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Microfluidics-based microbubbles in methylene blue solution for photoacoustic and ultrasound imaging

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ABSTRACT

Contrast agents which can be used for more than one bio-imaging technique has gained a lot of attention from researchers in recent years. In this work, a microfluidic device employing a flow-focusing junction, is used for the continuous generation of monodisperse nitrogen microbubbles in methylene blue, an optically absorbing organic dye, for dual-modal photoacoustic and ultrasound imaging. Using an external phase of polyoxyethylene glycol 40 stearate (PEG 40), a non-ionic surfactant, and 50\% glycerol solution at a flow rate of 1 ml/hr and gas pressure at 1.75 bar, monodisperse nitrogen microbubbles of diameter 7 microns were obtained. The external phase also contained methylene blue hydrate at a concentration of 1 gm/litre. The monodisperse microbubbles produced a strong ultrasound signal as expected. It was observed that the signal-to-noise (SNR) ratio of the photoacoustic signal for the methylene blue solution in the presence of the monodisperse microbubbles was 68.6\% lower than that of methylene blue solution in the absence of microbubbles. This work is of significance because using microfluidics, we can precisely control the bubbles’ production rate and bubble size which increases ultrasound imaging efficiency. A uniform size distribution of the bubbles will have narrower resonance frequency bandwidth which will respond well to specific ultrasound frequencies.

Keywords: multi-modal, contrast agents, microfluidics, flow-focusing junction, monodisperse microbubbles, photoacoustic, ultrasound.

1. INTRODUCTION

Multimodal imaging techniques integrated with photoacoustic imaging \cite{1-7} has emerged as the next big tool for clinical diagnostics. Photoacoustic imaging is one of the fastest growing biomedical imaging modalities and is also safe as it uses non-ionizing ultrasonic waves \cite{7}. Photoacoustic imaging overcomes high degree of scattering of optical photons which makes deeper imaging in the biological tissues possible. As long as the photons are converted into heat, both scattered and unscattered photons can be used to obtain photoacoustic signals which are then received by ultrasonic transducers. As a result, it is able to offer rich optical contrast and ultrasonic resolution for functional \cite{7, 8} and molecular \cite{9-11} imaging. Furthermore, as both photoacoustic (PA) and ultrasound (US) imaging can be done by the same ultrasound transducer (UST), the instrumentation required for both imaging modalities are highly complementary.

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Dual-modal US/PA agents have found applications in identifying tumours in lungs [12], sentinel lymphs nodes [13-15], thyroid glands [16] etc., for imaging joints [17] and angiogenesis [18], among others. Recently, Jeon et.al [19] reported the generation of octafluoropropane microbubbles stabilized by phospholipids in a methylene blue solution as a dual modality contrast agent for ultrasound and photoacoustic imaging. In this work, we present a microfluidics-based flow-focusing device for the generation of monodisperse nitrogen microbubbles in methylene blue solution. Microfluidics-based devices have been used for the generation of monodisperse microbubbles[20] which show great promise as ultrasound contrast agents [21]. Monodisperse [22] microbubbles may significantly increase the capability of ultrasound imaging modalities. A monodisperse size distribution is of significance because the strength of the backscatter [23] depends on the resonant frequency response which in turn depends on the size of the microbubbles. A wide size distribution will attenuate the response of ultrasound transducers as these transducers operate with a narrow frequency bandwidth. Hence, a monodisperse size distribution is desirable. These microfluidic devices offer several advantages over bulk mixing methods (sonication, agitation, etc.) such as precise control over microbubbles production rate and diameter. Typically, phospholipid-coated [24] or albumin-coated [25] microbubbles are used as ultrasound contrast agents. However, Parhizkar et.al [26] reported that microbubbles coated with polyoxyethylene glycol 40 stearate (PEG 40), a non-ionic surfactant, in a glycerol solution, were stable for 150 days. In this work, we used the same surfactant for stabilizing the microbubbles.

2. METHODS

2.1 FABRICATION OF THE MICROFLUIDIC DEVICE AND SYNTHESIS OF MONODISPERSE MICROBUBBLES

A polydimethylsiloxane (PDMS)-based block containing the flow-focusing junction was fabricated using soft photolithography. The height of the microchannels in the PDMS block was 10 microns. SU8-2010 photoresist was used for fabricating the mould on which PDMS was poured for fabricating the block. Following this, holes were drilled for the inlets and outlets in the PDMS block using a puncher. Plasma oxidation was then used for bonding the PDMS blocks with a PDMS coated glass slide. Once the tubes were inserted at the inlets and outlets, additions PDMS was poured over the entire device to tightly seal the gaps and prevent leakages [27]. Addition PDMS was poured as the external phase contains 50% glycerol in water which is a viscous fluid and leakages were observed when the tubings were fixed on the PDMS blocks using epoxy.

![Figure 1. (a) Monodisperse nitrogen microbubbles generation from a 10 microns nozzle at a flow-focusing junction in a](https://www.spiedigitallibrary.org/conference-proceedings-of-spie)
PDMS-based microfluidic device (b) Produced bubble suspension collected from the outlet of the microfluidic device in the LDPE tube 1 hour after formation. The lighter coloured stabilized nitrogen microbubbles are seen floating over the methylene blue solution when the tube is held vertically (image on the left). When the tube is held horizontally (image on the right), the microbubbles float across the entire solution.

A nitrogen gas cylinder (Leeden NOX, Singapore) fitted with a gas pressure regulator (Spectron, Singapore) was used for generating the microbubbles. The external phase consisting of methylene blue (Sigma, Singapore), PEG-40 (Sigma, Singapore) and glycerol (Sigma, Singapore) were delivered by a dual-syringe Longer pump. The generation of the monodisperse microbubbles with 7 microns diameter at the flow-focusing junction was observed using Nikon Eclipse TE2000-S microscope fitted with a high-speed CMOS camera (Phantom Miro ex4).

2.2 PHOTOACOUSTIC AND ULTRASOUND IMAGING OF THE MICROBUBBLES

Photoacoustic imaging of the microbubble samples was done with a clinical ultrasound-photoacoustic imaging system. A clinical research ultrasound system (ECUBE 12R, Alpinion, South Korea) was used. In order to use the system in the PA mode, laser excitation needs to be provided with a trigger [28-30], which synchronizes the excitation of the laser and the signal acquisition by the ultrasound transducer. The sample containing tube was placed under water and a linear array ultrasound transducer L3-12 (128 element linear array with center frequency of 8.5 MHz, 95% fractional bandwidth, array element pitch 0.3 mm, and elevation height 4.5 mm) was placed over the tube for sending and receiving the signals. An optical parametric oscillator, OPO (Continuum, Surelite), pumped by a Q-switched 532-nm Nd:YAG laser, was used as an excitation source for PA spectrum measurements. The laser generates 5-ns duration pulses at 10-Hz repetition rate with wavelengths tunable from 670 to 2500 nm. Since methylene blue has maximum optical absorbance at 677 nm, the wavelength is fixed at 670 nm.

3. RESULTS AND DISCUSSIONS

To investigate the dual-modal imaging capability of the monodisperse microbubbles, we placed the sample in a low density polyethylene (LDPE) tube with an inner diameter of 1.68 mm and outer diameter of 2.42 mm. The microbubbles collected from the outlet of the microfluidic device [Fig. 1(a)] is shown in Figs. 1 (b),(c) while the experimental imaging results are shown in Figs. 2 and 3. From our observation, the microbubbles retained their stability for atleast 2 hours when the tube was held horizontally. This is because holding the tube horizontally increase the inter-bubble spacing [24] as a result of which the nitrogen microbubbles cannot come in contact with each other for Ostwald ripening [31] to take place. Thereafter, bubbles do not burst or dissolve in the methylene blue solution. However, distinct coalescence of the bubbles was observed very easily with the naked eye in the LDPE tubes after 12 hours which finally will lead to the bursting of the bubbles.

The stability of the microbubbles as an ultrasound contrast agent is an important area of concern because losses of microbubbles occur during storage, administration and circulation. Talu et.al [31] reported that monodisperse nitrogen microbubbles retained their stability for atleast 9 hours. Moreover, Parhizkar et.al [26] reported that 50% glycerol solution with PEG-40, a non-ionic surfactant, enabled bubbles to be stable for 150 days. Hence, in our experiments as well, we synthesized nitrogen microbubbles in 50% glycerol and PEG-40 to enhance the stability of microbubbles in methylene blue solution. We compared the US and PA signals for microbubbles in methylene blue solution, DI water and just methylene blue solution in the tubes. Presence of microbubbles reduced the photoacoustic signal which correlates well with that reported in literature [19]. As shown in Fig. 2, the PA signal in (b) is lower than that of (f). The presence of the microbubbles increases the ultrasound intensity in Fig. 2(a) compared to (c) and (e). In Figs. 2(c) and (e), only the edges of the tube are visible as DI water and methylene blue cannot reflect
ultrasound waves. Also, no PA signal was observed in Fig. 2(d) as DI water has poor absorbance at 670 nm.

Fig. 2 Raw experimental images on the ECUBE system obtained for ultrasound (US) and photoacoustic (PA) imaging where (a), (b) shows the microbubbles in methylene blue solution at 1gm/L; (c), (d) shows DI water; (e), (f) shows methylene blue solution at a concentration of 1 gm/L.

Fig. 3 Beam formed images obtained for ultrasound (US) and photoacoustic (PA) imaging where (a), (b) shows the microbubbles in methylene blue solution at 1gm/L; (c), (d) shows DI water; (e), (f) shows methylene blue solution at concentration of 1 gm/L.
Beamformed images are shown in Fig. 3. The SNR values shown here were defined as the amplitude of the PA signal from agents within the tube divided by the standard deviation of the background noise, \( \text{SNR} = \frac{V}{n} \), where \( V \) is the PA signal amplitude and \( n \) is the standard deviation of the background noise. The SNR values of the PA signal of the methylene blue solution is 1197 and 2018 in the presence and absence of the microbubbles, respectively. Similarly, a 10-fold increase in the US signal is observed in the presence of the microbubbles.

4. CONCLUSION

Monodisperse nitrogen microbubbles of diameter 7 microns were generated in a microfluidic flow-focusing junction in methylene blue solution, which is an optically absorbing organic dye. We demonstrated that these dual modality contrast agents can be incorporated with photoacoustic and ultrasound imaging systems. The monodisperse microbubbles showed significant increase in the ultrasound signal intensity. Moreover, presence of nitrogen microbubbles in the methylene blue solution reduced the photoacoustic signal as compared to the methylene blue solution without the microbubbles. The microbubbles-based dual contrast agents involving photoacoustic imaging are of interest because the absence of microbubbles increases the photoacoustic signal. This will give spatio-temporal information of the microbubbles in the blood vessels which may have theranostic applications. With monodisperse microbubbles, the resonance frequency bandwidth is narrower so these bubbles will show strong echogenicity to certain ultrasound frequencies.

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