Generation of a droplet inside a microbubble with the aid of an ultrasound contrast agent: first result

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Running title: Ultrasonic antibubble generation

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Abstract

New ultrasound contrast agents that incorporate a therapeutic compound have become of interest. Such an ultrasound contrast agent particle might act as the vehicle to carry a drug or gene load to a perfused region of interest. The load could be released with the assistance of ultrasound. Generally, an increase in shell thickness increases the acoustic amplitude needed to disrupt a bubble. High acoustic amplitudes, however, have been associated with unwanted effects on cells. It would be interesting to incorporate a droplet containing drugs or genes inside a microbubble carrier. A liquid core surrounded by a gas encapsulation has been referred to as antibubble. In this paper, the creation of an antibubble with the aid of ultrasound has been demonstrated with high-speed photography.

Letter

Ultrasound contrast agents

Blood does not scatter clinical ultrasound well. In order to image and measure perfused areas of the human body with ultrasound, therefore, a contrast agent has to be injected that acts as a marker for the blood. Ultrasound contrast agents typically consist of low solubility gas microbubbles, encapsulated by a stabilizing biodegradable shell. With mean diameters below 6 μm, these microbubbles are small enough to pass through the capillary system. The microbubbles oscillate linearly when insonified at low acoustic amplitudes, but demonstrate nonlinear oscillating behavior at higher amplitudes. This harmonic behavior allows for the discrimination between perfused areas and surrounding tissue. At high acoustic amplitudes, nonlinear behavior of such microbubbles other than oscillation has been observed, such as fragmentation, contents release, and jetting [1-6]. As a result, encapsulated microbubbles have been investigated for their potential applications in ultrasound-assisted drug and gene delivery.

Ultrasound-assisted drug delivery

It has been proven, that the cellular uptake of drugs and genes is increased, when the region of interest is under ultrasound insonification, and even more when a contrast agent is present [7-

17]. This increased uptake has been attributed to the formation of transient porosities in the cell membrane, which are big enough for the transport of drugs into the cell (sonoporation). Owing to this technique, new ultrasound contrast agents that incorporate a therapeutic compound have become of interest. Such an ultrasound contrast agent particle might act as the vehicle to carry a drug or gene load to a perfused region of interest. The load could be released with the assistance of ultrasound. Combining ultrasound contrast agents with therapeutic agents may lead to a simple and economic method to instantly cure upon diagnosis, using conventional ultrasound scanners.

Microbubble carriers

The simplest microbubble carrier would be a therapeutic compound in the gas phase encapsulated by a shell to prevend its dissolution. Because of the impedance difference between the gas and surrounding blood, the therapeutic compound would be acoustically detectable and controllable. For example, the vasodilator nitric oxide has been proposed for incorporation in microbubbles [18]. More complicated carriers involve attaching a therapeutic compound to the microbubble shell, or incorporating a therapeutic compound inside the microbubble. An advance of the former method has been the *in vivo* delivery of a virus vector attached to albumin-encapsulated microbubbles [19]. A recent advance of the latter is the *in vitro* and *ex vivo* delivery of microbubbles consisting of an outer lipid layer, an oil layer into which a therapeutic compound can be dissolved, and a gas core [20]. When attaching or incorporating a therapeutic compound to an encapsulated microbubble, its physical shell properties, *e.g.*, shell stiffness and shell friction, are affected. A higher shell stiffness increases the bubble resonance frequency, whereas a higher shell friction increases the damping of the oscillating bubble. Generally, an increase in shell thickness increases the acoustic amplitude needed to disrupt a bubble [21]. High acoustic amplitudes, however, have been associated with unwanted effects on cells [22,23]. It would be interesting to incorporate a droplet containing drugs or genes inside a microbubble carrier. A liquid core surrounded by a gas encapsulation has been referred to as antibubble [24]. Incorporating a liquid droplet inside a microbubble is, however, technically challenging.

Jetting

Such a droplet inside a bubble may be generated with the jetting phenomenon: The collapse of a bubble near a free surface produces a liquid jet [25], which may break up into one or several droplets [26]. Early high-speed photographs of jets have been shown by Benjamin and Ellis [27], Crum [28, page 534], and Lauterborn [29,30]. High-speed photographs of jets breaking up have been presented in [31]. The length of the jet and the bubble pinch-off are related to the jet velocity [32], which in turn is related to the collapsing bubble size [33,34]. Ultrasound-induced jetting has been observed with ultrasound contrast agent microbubbles with a thin elastic shell [4]. We have also observed jetting with free gas microbubbles that had been released from the thick-shelled ultrasound contrast agent Quantison™ [6].

Quantison™

Quantison™ (Upperton Limited, Nottingham, UK) consists of human serum albumin-encapsulated air bubbles with a mean diameter of 3.2 μm. Shell thicknesses are between 0.2 and 0.3 μm [35]. The pressure field exerted by a Quantison™ microbubble is very low compared to the ultrasonic field, because of its low oscillation amplitude [36]. A released bubble, however, will generate a very high pressure, because of its high volumetric acceleration during collapse. Therefore, a collapsing microbubble near a bigger one is expected to generate instabilities on the surface of the bigger one. Fig. (**1**) illustrates the difference in oscillation behavior between Quantison™ microbubble and a free microbubble, insonified by a 0.5-MHz continuous wave with 20 kPa and 300 kPa peak acoustic pressures, respectively. At low acoustic amplitude the Quantison™ bubble oscillates linearly, whereas the free gas bubble shows harmonics. At high acoustic amplitude, the Quantison™ bubble oscillates nonlinearly as well, with moderate excursion amplitudes, whereas the free gas bubble has oscillation amplitudes over 5 times its equilibrium size.

Experimental setup

A 5 ml vial containing Quantison™ was vigorously stirred. The agent was inserted without further dilution through a syringe into a CUPROPHAN® RC55 cellulose capillary (Membrana GmbH, Wuppertal) with a 200 μm inner diameter and an 8 μm wall thickness. The capillary was positioned in a container in the acoustical focus of a V389-SU 0.5-MHz single-element transducer (Panametrics Inc., Waltham, MA), and in the optical focus of a high-numerical aperture microscopic system, connected to the high-speed Brandaris 128 camera [37]. Freely

flowing microbubbles were insonified with a burst of 8 cycles, 0.5-MHz ultrasound. Peak-negative acoustic pressures up to 1.3 MPa were applied. In this regime, less than 10% of the Quantison™ microbubbles demonstrate gas release [36]. Our experimental setup for taking high-speed photographs of microbubbles was more extensively described in [4]. A detailed description of the optical part of the system has been given in [37]. The experiment was repeated 12 times. In one experiment, we observed the formation of a drop inside a gas bubble. Here, we present this first result of generation inside a microbubble.

Droplet formation

Fig. (**2**) shows a high-speed photographic sequence of a free (unencapsulated) gas microbubble with a diameter of 30 μm close to a 7.5 μm Quantison™ microbubble that is slightly out of optical focus. Frame 0 was captured prior to ultrasound arrival, whereas frames 1–10 were captured after ultrasound has arrived. During insonification with 0.5-MHz ultrasound and a 0.8-MPa peak-negative acoustic pressure, the surface of the free gas microbubble close to the Quantison™ microbubble becomes instable, followed by the generation of a liquid drop inside the gas microbubble. The jet formation and the bubble pinch-off are similar to the observations of jet break-up in [31]. We attribute the surface instability to the behavior of released gas from the Quantison™ microbubble. Although the — much larger than resonance — free gas bubble has been observed in contraction phase in frame 1, the — much smaller than resonance — released gas bubble causing the jet must have oscillated out of phase with it and therefore been inexpansion phase [21].

Stability

In a subsequent multi-exposure recording after 100 ms, only the gas bubble could be detected, indicating a very low stability of the antibubble. The presence of a surfactant on the interfaces might lead to an improved stability of an antibubble [24].

Conclusion

Ultrasound contrast agent particle might act as the vehicle to carry a drug or gene load to a perfused region of interest. The load could be released with the assistance of ultrasound. It would be interesting to incorporate a droplet containing drugs or genes inside a microbubble

carrier. In this paper, the creation of an antibubble with the aid of ultrasound has been demonstrated with high-speed photography. Techniques for on demand antibubble creation will be needed for future applications in therapy.

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List of figures

Figure 1: Simulated normalized radius–time curves of microbubbles with a 7.5 μm diameter under 0.5-MHz continuous insonification: free gas (solid line) and Quantison™ (dotted line). Upper frame: peak acoustic amplitude 20 kPa, lower frame: 300 kPa. For Quantison™, the shell stiffness has been approximated by $25/8\pi$ kg s^{-2} [38].

Figure 2: Ultrasound-induced formation of an antibubble (A) and a schematic representation thereof (B). Each frame corresponds to a 71×52 μm^2 area. Frame 0 was captured before ultrasound arrival. Frames 1–10 cover 5 μs, captured during insonification. This corresponds to four frames per ultrasonic cycle. The depth-of-field is approximately 5 μm, causing a stacked representation of three-dimensional microobjects. In frame 0, a big free gas microbubble with a 30 μm diameter is seen just below a 7.5 μm Quantison™ microbubble that is slightly out of optical focus. After ultrasound arrival, between frames 0 and 1, presumably gas release takes place from the Quantison™ microbubble. This new microbubble is seen to expand and contract in the following frames. The two microbubbles interact, leading to an instability at the surface of the big free gas microbubble (frame 2). This instability has the form of a re-entrant jet protruding into the gas microbubble. The inward protrusion grows until frame 4. Between frames 5 and 8, the cylindrical protrusion drains. In frame 9, a 5 μm droplet is left inside the gas microbubble, while the protrusion retracts. Frame 10 shows the resulting antibubble: a spherical liquid core inside a spherical gas encapsulation.

Figure 3: Multi-exposure recording, captured 100 ms after Fig. (**2**). The frame corresponds to a 71×52 μm^2 area. The overlapping frames show only the free gas bubble, without the presence of a droplet inside.

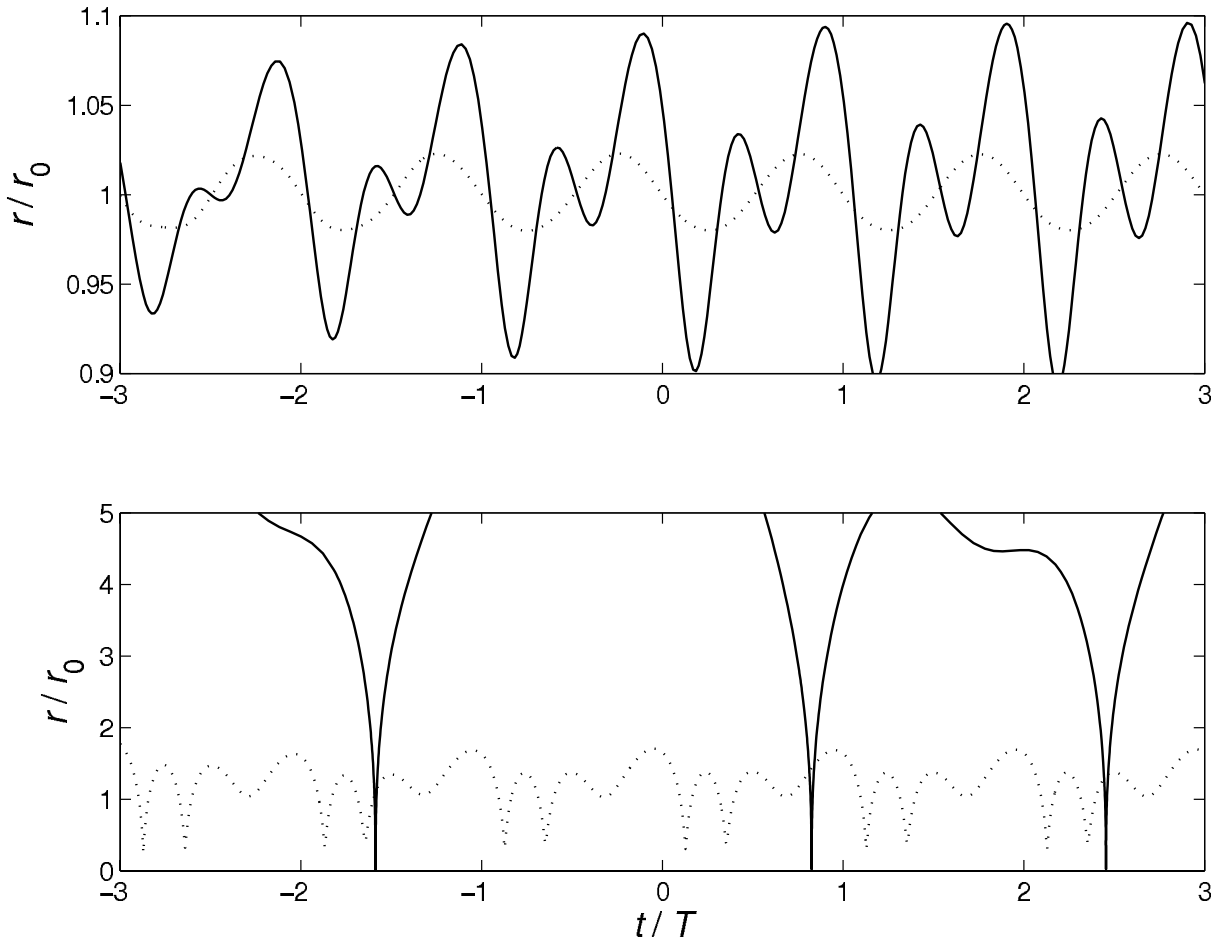

Figure 1

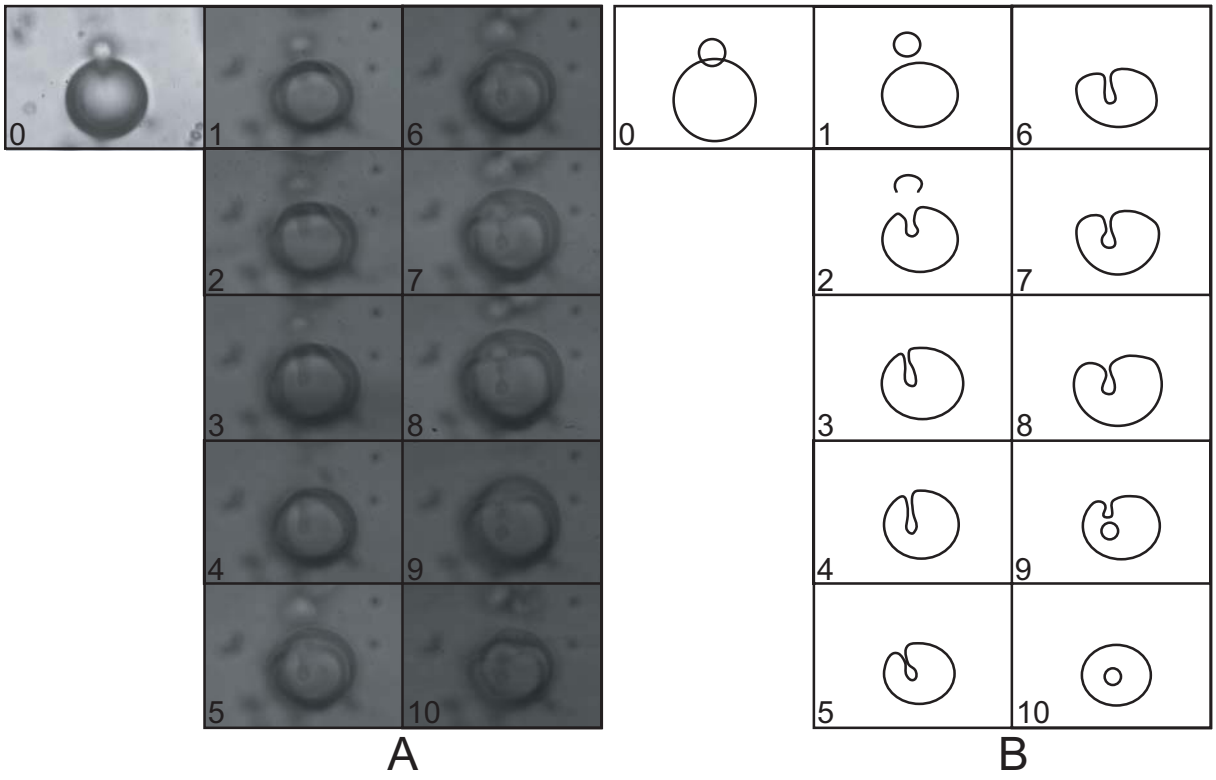

Figure 2

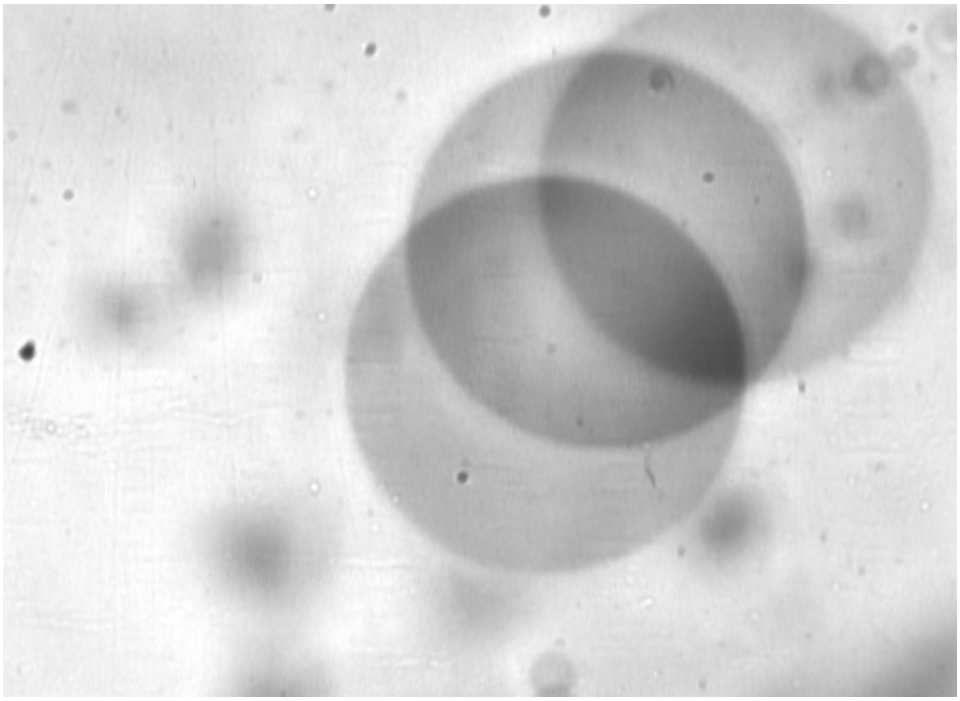

Figure 3