GIANT RESPONSE OF MICROBUBBLE CONTRAST AGENTS OSCILLATION IN A HIGH-INTENSITY FOCUSED ULTRASOUND FIELD

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Abstract. The interaction of microbubble contrast agents (BR14) with high-intensity focused ultrasound is investigated by high-speed photography. The experiments were conducted at an ultrasound frequency of 1.08 MHz with a peak negative pressure of 1.8 MPa. The microbubble oscillations run on a giant response with a long expansion phase followed by a violent collapse. The implications in high-intensity focused ultrasound surgery are also discussed.

Key words: cavitation, microbubble contrast agents, giant oscillations.

1. INTRODUCTION

There is an increasing interest in understanding the interaction of microbubble contrast agents with ultrasound waves. Hypotheses of potential benefits from these interactions suggested that microbubble contrast agents loaded with therapeutic substances could be targeted for destruction with ultrasound and thus enhance diffusion-mediated delivery by increasing localized concentration of the substances. The ability to increase tissue permeability and concomitantly augment localized drug concentrations through targeted microbubble destruction has fueled interest in developing efficient methods for delivering drugs and genetic material. Most investigators who have used ultrasound contrast agents for therapeutic applications worked with perfluorocarbon bubbles stabilized by an albumin or lipid shell [1]. The main advantage of this type of contrast agents is their fragility when exposed to ultrasound. Several studies have been conducted on the behaviour of microbubble contrast agents. Chomas et al. [2], de Jong et al. [3],

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Bouakaz et al. [4], and Doinikov et al [5] describe the different effects of ultrasound on microbubbles and demonstrate these effects by experiments using high-speed photography. Depending on the applied ultrasound amplitude and frequency, effects such as stable oscillation of microbubbles, inertial cavitation, coalescence, fragmentation, ultrasound induced damage of the shell causing gas to escape from microbubbles, and jetting are described. The effects of various factors including the ultrasound driving frequency, pulse length, peak negative pressure, bubble size and shell properties on the fragmentation of microbubbles were also investigated by Chomas et al. [5] and Bloch et al. [6]. Microjet formation during collapse of microbubbles in the vicinity of a boundary was experimentally observed by Prentice et al. [7]. They also noted that the jetting and the microbubble translation towards the boundary are dependent on the relative distance between microbubble and boundary.

In this article we describe experimental investigations on the interaction of microbubble contrast agents with high-intensity focused ultrasound. Previous *in vivo* investigations have indicated that microbubble contrast agents increase the ablation efficiency of high-intensity focused ultrasound [8, 9]. We found that the microbubble oscillations run on a giant response with a long expansion phase followed by a violent collapse. In the expansion phase the maximum microbubble radius grows rapidly to several ten times their original size while the rapid compression yields fragmentation of the microbubble.

2. METHODS AND MATERIALS

All experimental investigations were performed with a third-generation contrast agent, BR14 (Bracco Diagnostics, Geneva, Switzerland). BR14 consists of perfluorocarbon-containing microbubbles stabilized by a phospholipid monolayer. The mean diameter of the microbubbles is between 2.5 to 3.0 μm. The diluted contrast agent solution is pumped through 100 μm syringe needle with a microinjector. High-intensity continuous wave ultrasound is generated by a 2 mm thick concave piezo-ceramic transducer with an outer diameter of 80 mm and a focal length of 80 mm (Fig. 1). A linear amplifier (E&I 2100L) takes a sinusoidal signal from a function generator (NF Corporation WF 1974) and drives the transducer at a frequency of 1.08 MHz. In the degassed water (2 ppm in O_2 concentration), ultrasound is gathered at the focus of the transducer where an aluminium wall is placed. Then, in the localized focal volume near the wall surface, high amplitude pressure fluctuations (peak negative pressure 1.8 MPa) are obtained. The peak negative pressure was measured by using a PVDF needle hydrophone (Imotec Messtechnik, type 80-0.5-4.0) with a rise time of 25 ns, and a sensitivity of 10.53 MPa/V (calibrated by the manufacturer up to a frequency of 10 MHz). We also note that, for this value, no cavitation activity was observed in the absence of BR14 microbubbles. The microbubble dynamics were recorded with a

high-speed image converter camera (DRS Hadland, Imacon 200) using parallel illumination provided by a cw Nd:Yag laser (λ = 532 nm). Independent of the interframing time, the exposure time on the fluorescent screen of the image converter camera is 5 ns. The image on the fluorescent screen was recorded with an intensified scan ICCD camera system (Photometrics AT200A) with a 1 360 × 1 024 pixel resolution per frame. The signal from the ICCD camera was then digitized with 12-bit resolution (256 grey levels) and passed to a computer. A Nikon lens (Ai-Micro Nikkor, 105 mm, F2.8S) with 10 close up rings enabled a field view of 2.56 mm × 2.09 mm.

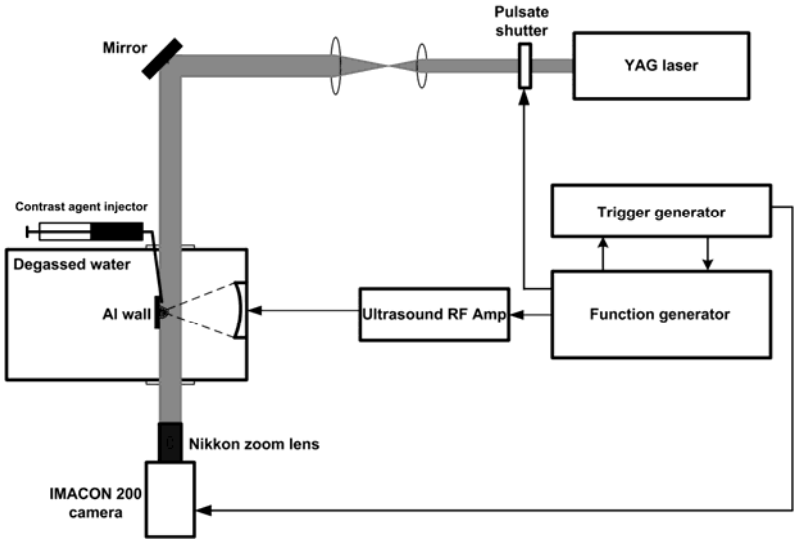

Fig. 1 – Experimental arrangement for the investigation of the behaviour of a microbubble contrast agent in high-intensity focused ultrasound field.

3. RESULTS AND DISCUSSION

Figure 2 shows the oscillation of a microbubble situated at a distance of 770 μm from the wall. The microbubble motion is characterized by a long expansion phase, that last for at least 10 μs, followed by a rapid compression. During the collapse phase, when the kinetic energy of the bubble surpasses its surface energy, the microbubbles fragment into a number of smaller cavities. The images obtained in this figure do not clearly show the compression of the bubble prior to fragmentation due to the limited temporal resolution, although it clearly shows the resulting fragments. Up to three fragments can be seen in frame 8. No translation of the microbubble towards or away from the wall was observed. The experimental microbubble radius was calculated using the long and short radii of the elliptically

shaped bubble, measured from the photographic frames, $R = [(R_{long}(R_{short})^2]^{1/3}$. The maximum bubble radius is 132 ± 10 μm (frame 3), i.e. 50 times larger than the equilibrium radius of the microbubbles.

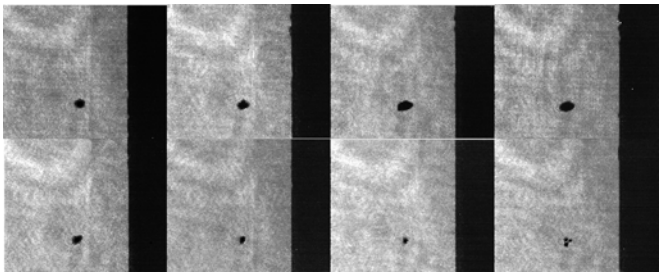

Fig. 2 – Photographic series of the interaction of a BR14 microbubble contrast agent with high-intensity focused ultrasound. Frame interval 2 μs, frame size 2.56 mm × 2.09 mm.

Figure 3 illustrates the behaviour of three microbubbles situated at various distances from the wall. Microbubble "a" is situated at a distance of 725 μm from the wall while the distance between microbubble "b" and the wall is 170 μm. The third microbubble is attached to the wall. As in the previous case, a long expansion phase followed by a violent collapse are the main features of the microbubble dynamics. The maximum radius of the microbubble situated farthest from the rigid wall is 120 ± 10 μm (approximately 40 times larger than the equilibrium radius of the microbubbles). The microbubble "b" expands to a smaller value of the maximum radius, probably because it is outside the focal volume of the ultrasound. There is no migration of the microbubbles situated far from the wall and no clear evidence for the formation of a microjet during microbubble collapse was found in these cases. Only the attached bubble shows interaction with the rigid boundary, including jetting (frames 12 and 13). Jetting behaviour has been previously noticed in micrometer-sized shelled and unshelled bubbles situated close to a rigid boundary [7, 10]. The oscillation time of both microbubbles situated far from the wall is about 24 μs. The microbubble attached to the wall has a larger oscillation time which is most likely a consequence of the interaction with a rigid boundary. It is well known from studies with laser generated bubbles that a rigid wall in close proximity to a bubble results in a lengthening of the oscillation period with about 20% [11]. The maximum collapse velocity was obtained in the case of the microbubble attached to the rigid wall and is about 30 m/s (mediated between frames 12 and 13). We note, however, that this value is only a lower estimate of the collapse velocity because the temporal resolution is not sufficient to resolve the final collapse phase where the microbubble achieves the minimum radius.

Figure 4 shows the oscillation of two microbubbles that are attached to the rigid boundary. The maximum radius of these bubbles are 120 ± 10 μm (frame 9), and fragmentation of both microbubbles can be observed starting with frame 10.

The shape of the lower bubble in frame 2 and the shape of the upper bubble in frame 6 suggest the formation of a liquid jet directed towards the boundary. We also note that no shock wave emission from the collapsing microbubbles have been observed, probably as a consequence of microbubble fragmentation. Shock wave emission from un-encapsulated microbubbles were previously observed using the same experimental set-up and similar ultrasound parameters [12].

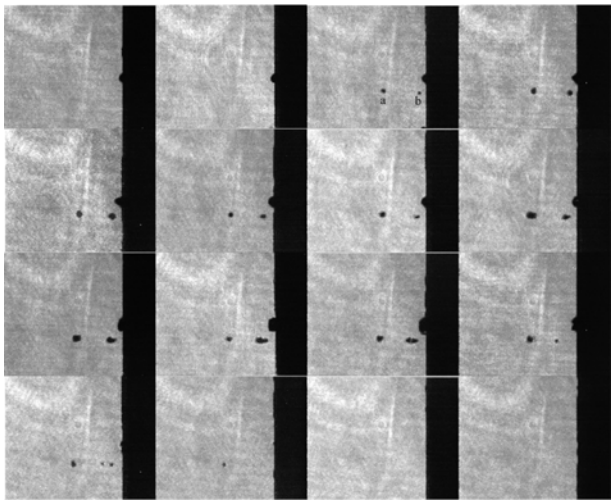

Fig. 3 – Photographic series of the interaction of three BR14 microbubble contrast agents with high-intensity focused ultrasound. Frame interval 2 μs, frame size 2.56 mm × 2.09 mm.

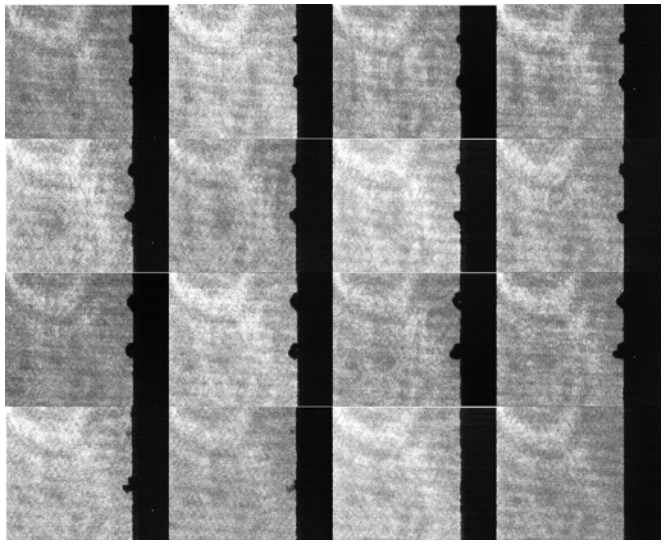

Fig. 4 – Photographic series of the interaction of three BR14 microbubble contrast agents with high-intensity focused ultrasound. Frame interval 2 μs, frame size 2.56 mm × 2.09 mm.

When unencapsulated bubbles of different sizes are subject to only a very small acoustic pressure amplitude, they will respond with small oscillations about their equilibrium radius. Going up in the driving amplitude will bring out the effects of nonlinearity manifesting themselves in a non-sinusoidal bubble oscillation and in the occurrence of several resonances. At even larger oscillation amplitudes, the bubble can be elongated from its equilibrium radius to arbitrarily large radius values and can be compressed down to near zero radius [13]. The present experimental investigations show a dramatic increase in the response of BR14 microbubbles in a high-intensity focused ultrasound field. Physically, this giant response comes about through the instability of the bubble, when the static pressure is overcome by the sound pressure during part of the sound cycle.

Bloch et al. [6] also investigated the behaviour of BR14 microbubbles at values of the peak negative pressure similar to that used in the present experiment. The microbubbles were exposed to 2.25 MHz, two-cycle pulses at peak negative pressures ranging from 180 kPa to 1.42 MPa. The maximum bubble radius obtained at the peak negative pressure of 1.42 MPa was about 20 μm, i.e. six times smaller than in this experiment. We note, however, that the giant response strongly depends on frequency. The peak reduces with increasing frequency because the duration of the inertial instability gets smaller in proportion to the smaller period of the driving field [13–15]. Thus, low frequency more easily leads to a large bubble expansion and strong collapse, high frequencies to more collapses in a fixed amount of time [14, 15]. Pressure-wave-excited contrast agent bubbles (Levovist) in the vicinity of rat kidney fibroblast cells were investigated by Wolfrum et al. [16]. Their results showed that even at moderate peak negative pressure amplitudes of less than 2 MPa, the contrast agent bubbles expand to more than 30 times their original radius.

In a recent paper, McDannold and co-workers [17] investigated, in rabbits, whether the combination of ultrasound contrast microbubbles and high-intensity focused ultrasound could induce lesions in the brain. The lowest values for the production of lesions with the microbubbles were 1.2 W for 10-second pulsed sonications and 0.6 W for 20-second pulsed sonications, almost 12 times lower than a previously determined threshold for lesion creation. The 50% probability for inducing tissue necrosis required a temperature increase of 5.9°C, approximately half the 11.4°C threshold established in prior experiments. Interestingly, the temperature measured with the microbubbles present in the ultrasound focus appeared to be below the threshold for thermal damage, indicating that the lesions induced during high-intensity focused ultrasound with contrast microbubbles are caused by other cavitational bioeffects. The present results indicate that the giant response of microbubble oscillations in a high-intensity focused ultrasound field may be a potential source of tissue damage because tissue close to the microbubble wall can be compressed and stretched beyond the damage threshold.

4. CONCLUSION

The interaction of microbubble contrast agents (BR14) with high-intensity focused ultrasound is investigated by high-speed photography. The experiments were conducted at an ultrasound frequency of 1.08 MHz with a peak negative pressure of 1.8 MPa. The present results indicate that the microbubble oscillations run on a giant response with a long expansion phase followed by a violent collapse. In the expansion phase the maximum microbubble radius grows rapidly to several ten times their original size while the rapid compression yields fragmentation of the microbubble. The giant response of microbubble oscillations in a high-intensity focused ultrasound field may be a potential source of tissue damage because tissue close to the microbubble wall can be compressed and stretched beyond the damage threshold.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0079.

Received on September 29, 2013

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