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A magnetic droplet vaporization approach using perfluorohexane-encapsulated magnetic mesoporous particles for ultrasound imaging and tumor ablation

Zhaogang Teng [a, e, 1], Ronghui Wang [c, 1], Yang Zhou [c], Michael Kolios [d], Yanjie Wang [d], Nan Zhang [c], Zhigang Wang [c], Yuanyi Zheng [a, b, c, *], Guangming Lu [a, e, **]

[a] Department of Medical Imaging, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, 210002 Jiangsu, PR China
[b] Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai 200233, PR China
[c] Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, PR China
[d] Department of Physics, Ryerson University, Toronto, Ontario M5B 2K3, Canada
[e] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093 Jiangsu, PR China

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ABSTRACT

Phase change agents consisting of low boiling point perfluorocarbon (PFC) compounds have attracted increasing attention for ultrasound contrast-enhanced imaging and tumor therapy. However, the refraction, acoustic shadowing, reverberation, or limited penetration depth hamper their practical applications through previously reported acoustic droplet vaporization (ADV) or optical droplet vaporization (ODV) technique. Herein, we demonstrate a magnetic droplet vaporization (MDV) approach by loading perflurohexane (PFH) in magnetic mesoporous particles with a hollow space to carry out ultrasound imaging and tumor ablation. *In vitro* and *in vivo* magnetic thermal effects show that magnetic energy can be efficiently transformed into thermal energy by the PFH-encapsulated magnetic mesoporous particles, and then leading to vaporization of the loaded PFH. Owing to the generation of the PFH gas bubbles, the ultrasound signals are greatly improved in both harmonic mode and B mode. Simultaneously, anti-cancer experiments demonstrate that the tumor can be ablated after treating with the MDV method for 4 days, demonstrating highly efficient anti-cancer effects.

1. Introduction

Ultrasound imaging is a widely used and cost-effective clinical diagnostic modality. Ultrasound contrast agents consisting of gas microbubbles can provide a significant acoustic impedance mismatch with aqueous media and oscillate in response to ultrasound pulses to generate non-linear signals, and thus allow to get high quality image, early screen of severe pathologies, and detect the molecular markers on the vascular endothelium and other intravascular targets [1–3]. However, the micrometer sized gas bubbles cannot infiltrate the normal endothelial cells gap or the leaky tumor vasculature with pore size of less than 700 nm, which limits their applications in extravascular imaging [2,4–6]. Recently, phase change contrast agents consisting of low boiling point perfluorocarbon (PFC) compounds have attracted more and more attention because of their vessel penetrated nanometer size and the capability to generate gas bubbles and radiation force under external trigger [7–10]. Owing to the unique properties, phase change contrast agents are appealing for cancer imaging and therapy, such as extravascular ultrasound imaging [11], and enhancement of high intensity focused ultrasound (HIFU) treatment [12,13]. Previously, vaporization of the PFC liquid was realized by using a technique called acoustic droplet vaporization (ADV) [8,13–16], in which the cavitation effect of ultrasound pressure waves induces the liquid nanodroplets to form gas bubbles. However, refraction, acoustic shadowing, and reverberation exist while

* Corresponding author. Department of Medical Imaging, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, 210002 Jiangsu, PR China.
** Corresponding author. Department of Medical Imaging, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, 210002 Jiangsu, PR China.
E-mail addresses: zhengyuanyi@gmail.com (Y. Zheng), cjr.luguangming@vip.163.com (G. Lu).
[1] Z. Teng and R. Wang contributed equally to this work.

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ADV is carried out by using ultrasound. Hence, ADV meets great challenges when it is applied to lesions hidden behind gas-bearing bowel or bones, such as a liver lesion adjacent to a rib. Alternatively, a method termed optical droplet vaporization (ODV) was developed to trigger the vaporization of PFC by using laser irradiation [7,17,18]. However, the interferences of photons with tissues and surrounding media lead to limited penetration depth of only a few centimeters and hamper their effectiveness in theranostics [19–21]. Therefore, it is highly desirable to develop PFC vaporization method with excellent penetration for tumor imaging and therapy.

The tissue penetration of electromagnetic energy is almost not limited. Theoretically, at 500 kHz for example, 99% of energy can penetrate into 15 cm of tissue and be transferred to thermal energy by magnetic nanoparticles [22,23]. This thermal energy generation technique *via* magnetic particle mediators has been used to release drugs with controlled manner [24], remotely regulate protein production *in vivo* [25], and control temperature-sensitive ion channels [26]. In addition, the heat converted by magnetic materials also has been explored to kill cancer cells [27–29]. These unique properties and exciting applications of the magnetic nanoparticles inspire us to develop magnetic droplet vaporization (MDV) approach to vaporize phase change contrast agents for ultrasound imaging and thermal treatment of cancer.

In order to realize the idea of MDV, the compound PFC should be loaded into magnetic materials. However, previously reported phase change contrast agents are generally prepared by encapsulating PFC droplets within lipid, surfactant, or amphiphilic copolymer shells [13,30–32]. The relatively low strength, low heat-resistance, and broad size distribution of the organic molecule stabilized PFC droplets hamper their circulation and acoustic contrast properties. Moreover, they cannot provide magnetic thermal transfer capability for the proposed MDV approach. Recently, hollow mesoporous silica has been subjected to extensive research on nanooncology because of their unique characteristics such as high surface area, uniform pore size, large void space, and good biocompatibility [12,33–40]. Furthermore, their interior spaces can be further functionalized through encapsulating magnetic particles, *viz*, creating magnetic particles embedded hollow mesoporous silica [37,41–43]. The composite particles possess not only large void spaces and permeable outer mesoporous shells but also unique magnetic properties, thus making them ideal candidates to load PFC liquid and then vaporize it upon exposure to an external alternating current (a.c.) magnetic field.

Herein, we introduce the MDV approach by loading perflurohexane (PFH), a highly biocompatible PFC compound with an appropriate phase transition temperature of 56 °C, in magnetic mesoporous particles with a hollow space (denoted as MPs) for the first time to carry out ultrasound imaging and tumor ablation. The PFH-encapsulated MPs (denoted as MDs) successfully integrate the merits of structural stability, accessible mesochannels, excellent magnetic heating capability, and temperature-sensitive property of PFH in one. After exposing to an a.c. magnetic field, the electromagnetic energy can be transformed into thermal energy by the MDs, and PFH are vaporized to form bubbles for enhanced ultrasound imaging. Simultaneously, magnetic thermal produced by the MDs can be used for tumor ablation through the proposed MDV approach.

2. Materials and methods

2.1. Materials

TEOS, cetyltrimethylammonium bromide (CTAB), concentrated ammonia aqueous solution (25 wt%), and anhydrous ethanol were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Perfluorohexane (PFH) was purchased from Sigma-Aldrich Co. LLC (Germany). Millipore water with a resistivity of 18.2 MΩ cm was used in all experiments. RPMI 1640 medium, heat-inactivated fetal bovine serum (FBS), and penicillin-streptomycin solution were purchased from Gibco Laboratories (Invitrogen Co, Grand Island, NY, USA). Cell counting kit-8 (CCK-8) was purchased from Nanjing Keygen Biotech. Co., Ltd. (Nanjing, China). The human embryo kidney 293T cell line and MDA-MB-231 breast cancer cell line were obtained from American Type Culture Collection (ATCC).

2.2. Preparation of perfluorohexane-encapsulated magnetic droplets

The MPs were first prepared according to previously reported method [37]. First, superparamagnetic Fe_3O_4 particles composed of plentiful nanocrystals were prepared *via* a solvothermal approach by using citrate groups as stabilizer [44]. In brief, $FeCl_3$ (0.65 g), trisodium citrate dehydrate (0.20 g), and sodium acetate (1.20 g) were dissolved in ethylene glycol (20 mL) under magnetic stirring. The obtained homogeneous mixture was transferred to a Teflon-lined stainless-steel autoclave with a capacity of 50 mL. The autoclave was heated in an air flow electric oven at 200 °C for 10 h. After cooling down to room temperature, the obtained Fe_3O_4 particles were washed with water for ten times, and dried in vacuum at 60 °C for 12 h. The superparamagnetic Fe_3O_4 particles with citrate groups are highly re-dispersible and can be easily coated with silica through a sol-gel process [45]. Thus, 0.16 g of the Fe_3O_4 particles were dispersed in a mixture of ethanol (100 mL), deionized water (2.0 mL), and concentrated aqueous ammonia solution (3.4 mL, 25 wt%). After adding 280 μL of TEOS and stirring at 40 °C for 24 h, nonporous amorphous silica coated Fe_3O_4 nanoparticles (Fe_3O_4@nSiO_2) were obtained and washed five times with water. The prepared Fe_3O_4@nSiO_2 spheres were re-dispersed in a mixed solution containing CTAB (0.32 g), deionized water (110 mL), ethanol (60 mL), and concentrated aqueous ammonia solution (2.0 mL, 25 wt%). After adding 2.0 mL of TEOS and stirring at 35 °C for 24 h, the product was collected and washed five times with water. Then, the products were incubated in water (160 mL) at 70 °C for 12 h. To remove the CTAB templates, the as-synthesized materials were extracted three times in an ethanol solution (240 mL) containing concentrated HCl (480 μL, 37%) at 60 °C for 3 h. The template-removed MPs were washed with ethanol three times and dried under vacuum. The MPs were weighed (40 mg) and transferred into a 2 mL vials. Then, the vials were capped under vacuum and 1 mL of PFH was added into the vials and placed under 4 °C for another 24 h. Finally, these vials were sonicated in cleaning bath at 4 °C for 1 min after injection of 1 mL saline into the vials and MDs dispersed in 1 mL of saline were obtained.

2.3. Characterization

Transmission electron microscopy (TEM) images were taken using an HT7700 microscope (Hitachi, Tokyo, Japan) operated at an accelerating voltage of 100 kV. The samples were dispersed by ultrasonic in ethanol and dropped on a carbon-coated copper grid for TEM observation. Nitrogen sorption isotherms were measured using a Micromeritics Tristar 3000 analyzer at −196 °C. The samples were degassed at 180 °C for 6 h before the measurements. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface area (S_{BET}) using the adsorption data at p/p_0 = 0.05–0.15. Pore size analysis was performed by Barrett–Joyner–Halenda (BJH) method from the adsorption branch of isotherm. The total pore volume (V_{total}) was estimated from the adsorbed amount at p/p_0 = 0.995. X-ray diffraction (XRD) pattern