Nanoparticle-Shelled Microbubbles Used for Medical Ultrasound Nonlinear Imaging

Fang Yang *, Huating Cui, Yang Liu, Li Yang, Yixin Li, Ping Chen, Ning Gu*

Jiangsu Laboratory for Biomaterials and Devices, State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, 2 Sipailou, Nanjing, 210096, China

Abstract

The nonlinear property of microbubbles is important for ultrasound contrast imaging. Many technologies have been proposed to enhance the harmonic and subharmonic ability of the microbubbles. In this study, microbubbles with inclusion of different concentration of Fe3O4 nanoparticles in the shell were prepared and their acoustic signals were investigated. Results have shown that the acoustic scattering cross-section of microbubbles would change with the different concentration of Fe3O4 nanoparticles inclusion. When the Fe3O4 concentration is 86.47 \( \mu \text{g/ml} \), the microbubbles possess best harmonic acoustic response and maintain better in vitro nonlinear contrast imaging effect and longer life time. Therefore, by optimizing the nanoparticle concentration in the shell of microbubbles can make the microbubbles multifunctioned and at the same time retaining the excellent nonlinear acoustic response property.

1. Introduction

As a non-invasive, low cost and real time imaging technology, ultrasound (US) is the most frequently used imaging modality worldwide. However, the conventional ultrasound is limited in clinical application because of its low sensitivity and resolution. The introduction of ultrasound contrast agents (UCAs) and relative imaging technologies makes the ultrasound images with higher contrast-tissue ratio (CTR) and opens a new era for...
ultrasound diagnostic imaging [1]. In recent decades, gas-filled, encapsulated microbubbles (MBs) have been developed as the most commonly used UCAs. Besides enhancing the ultrasound imaging, the shell provides the platform for further functionalizations of MBs to hybrid imaging contrast agents [2]. Especially nanoparticle in the MBs has been shown to provide flexible and useful potential applications in multi-modal medical imaging. For example, it has been shown that by adding Fe$_3$O$_4$ superparamagnetic iron oxide nanoparticles (SPIO) on the MBs could develop US/ magnetic resonance double-modality contrast agents [3-5]. Deposition of CdTe quantum dots (QDs) onto microbubble shells can be developed as novel US/ fluorescent imaging bi-mode contrast agents [6]. Gold nanoparticles in the MBs can be applied to photoacoustic imaging [7]. These novel emerging multimodal hybrid MBs give good promising for obtaining complementary diagnostic information based on one microbubble carrier platform. However, the major challenges in developing these nanoparticle-shelled MBs are the different functional requirements needed to match. Especially, whether or not when the nanoparticle are assembled on the shell of MBs, MBs could still maintain the adequate ultrasonic echo signal.

The objective of this work is to investigate the nonlinear properties of MBs with and without inclusion of SPIO. First, based on the dynamics scattering theory of MBs under the exposure of ultrasound, the scattering intensity of MBs increases with the increase of SPIO concentration from 0 to 86.47 $\mu$g/ml and then decreases when SPIO concentration increased from 86.47 to 191.46 $\mu$g/ml. Thus the SPIO concentration inclusion in MBs has been optimized to have good acoustic response. Second, the power spectra results indicate that when SPIO concentration is 86.47 $\mu$g/ml, SPIO-inclusion MBs possess better fundamental and harmonic signal. Finally, the in vitro nonlinear contrast imaging demonstrates better imaging enhancement effect when the SPIO concentration is 86.47 $\mu$g/ml.

2. Methods

2.1. Preparation and characterization of nanoparticle-shelled microbubbles

MBs, SPIO-inclusion MBs were prepared according to the previous methods [8, 9]. By adding different amount of Fe$_3$O$_4$ nanoparticles (0, 24.16, 28.77, 45.65, 50.42, 52.87, 64.95, 70.23, 86.47, 116.41, 121.16, 124.56, 126.13, 145.24, 161.87, 182.22, 191.46 $\mu$g/ml), 17 samples were obtained for the experiments. The morphology and structure of the MBs were studied by scanning electron microscope (SEM). The presence of SPIO in the MB shell was confirmed by means of an energy dispersive X-ray analysis (EDXA, FEI XL30, USA).

2.2. Simulation calculations and acoustic reflection and scattering measurement

Microbubble scattering can be quantified by scattering cross-section $\sigma_s$, which can be determined by the shell viscoelastic properties $\chi$ and $\kappa$, when the bubble size $R_0$ and excitation frequency $\omega$ are set. In the experiment, the initial values of $\chi$ and $\kappa$ were set at first and then the attenuation spectrum was calculated theoretically. The values of $\chi$ and $\kappa$ were estimated by adjusting their values to minimize summed square difference between the measured and calculated spectrum.

The acoustic attenuation spectrum of MBs was measured using an ultrasound spectroscopy method shown in Fig. 2a. A broadband planar transducer (diameter 15mm, 3 MHz, Panametrics, USA) was used as both the transmitter and receiver. The attenuation was measured in the frequency range 1.5–27.5 MHz in 0.5 MHz increments. Ultrasound pulses were generated by an arbitrary waveform generator (WW2572A, Tabor Electronics) and amplified (150A, ENI) before reaching transducer. An aluminium plate was placed at the focus of each transducer to serve as a reflector to generate echoes that were detected by the same transducer. Echoes were then amplified and bandpass-filtered before being digitized (400 MHz sampling frequency, Agilent Technologies) for post-process analysis. For the statistical analysis, the sound exposures are repeated three times for one sample, and 10 echoes are collected for each exposure.

Power spectra were calculated from fast Fourier transform (FFT). To reduce the measurement error, this procedure was repeated three times, and then the three spectra were averaged to obtain the acoustic attenuation spectrum of the sample.
2.3. *In vitro* nonlinear ultrasound imaging

A tissue-mimicking phantom with a 1 cm diameter channel within the phantom was used for sample loading, which is shown in Fig. 3f. The nonlinear ultrasound imaging was acquired and analyzed by using Vevo 2100 imaging system with an 18 MHz transducer (Visual Sonics, Canada) in nonlinear contrast mode.

3. Results and Discussion

3.1. Morphology characterization of nanoparticle-shelled microbubbles

The mean diameter of both MBs and SPIO-inclusion MBs (161.87 μg/ml Fe$_3$O$_4$) is 1.91± 0.11μm (Fig. 1a-1, 1b-1), which indicates that the inclusion of SPIO in the shell of MBs does not influence the diameter. But the roughness of the shell surface is different. Fig. 1b-2 is SEM image of the MBs with the SPIO, which demonstrates coarser surfaces than MBs without inclusion of SPIO (Fig. 1a-2). The EDXA spectrum also clearly reveals that SPIO-inclusion MBs consist of Fe element in the microbubble samples (Fig. 1b-3).

![Fig. 1. Schematic diagram, size distribution, SEM images and EDXA spectrum of MBs without SPIO (a, a-1, a-2, a-3) and with SPIO (b, b-1, b-2, b-3) respectively.](image)

3.2. Shell properties and scattering cross-section

Table 1 shows the values of the estimated shell viscoelastic parameters for three typical microbubble samples. With the increase of the SPIO inclusion concentration, the viscoelastic parameters decrease at first and then increases. The decrease of the stiffness as the inclusion amount increases at the beginning (0- 86.47 μg/ml) appears to be counter-intuitive. However, some theoretical study predicted that the rigid inclusion may either stiffen or soften the membrane, which depends on the concentration of the inclusion in the membrane [10]. Thus, the mechanism of the inclusion-induced membrane softening may be that, on insertion of inclusions, a lateral flow of polymer molecules must take place which lead to the reduce of the molecules participating in the bending deformation.

| Sample                | Inclusion concentration (μg/ml) | Elasticity parameter $\chi$ (N/m) | Viscosity parameter $\kappa_s (\times 10^9 N)$ |
|-----------------------|---------------------------------|-----------------------------------|-----------------------------------------------|
| MBs                   | 0                               | 1.02±0.02                         | 11.21±0.50                                    |
| SPIO-inclusion MBs1   | 86.47                           | 0.86±0.01                         | 8.90±0.31                                     |
| SPIO-inclusion MBs2   | 161.87                          | 0.98±0.02                         | 10.44±0.62                                    |
Fig. 2c shows the changing trend of the cross-section of the 17 samples. Several parameters such as microbubble concentration, microbubble size, excitation frequency and shell viscoelastic properties together determine the scattering response. In this experiment, the scattering cross-section is calculated based on the suggestion that all the conditions of the sample are the same except for the shell properties. Therefore, the contribute factors to the changing scattering cross-section can only be the shell properties. When the viscosity decreases and the resonance frequency gets close to the excitation frequency, the scattering increases. As the inclusion concentration increases, there are two contributors to the changing trend of the sample’s scattering cross-section: (1) the viscosity coefficient decreases and then increase (Fig. 2b); (2) the resonance frequency of most microbubbles in the sample gets close to the excitation frequency at first and then becomes to deviates the excitation frequency (Fig. 2d).

3.3. Acoustic scattering signals and in vitro nonlinear ultrasound imaging

The backscattered RF signals from MBs and the power spectra are obtained and displayed in Fig. 3a-e. Results show that the fundament, second and third harmonic components are evident for the three typical microbubble samples. The amplitudes of the second and third harmonic for SPIO-inclusion MBs are higher (about 5 or 10 dB) than that of MBs without SPIO, especially when SPIO concentration is 86.47 μg/ml (SPIO-inclusion MBs1). The amplitudes of the fundamentals, second and third harmonics are statistically analyzed and compared, the histogram of the statistical results is shown in Fig. 3e. The harmonic backscatter signal is higher for SPIO-inclusion MBs than MBs without SPIO. And the amplitudes of second and third harmonic for the SPIO-inclusion MBs1 (86.47 μg/ml) are higher than SPIO-inclusion MBs2 (161.87 μg/ml). The reason may be that the deposition of nanoparticles on the surface of the microbubble alters the shell’s viscoelastic property and thus to effect the microbubble’s acoustic response behavior (Fig. 2). Further, nanoparticles embedded in the shell of microbubble restrict the microbubble compression and make the oscillation asymmetrically with compression-obstructed.

The plots of average pixel intensity over ROI versus time for the MBs, SPIO-inclusion MBs1 and SPIO-inclusion MBs2 are shown in Fig. 3g, which further confirms that when SPIO concentration is 86.47 μg/ml, the nonlinear ultrasound imaging shows the enhanced contrast imaging and longer life time.
Fig. 3. The backscattered RF signals from MBs (a), SPIO-inclusion MBs1 (b), and SPIO-inclusion MBs2 (c). Power spectrum of these three types of MBs (d). Amplitudes for the fundamental, second and third harmonic from these three types of MBs (e). (f) Schematic diagram of *in vitro* imaging. (g) Changes of average pixel intensity for three types of microbubbles vs. time. (h-1, h-2, h-3) are the *in vitro* nonlinear images.

**Conclusion**

In summary, the nonlinear acoustic signal response and contrast imaging ability of SPIO inclusion MBs are explored. By studying the viscoelastic properties of the SPIO-inclusion MBs, results show that when the optimized Fe$_3$O$_4$ concentration is 86.47 µg/ml, the MBs have the good acoustic scattering cross-sections. Both the power spectra and *in vitro* nonlinear ultrasound imaging results demonstrate that the MBs with SPIO possess excellent properties to reflect sound wave at the frequency of fundamental and harmonic, especially, when MBs inclusion with SPIO concentration is 86.47 µg/ml.

**Acknowledgements**

The authors acknowledge the support from National Key Basic Research Program of China (2013CB733804), National Natural Science Foundation of China (31370019), Foundation for the Author of National Excellent Doctoral Dissertation of PR China (201259), Fundamental Research Funds for the Central Universities.

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