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Sonic cracking of calcium carbonate-encapsulated microbubbles observed at moderate acoustic amplitudes

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Abstract: Theranostic agents are materials that act both as tracers during diagnostic imaging and as vehicles carrying and releasing therapeutics during treatment. Ultrasound-triggered theranostic agents comprise shell-encapsulated microbubbles that pulsate during low-amplitude ultrasonic imaging and release their payload upon higher-amplitude sonication whilst simultaneously assisting in the permeation of target tissue. High-amplitude release may be undesirable due to unwanted side effects related to inertial cavitation. However, low-amplitude release from microbubbles typically requires thin encapsulating shells, which in turn may be permanently disrupted under diagnostic imaging conditions. The purpose of this study was to investigate the suitability for theranostic applications of a novel microbubble agent with thick shells composed of calcium carbonate.

Hydrophobised calcium carbonate-encapsulated microbubbles of radii between 1.0 μm and 11 μm were subjected to short ultrasound pulses of 1-MHz ultrasound at acoustic amplitudes of 0.5 MPa or less, which corresponds to low mechanical indices. During sonication, high-speed video footage was recorded at a frame rate of ten million frames per second.

We observed pulsations but no gas release at a 0.1-MPa amplitude and intra-encapsulation fragmentation during sonication at a 0.3-MPa amplitude. At 0.5-MPa amplitude sonication, release was observed from more than 70% of the microbubbles in the field of view. This finding indicates that the microbubbles were stable scatterers during 0.1-MPa sonication, but instable vehicles during 0.5-MPa sonication.

The pressures used in this study to observe release were too low to allow for unwanted inertial cavitation. In conclusion, therefore, the microbubbles studied were a promising theranostic agent whose contents could be released at moderate acoustic amplitudes.

Keywords: Controlled release, $CaCO_3$, low-MI sonication, LIFU, toothpaste.

1 Introduction

Instant treatment upon diagnosis is becoming feasible owing to the development of theranostic agents [1]. Theranostic agents are materials that act both as tracers during diagnostic imaging and as vehicles carrying and releasing therapeutics during treatment. Ultrasound-triggered theranostic agents comprise microbubbles, whose shell encapsulations prevent them from rapidly dissolving [2–4]. These microbubbles pulsate during low-amplitude sonication [4], but they demonstrate highly irregular behaviour behaviour at high amplitudes, including contents release [5]. Microbubble pulsations near cells have been reported to increase membrane permeation, a process referred to as sonoporation [6–8]. The payload of the theranostic agent may be in the gas phase [9], or may be composed of a more complicated structure [10–12].

Overall, theranostic microbubbles are preferably stable cavitation sources at low acoustic amplitudes, only to be destroyed at therapeutic amplitudes [13]. Microbubbles with thin, *i.e.*, with less than 50-nm thickness, flexible shells are readily disrupted however [14]. Microbubbles with thick, *i.e.*, with greater than 200-nm thickness, shells are disrupted in regimes associated with intertial cavitation [15]. The disruption of such microbubbles has been referred to as sonic cracking [16, 17].

In a previous study, sonic release from two types of albumin-encapsulated microbubble ultrasound contrast agents was quantified [15]. Even at very low acoustic amplitudes, corresponding to a 0.5 mechanical index, only a minority of the microbubbles observed demonstrated release [15]. The pur-

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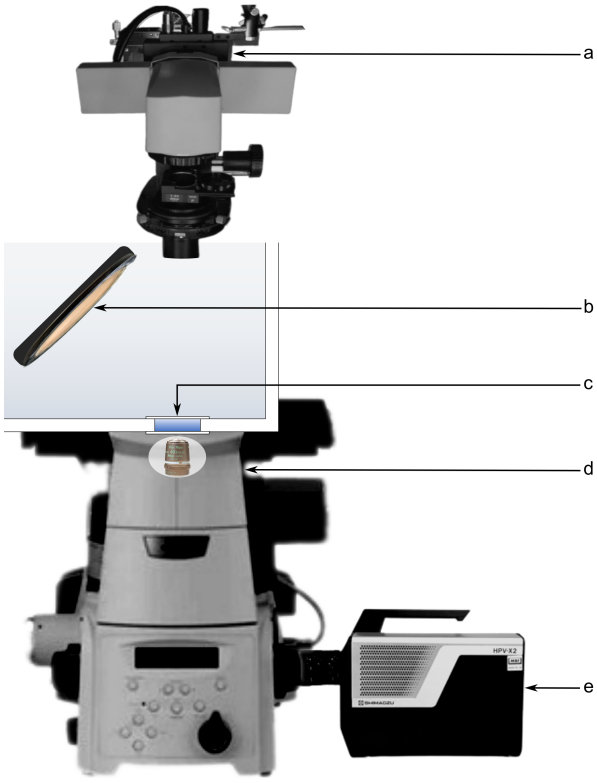

Fig. 1: Frontal schematic overview of the experimental setup, showing a light source (a), a transducer (b), a sonication compartment (c), an inverted microscope (d), and a high-speed camera (e).

pose of the present study was to investigate the suitability for theranostic applications of a novel microbubble agent with thick shells composed of calcium carbonate, by quantifying pulsation response and gas release resulting from pulsed sonication.

2 Materials and methods

CALOFORT® U calcium carbonate ($CaCO_3$) particles (Specialty Minerals Inc., Bethlehem, PA, USA) were hydrophobised [18]. In brief, 5% $CaCO_3$ particles were dispersed in distilled water containing 10% of GLUCIDEX® maize maltodextrin dextrose equivalent of 2 (Roquette Frères, Lestrem, France) and this dispersion was mixed with a 0.2% sodium stearoyl lactylate (TCI Europe N.V., Zwijndrecht, Belgium) solution in a 1:1 ratio.

Subsequently, emulsions were made by adding 10% cyclooctane (TCI Europe N.V.) and homogenising this using a T18 ultraturrax (Ika Werke GmbH & CO. KG, Staufen, Germany).

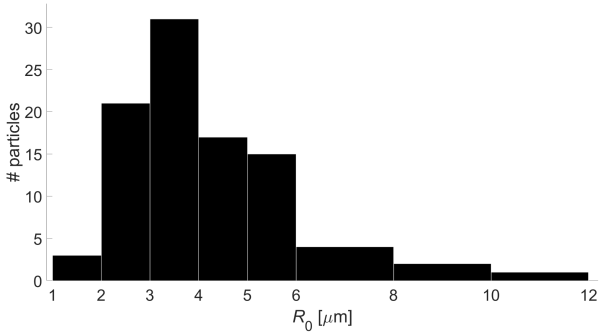

Fig. 2: Size distribution of $CaCO_3$-encapsulated microbubbles pre-sonication.

The emulsions were frozen for 24 hours at −60°C and subsequently freeze-dried for 24 hours.

Quantities of 5 mg of freeze-dried material were reconstituted in 5 ml of degassed distilled water (FUJIFILM Wako Pure Chemical Corporation, Chuo-ku, Osaka, Japan). For each experiment, 200 μl of reconsituted dispersion was pipetted into a cylindrical compartment of 8-mm diameter and 2-mm height before being closed with an 18×18 mm Thickness No. 1 Micro Cover Glass (Matsunami Glass Ind.,Ltd., Kishiwada-shi, Osaka, Japan) and sealed with No.600M cloth tape (Sekisui Chemical Co., Ltd., Kita-ku, Osaka, Japan).

The compartment was part of a 244×145×76-mm^3 Perspex container that was positioned on top of an Eclipse Ti inverted microscope (Nikon Corporation, Minato-ku, Tokyo, Japan) with an S Plan Fluor ELWD 40×/0.6 objective lens focused at the bottom of the cover glass. The microscope was attached to an HPV-X2 high-speed camera (Shimadzu, Nakagyo-ku, Kyoto, Japan) [19], operating at frame rates equal to ten million frames per second during sonication with a 3-cycle pulse at a 1-MHz centre frequency and a peak-negative pressure of up to 0.5 MPa.

The pulse was generated by a custom-built focused ultrasound transducer of 65-mm diameter that was placed under a 45° angle relative to the cover glass surface and at a 60-mm distance from the optical focus that had been calibrated in a separate setup [20]. A line drawing of the experimental setup is shown in Figure 1.

The video data were exported to be processed offline using MATLAB® (The MathWorks, Inc., Natick, MA, USA). Segmentation was done automatically using Otsu's method for adaptive thresholding [21]. For each experiment, pre-sonication microbubble resting radii were determined from the first frame of the video recording.

Fifty nine experiments were performed, from which datasets of 94 unique microbubbles were generated. The size distribution of these unique microbubbles is shown in Figure 2.

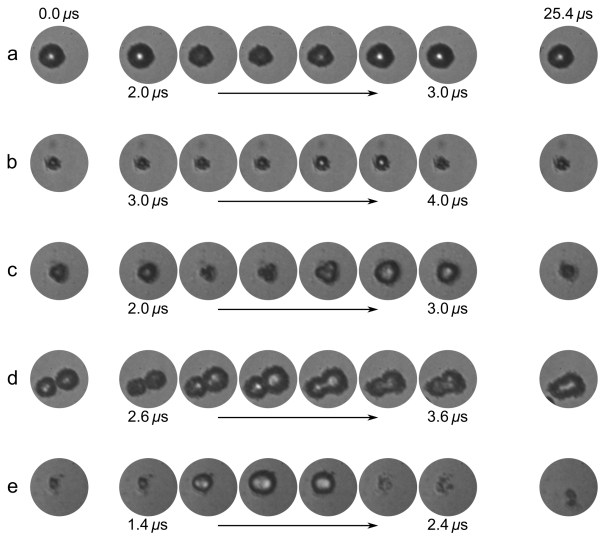

Fig. 3: High-speed footage of typical dynamic microbubble behaviour at different acoustic amplitudes: pulsation at a peak-negative pressure of 0.1 MPa (a), unsuccessful release at 0.1 MPa (b), intra-encapsulation fragmentation at 0.3 MPa (c), coalescence at 0.3 MPa (d), and successful gas release at 0.5 MPa (e). Each circular photo frame corresponds to a 15-μm diameter.

3 Results and discussion

Figure 3 shows examples of typical dynamic phenomena of microbubbles sonicated at a very low acoustic amplitude of 0.1 MPa, a moderately low acoustic amplitude of 0.3 MPa, and an intermediate acoustic of 0.5 MPa. Pulsations were observed at all acoustic amplitudes used. Even at very low amplitudes, the pulsations were visible. Not all encapsulations were visibly uniform. During pulsations at very low amplitudes, gas was observed to pass through the encapsulation without breaking free. At moderately low amplitudes, fragmentation of microbubbles was observed inside the confinements of the shell encapsulation. This phenomenon was to our knowledge not previously described elsewhere. At intermediate amplitudes, gas release from the microbubble encapsulation was observed, similar to footage shown in literature [15]. However, in literature, sonic cracking of thick-shell-encapsulated microbubbles required high amplitudes, less recommended for diagnosis [15]. Another phenomenon observed at moderately low and intermediate amplitudes was coalescence of encapsulated microbubbles. The supporting footage shows that the encapsulations rearranged themselves around the coalesced bubble. This indicated that the shell material behaved less as a rigid solid material but more like a flexible agglomerate accumulated on the interfaces.

Figure 4 shows the percentages of microbubbles released as a function of acoustic amplitude. The thick-shell-

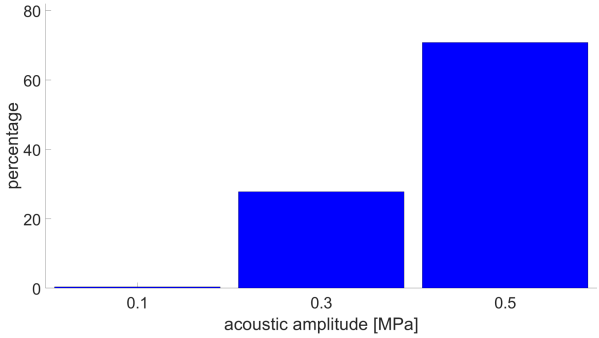

Fig. 4: Percentage of microbubbles released as a function of acoustic amplitude.

encapsulated microbubbles used in this study had a release rate of less than 1% at very low amplitudes, less than 30% at moderately low amplitudes, and greater than 70% at intermediate amplitudes. Compared to release rates published with different agents [15], our values were closer to those preferred for theranostic agents.

Figure 5 shows microbubble radius as a function of time of a released microbubbles post-sonication. Both microbubble were observed to resonate. The cause of this newly observed phenomenon has been attributed to energy transfer during either the collapse phase of the mother bubble to the daughter microbubble, or by an energy surplus following coalescence.

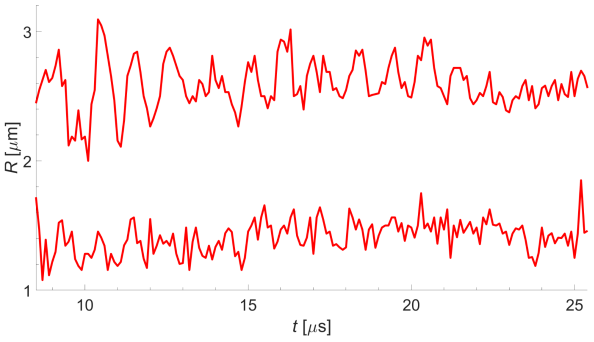

Fig. 5: Radius as a function of time of two microbubbles showing post-sonication pulsation behaviour.

4 Conclusions

The pressures used in this study to observe release were too low to allow for unwanted inertial cavitation. In conclusion, therefore, the microbubbles studied were a promising theranostic agent whose contents could be released at moderate acoustic amplitudes.

Author Statement

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