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# Superhydrophobic silica nanoparticles as ultrasound contrast agents

Qiaofeng Jin [a,1], Chih-Yu Lin [b,1], Shih-Tsung Kang [a], Yuan-Chih Chang [c], Hairong Zheng [d], Chia-Min Yang [b,*], Chih-Kuang Yeh [a,*]

[a] Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan
[b] Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan
[c] Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan
[d] Paul C. Lauterbur Research Center for Biomedical Imaging, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen China

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ABSTRACT

Microbubbles have been widely studied as ultrasound contrast agents for diagnosis and as drug/gene carriers for therapy. However, their size and stability (lifetime of 5–12 min) limited their applications. The development of stable nanoscale ultrasound contrast agents would therefore benefit both. Generating bubbles persistently in situ would be one of the promising solutions to the problem of short lifetime. We hypothesized that bubbles could be generated in situ by providing stable air nuclei since it has been found that the interfacial nanobubbles on a hydrophobic surface have a much longer lifetime (orders of days). Mesoporous silica nanoparticles (MSNs) with large surface areas and different levels of hydrophobicity were prepared to test our hypothesis. It is clear that the superhydrophobic and porous nanoparticles exhibited a significant and strong contrast intensity compared with other nanoparticles. The bubbles generated from superhydrophobic nanoparticles sustained for at least 30 min at a MI of 1.0, while lipid microbubble lasted for about 5 min at the same settings. In summary MSNs have been transformed into reliable bubble precursors by making simple superhydrophobic modification, and made into a promising contrast agent with the potentials to serve as theranostic agents that are sensitive to ultrasound stimulation.

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## 1. Introduction

Microbubbles (MBs) ranging in size from 1 to 10 μm have not only been explored extensively as contrast agents for use in ultrasound-based diagnosis but also shown to have substantial potentials in ultrasound molecular imaging [1–4], ultrasound targeted drug/gene delivery [5,6], thermal tissue ablation [7,8], and sonothrombolysis [9–11] for therapeutic purposes. However, their micron size and poor stability have greatly hindered their application as theranostic agents. The development of stable nanoscale ultrasound contrast agents would therefore be hugely beneficial.

One possible strategy for addressing the issues mentioned above is to make smaller nanobubbles [12–14] or gas liposomes [15–17]. However, nanobubbles are not ideal ultrasound contrast agents because their contrast efficiency is lower and lifetime is shorter than those of MBs [18]. A second strategy is to design nanoscale bubble-precursors, which are usually metastable agents capable of being converted into MBs when exposed to a physical or chemical stimulus [19,20]. Among the most promising candidates are phase-shift nanodroplets constructed from liquid perfluorocarbons, which could potentially overcome the size and stability issues simultaneously [21–24]. Unfortunately, the Laplace pressure associated with their nanoscale size results in an extremely high vaporization threshold [25]. Although Sheeran et al. developed an ingenious condensation technique using low-boiling-point perfluoropropane and perfluorobutane and lowered the threshold to about 4 MPa [26], there is a trade-off between the acoustic vaporization threshold and stability. Another solution is to generate MBs in situ based on chemical reactions of solid nanoparticles (NPs) with a surrounding medium (e.g. tumor interstitial fluid) [27,28]. However, it is very difficult to control the chemical reactions involved in the process of bubble production.

Recently, it has been found that the interfacial nanobubbles (INBs) on a hydrophobic surface have a much longer lifetime (orders

* Corresponding authors at: Department of Chemistry, National Tsing Hua University, No. 101, Section 2, Kuang-Fu Road, Hsinchu 30013, Taiwan (C.-M. Yang). Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, No. 101, Section 2, Kuang-Fu Road, Hsinchu 30013, Taiwan (C.-K. Yeh).
*E-mail addresses:* cmyang@mx.nthu.edu.tw (C.-M. Yang), ckyeh@mx.nthu.edu.tw (C.-K. Yeh).
[1] Qiaofeng Jin and Chih-Yu Lin contributed equally to this work.

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of days) than the bulk nanobubbles (orders of microseconds) [29–33]. In addition, many studies found that these INBs can nucleate the formation of bubbles during ultrasound exposure [34–36]. Furthermore, the INBs trapped by superhydrophobic pits can nucleate cavitation hundreds of times and thereby greatly improve sonochemical productivity [37,38]. However, there has been little research taking full advantage of the stability of INBs as gaseous bubble-precursors to develop a stable nanoscale ultrasound contrast agent. Yildirim et al. very recently applied mesoporous silica nanoparticles (MSNs) with a hydrophobic interior to produce bubbles for use as ultrasound contrast agents under an extremely high acoustic pressure (9.87 MPa at a frequency of 1.1 MHz) [39], corresponding to a mechanical index (MI) of 9.4, which far exceeds the FDA safety guidance ($M \leqslant 1.9$) for diagnosis purpose. Noted that MI can be used as an estimate for the degree of bio-effects.

Herein we propose producing MBs from INBs using a clinically available transducer to generate MBs in situ in a switchable manner, which is considered accessible when combining the characteristics of high surface area and superhydrophobicity. Therefore, MSNs were selected in this work due to their large surface areas, biocompatibility, and adjustable surface properties, which render various biomedical applications such as drug delivery [40–43], cancer-targeting [44–46], and multimodality imaging [47,48]. As schemed in Fig. 1, superhydrophobic MSNs are designed to adsorb INBs on their surfaces and in their mesopores, since such bubble-precursors could remain stable until being converted into MBs under exposure to acoustic pressure above a certain MI. MSNs (MCM-48 type) with different levels of hydrophobicity were prepared to demonstrate our idea.

## 2. Experimental section

### 2.1. Materials

Benzylcetyldimethylammonium chloride (BCDAC) and diethylene glycol hexadecyl ether ($C_{16}E_2$) were purchased from Sigma Aldrich, and tetraethoxysilane (TEOS) was obtained from Acros. Hydrogen chloride (HCl) and ammonia (28%) were purchased from Showa. Ethanol and toluene were purchased from Echo Chemical. Trimethylchlorosilane (TMCS) was provided by Lancaster, and perfluorodecyltriethoxysilane (PFDTS) was purchased from Alfa Aesar. Sonovue MBs were purchased from Bracco. All of the buffers and suspensions were prepared with MilliQ water (18.2 MΩ).

### 2.2. Preparation of MSN, M-MSN and F-MSN

The parent MSN (MCM-48 type) was synthesized using the method in our previous publication [49]. Briefly, 0.74 g of BCDAC, 0.26 g of $C_{16}E_2$, 21.4 mL of 0.4 M NaOH, and 575 mL of water were added to a polyethylene bottle and stirred at 35 °C overnight. TEOS (5.98 mL) was then injected with a rate of 7.5 mL per hour, followed by aging at 90 °C for 24 h. The MSN was filtrated, washed with water and acetone, and dried under ambient conditions. The surfactants were removed by repeated ion exchange in a dilute HCl-ethanol solution at 35 °C. The MSN (0.1 g) was heated at 150 °C in vacuum for 12 h to remove the adsorbed water and then dispersed in a solution containing 1 mL of silane (using TMCS to produce M-MSN and PFDTS to produce F-MSN) and 10 mL of toluene. The mixture was stirred at 25 °C for 1 h (M-MSN) or 100 °C for 48 h (F-MSN), and the solid was collected by filtration, washed repeatedly with ethanol, and finally dried at 60 °C for 12 h.

#### 2.2.1. Preparation of SS and F-SS

Nonporous Stöber silica nanoparticles (SS) were prepared by Stöber process [50], Ethanol (65.5 mL), 6.7 mL of ammonia, and 2.9 mL of water were mixed and then stirred at 35 °C for 30 min, followed by the direct addition of 5 mL of TEOS and then stirring for another 2 h. Sample (SS) was then collected by filtration, washed with water and acetone, and dried at 60 °C. The SS was heated at 150 °C in vacuum for 12 h to remove the adsorbed water, and then dispersed in a solution containing 0.1 mL of PFDTS and 10 mL of toluene. The mixture was stirred at 100 °C for 48 h, and the resultant F-SS was collected by filtration, washed repeatedly with toluene and ethanol, and then dried at 60 °C for 12 h.

#### 2.2.2. Characterization of nanoparticles

X-ray diffraction patterns were recorded on a Mac Science 18MPX diffractometer using Cu Kα radiation. SEM images were obtained using a field-emission JEOL JSM-7000F microscope operating at 10 kV, with the samples coated with 5 nm of platinum before measurements. Nitrogen physisorption isotherms were measured at 77 K using a Quantachrome Autosorb-1MP instrument. The pore volumes were evaluated at a relative pressure of 0.95, and the adsorption branches in the relative pressure range of 0.05–0.30 were used to calculate surface areas by applying the Brunauer-Emmett-Teller method. TEM images were obtained using a JEOL JEM-2010 microscope operating at 200 kV. Solid-state $^{29}Si$ MAS NMR spectra were measured on a Bruker DSX400WB spectrometer using a 7-mm probe. FTIR spectra were analyzed using the Bruker Tensor 27 device. The size distributions and ζ-potentials of the nanoparticles (NPs) were measured using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). Their contact angles of as-prepared surfaces using various NPs were measured by using a contact angle analyzer (FTA-1000B, First Ten Angstroms, VA, USA). All of the samples were diluted with 18.2-MΩ water filtered through a 0.22-μm syringe filter.

#### 2.2.3. The preparation of INBs and in vitro ultrasound imaging under various conditions

The INBs are supposed to automatically appear on the surface of mesoporous NPs after suspending the NPs in water and do not require additional preparing process if the NPs were superhydrophobic. In vitro ultrasound imaging of all kinds of silica NPs was performed under various buffer conditions. An acoustically transparent phantom made of agarose gel (1.5%, w/v) with a 6-mm diameter hollow cylinder chamber was used to hold the NP suspension for the in vitro experiments. Various aqueous NP suspensions (100 μg $mL^{-1}$) were prepared, and their contrast intensities were obtained using a Philips CX50 ultrasound system with a linear-array transducer (model L3-12) having a center frequency of 7.5 MHz (dynamic range = 50 dB, gain = 50). The time–intensity curves and NP concentration responses were measured up to 30 min, and the ultrasound signal intensities of various samples were measured by calculating the relative average intensity in the region of interest (ROI) in the phantom chamber. Three 2-s videos (Video s1 is representative of a 100 μg $mL^{-1}$ F-MSN sample in DI water) were recorded at 60 frames per second for each sample, and the signal intensity in the ROI was analyzed using MATLAB (Mathworks).

## 3. Results

### 3.1. Characterization of NPs

The MSN displayed a sharp powder X-ray diffraction (PXRD) pattern corresponding to high-ordered cubic Ia3d symmetry, while no peaks were observed in the XRD diffraction patterns of the nonporous SS (Fig. 2a). The mesostructure remained after functionalization with trimethylsilyl (TMS) or perfluorodecyl (PFD) groups. The nitrogen physisorption isotherm (Fig. 2b) of MSN features H4-type hysteresis loop with a sharp step at relative pressure ($P/P_0$) of

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