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Creating antibubbles with ultrasound

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Abstract—Ultrasound contrast agents have been investigated for their potential applications in local drug and gene delivery. A microbubble might act as the vehicle to carry a drug or gene load to a perfused region of interest. The load has to be released with the assistance of ultrasound. We investigate the suitability of antibubbles for ultrasound-assisted local delivery. As opposed to bubbles, antibubbles consist of a liquid core surrounded by a gas encapsulation. Incorporating a liquid drop containing drugs or genes inside an ultrasound contrast agent microbubble, however, is technically challenging.

An ultrasound-insonified microbubble generates a pressure field that is inversely proportional to the distance from the microbubble. Therefore, an oscillating contrast agent microbubble may create a surface instability with a relatively big bubble at a short distance. For big enough instabilities, a drop may be formed inside the big bubble.

Three different contrast agents were subjected to 0.5 MHz ultrasound, with mechanical indices >0.6 . The contrast agents were inserted through an artificial capillary which led through the acoustic focus of the transducer. High-speed photographs were captured at a speed of 3 million frames per second and higher. We observed that ultrasound contrast microbubbles below resonance size may create visible surface instabilities with bubbles above resonance size. With an albumin-shelled contrast agent, we induced a surface instability that was big enough to create an antibubble inside a free (unencapsulated) gas bubble with an 8 micron diameter. The surface instability has been attributed to the presence of a contrast microbubble with a 3 micron diameter. This instability has the form of a re-entrant jet protruding into the gas bubble. The inward protrusion grew and subsequently drained, leaving a droplet with a five micron diameter inside the bubble. In a subsequent recording after 100 ms, only the gas bubble could be detected. Thus, the lifetime of the antibubble was less than 100 ms. The presence of a surfactant on the interfaces might lead to an improved stability of an antibubble.

I. INTRODUCTION

Ultrasound contrast agents consist of microscopically small bubbles in the micrometer range. These microbubbles oscillate linearly if insonified at low acoustic amplitudes, but demonstrate highly nonlinear behavior at relatively high acoustic amplitudes, such as rapid collapse, fragmentation and jetting [1]. Therefore, ultrasound contrast agents have been investigated for their potential applications in local drug and gene delivery.

A microbubble might act as the vehicle to carry a drug or gene load to a perfused region of interest. The load has to be released with the assistance of ultrasound.

Several schemes have been proposed to combine microbubbles with a therapeutic load:

A. Mixing microbubbles with a therapeutic agent

Tachibana *et al.* subjected cells in the presence of a photosensitive drug to continuous ultrasound, and noticed surface pores on the cells [2]. Inducing porosities in cells with ultrasound, generally referred to as sonoporation, has potential applications in (tumor) cell lysis and selective delivery of drugs and genes into cells.

Independently, it was demonstrated that moderate microbubble oscillations are sufficient to achieve rupture of lipid membranes, in a regimen in which the bubble dynamics can be accurately controlled [3]. This might enable the transport of drugs through the cell membrane.

The behavior of contrast agent microbubbles near cells has been recently studied by Kudo, Van Wamel, and Wolfrum [4], [5], [6].

The *in vitro* and *in vivo* transfection of endothelial cells with plasmid DNA mixed with ultrasound contrast agent has been demonstrated [7]. Accelerated cellular uptake of the radionuclide $^{111}\text{In-DOTA-Tyr}^3\text{-octreotate}$ takes place when microbubbles are present and under insonification [8].

B. Therapeutic compounds attached to microbubble shells

It has been put forward that targeted microbubbles with ligands attached to them may be applied for selective delivery to the areas where selective enhancement of the action of ultrasound would be required, and if such a bubble would contain plasmid DNA, enzyme, or another therapeutic agent, it might be released at the site of interest during insonification [9].

A recent advance in local gene delivery is the *in vivo* delivery of a virus vector, attached to albumin microbubbles [10].

C. Therapeutic compounds in the gaseous phase

Sonic cracking is the ultrasound-induced release of gas from thick-shelled microbubbles. Therapeutic compounds in the gas phase might be encapsulated with thick shells, to keep them from dissolving. At the region of interest, the shell can be cracked with ultrasound, releasing the content [11].

A few therapeutic compounds exist in the gaseous phase. We think of nitric oxide [12] and of gaseous anaesthetics.

D. Gas-filled lipospheres containing drugs

May *et al.* performed high-speed optical experiments on microbubbles with an outer lipid layer, an oil layer, and a gas core [13]. Such microbubbles may be applicable in local drug delivery, since their lipid-oil complex can carry bioactive substances at high concentrations [14]. The delivery of the contents of these microbubbles has been demonstrated *in vitro* and *ex vivo* [14].

E. Drug-filled antibubbles

A therapeutic agent inside the microbubble shell may react with the shell and damp the bubble oscillations. Therefore, it might be more suitable to have the therapeutic agent in the core of the microbubble, separated from the shell by a gaseous layer. Incorporating a liquid drop containing drugs or genes inside an ultrasound contrast agent microbubble, however, is technically challenging. We investigate the suitability of antibubbles for ultrasound-assisted local delivery.

As opposed to bubbles, antibubbles consist of a liquid core surrounded by a gas encapsulation (*cf.* Figure 1) [15]. 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 [16], which may break up into one or several droplets [17]. Early high-speed photographs of jets have been shown by Benjamin and Ellis [18], Crum [19, page 534], and Lauterborn [20], [21]. High-speed photographs of jets breaking up have been presented in [22]. The length of the jet and the bubble pinch-off are related to the jet velocity [23], which in turn is related to the collapsing bubble size [1]. Ultrasound-induced jetting has been observed with ultrasound contrast agent microbubbles with a thin elastic shell [1]. We have also observed jetting with free gas microbubbles that had been released from the thick-shelled ultrasound contrast agent Quantison™.

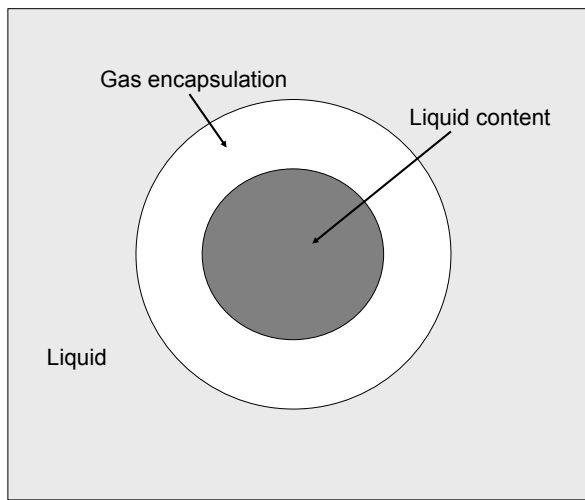


Fig. 1. An antibubble consists of a liquid core encapsulated by a gas shell.

Quantison™ (Upperton Limited, Nottingham, UK) consists of human serum albumin-encapsulated air bubbles with

a mean diameter of $3.2 \mu\text{m}$. Shell thicknesses are between 0.2 and $0.3 \mu\text{m}$ [24]. The pressure field exerted by a Quantison™ microbubble is very low compared to the ultrasonic field, because of its low oscillation amplitude [11]. A released bubble, however, will generate a very high-pressure, because of its high volumetric acceleration during collapse [25]. Therefore, a collapsing microbubble near a bigger one is expected to generate instabilities on the surface of the bigger one.

II. EXPERIMENTAL SETUP

A 5 ml vial containing Quantison™ (Upperton Limited, Nottingham, UK) was heavily stirred. The agent was inserted through an artificial capillary with a $200 \mu\text{m}$ diameter, without further dilution. The artificial capillary was positioned in a container in the acoustical focus of a V389-SU 500 kHz single-element transducer (Panametrics Inc., Waltham, MA), and in the optical focus of a high-numerical aperture microscopic system. An overview hereof is shown in Figure 2.

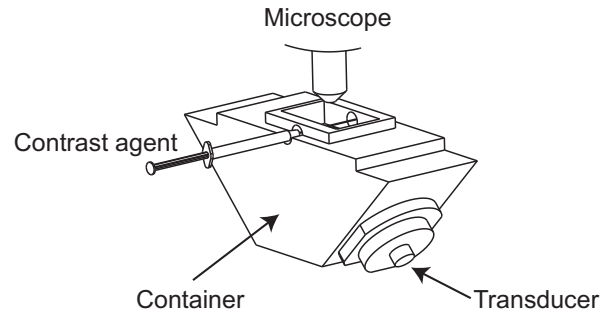


Fig. 2. Overview of the optical observation part of the experimental setup.

Freely flowing microbubbles were insonified with a burst of 8 cycles, 500 kHz ultrasound. Acoustic pressures up to 1.3 MPa were applied. In this regime, less than 10% of the Quantison™ microbubbles demonstrate gas release [11]. Our experimental setup for taking high-speed photographs of microbubbles was more extensively described in [1]. This experiment was performed 13 times. In one experiment, we observed the formation of a drop inside a gas bubble. Here, we present this first result of ultrasonic antibubble generation.

III. RESULT

Figure 3 shows a high-speed photographic sequence of a free (unencapsulated) gas microbubble with a diameter of $8 \mu\text{m}$ close to a $3 \mu\text{m}$ Quantison™ microbubble that is slightly out of optical focus. During insonification with 0.5 MHz ultrasound and a mechanical index $MI=1.1$, the surface of the free gas microbubble close to the Quantison™ microbubble becomes unstable, followed by the generation of a liquid drop inside the gas microbubble.

In frame 0, a big free gas microbubble with an $30 \mu\text{m}$ diameter is seen just below a $7.5 \mu\text{m}$ Quantison™ microbubble that is slightly out of optical focus. After ultrasound arrival, 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.

IV. DISCUSSION

The jet formation and the bubble pinch-off are similar to the observations of jet break-up in [22]. We attribute the surface instability to the behavior of released gas from the Quantison™ microbubble. In a subsequent recording after 100 ms, only the gas bubble could be detected. Thus, in this first attempt to generate an antibubble with the aid of an ultrasound contrast agent, the life-time of the antibubble was less than 100 ms. The presence of a surfactant on the interfaces might lead to an improved stability of an antibubble [15]. Techniques for on demand antibubble creation are needed for future applications in therapy.

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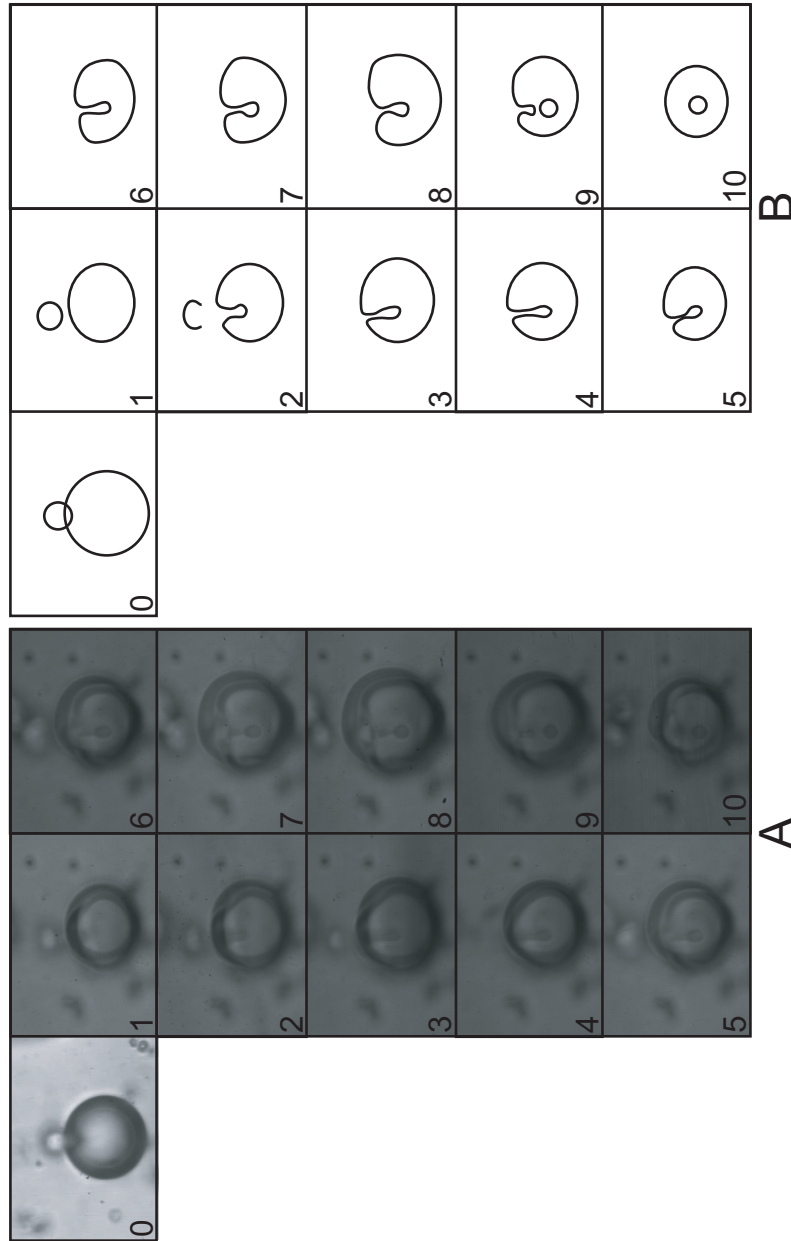


Fig. 3. Ultrasound-induced formation of an antibubble (A) and a schematic representation thereof (B). Each frame corresponds to a $71 \times 52 \mu\text{m}$ area. Frame 0 was captured before ultrasound arrival. Frames 1–10 cover $5 \mu\text{s}$, captured during insonification. This corresponds to four frames per ultrasonic cycle. The depth-of-field is approximately $5 \mu\text{m}$, causing a stacked representation of three-dimensional microobjects. In frame 0, a big free gas microbubble with an $30 \mu\text{m}$ diameter is seen just below a $7.5 \mu\text{m}$ Quantison™ microbubble that is slightly out of optical focus. After ultrasound arrival, 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 $5 \mu\text{m}$ 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.