was employed as the model anticancer agent in this study because it has demonstrated good stability in a test of US-induced release. The animals used in the study were purchased from the Laboratory Animal Center of National Yang-Ming University. All the animals were treated and housed following a protocol approved by the Institutional Animal Use and Care Committee of National Yang-Ming University.

2.1. Preparation of BSA MBs

An aqueous solution containing BSA (5% w/v), glucose (5% w/v), and glycerol (6 vol.%) was placed in a glass vial. This mixture was purged with SF_6 gas and sonicated with a probe-type sonicator (XL2000, Misonix Inc.) for 3 min. After sonication the milky suspension obtained was placed in a refrigerator to allow the MBs to separate by flotation. The layer of solution containing the MBs was withdrawn and redispersed into a GA solution (0.0375% w/v) with stirring for 2 h. The resulting suspension containing cross-linked MBs was centrifuged at 1000 r.p.m. for 10 min. After the supernatant had been removed the pristine MBs (PMBs) obtained were redispersed in 5 ml of deionized water.

2.2. Preparation of CHC/SPIO micelles and CHC/SPIO-decorated MBs

Hydrophilic SPIO nanoparticles were prepared following a well-known method using $FeCl_2$ as the precursor [26]. Next, lipophilic SPIO nanoparticles were obtained by the phase inversion method. Our group has reported the synthesis of CHC using N,O-carboxymethyl chitosan (NOCC) as a precursor elsewhere [25]. The as-prepared lipophilic SPIO nanoparticles were mixed with hexane (1 ml) and CHC aqueous solution (0.125% w/v). The mixture was placed in an ice bath and sonicated by probe sonication for 2 min, producing CHC/SPIO micelles. CHC/SPIO micelles loaded with a hydrophobic model drug were prepared by replacing hexane with a CPT/hexane solution (0.34 mg ml^{-1}) during this assembly process. The resulting CHC/SPIO micelles without and with drug loading could be collected by magnetic separation. Drug carrying capacity was determined by an extraction method. The drug payload was 0.067 wt.% (CPT weight/micelle weight). Various concentrations of CHC/SPIO micelle suspension (0, 52.08, 104.16, and 208.32 μg ml^{-1}) were added to the suspension containing cross-linked MBs, followed by mild stirring for 1 h to obtain CHC/SPIO-decorated MBs. The features of the drug vehicle samples are summarized in Table 1. Samples were freeze-dried and kept in vessels filled with SF_6 gas.

2.3. Material characterization

Microstructural observations were performed by optical microscopy (OM) (Olympus) and transmission electron microscopy (TEM) (Philips Tecnai 20) at 200 keV. The crystallographic phase of SPIO was identified by X-ray diffraction (XRD) (M18XHF, Mac Science, Tokyo, Japan) at a scanning rate of 4° (in units of 2θ min^{-1}) over a 2θ range of 20–70°. The magnetic properties of the CHC/SPIO-decorated MBs were characterized using a superconducting quantum interference device (SQUID) at a temperature of 300 K. The size distributions of the drug vehicles were measured by a particle size analyzer (Malvern, ZS90) using a dynamic light scattering (DLS) method (measurement capability 3–5000 nm). The SPIO and iron contents were confirmed by thermogravimetric analysis (TGA) (TA Instruments Q500) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Kontron S-35), respectively.

2.4. MRI characterization

In vitro MRI experiments were performed with a 7T MRI instrument (Bruker S300 Biospec/Medspec). 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 ($R_2 = 1/T_2$) was determined by

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