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Characterization and UltrasoundPulse Mediated Destruction of Ultrasound Contrast Microbubbles
The “quasi-stable” lipid shelled microbubble in response to consecutive ultrasound pulses

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Controlled microbubble stability upon exposure to consecutive ultrasound exposures is important for increased sensitivity in contrast enhanced ultrasound diagnostics and manipulation for localised drug release. An ultra high-speed camera operating at $13 \times 10^6$ frames per second is used to show that a physical instability in the encapsulating lipid shell can be promoted by ultrasound, causing loss of shell material that depends on the characteristics of the microbubble motion. This leads to well characterized disruption, and microbubbles follow an irreversible trajectory through the resonance peak, causing the evolution of specific microbubble spectral signatures. © 2012 American Institute of Physics. [http://dx.doi.org/10.1063/1.4746258]

The rapidly developing field of contrast-enhanced ultrasound spans application areas from molecular diagnostics to novel therapeutic techniques.1,2 The introduction of visco-elastic phospholipid shells to physically stabilise microbubbles over prolonged periods in vivo3 has enabled their safe and reproducible usage as contrast agents in diagnostic ultrasound, as a means to enhance the echogenicity of the blood pool and the microcirculation during ultrasound imaging.1 In modern diagnostic ultrasound applications, microbubbles are exposed to a series of short ultrasound pulses. Subsequent signal processing enables both tissue scatter cancellation and exploitation of the microbubble nonlinear properties. Their use as drug/gene delivery agents is also attractive, as they can promote (a) site specific targeting,4 (b) increased cell permeability and membrane permeation, and (c) deliver gene or other drugs safely in a variety of techniques.6,7 However, the mechanism of ultrasound mediated microbubble disruption and degradation remains unclear8–10 and this hinders the development of both advanced imaging modalities and efficient drug delivery strategies for an integrated theranostics approach.

The existing literature suggests that acoustic fields can be controlled to avoid damage to the microbubble shell, which will otherwise irreversibly lose structural integrity above the fragmentation threshold.11 However, the structural stability, and hence the stability of the backscattered signal, of the microbubble below such a threshold has not been interrogated until recently. New experimental techniques that enable detailed optical observation of the microbubble shell during oscillation hint at physical mechanisms such as buckling,12,13 lipid shedding14,15 and deflation,16 although the intricacies of ultra high-speed optical experiments limit the sample sizes obtained.

The study of the mechanisms that underlie this “quasi-stable” behaviour of microbubbles over a series of pulses is the aim of the present letter. A high speed optical camera was deployed to examine the response of single microbubbles to ultrasound exposure. Results show that as microbubbles decay they shed shell material and follow an irreversible trajectory through the resonance peak, leading to a well characterized evolution of oscillation amplitude. The amount of material removed from the shell is shown to be dependant upon the relative amount compression during each oscillation.

Perfluorocarbon-filled phospholipid coated microbubbles (Definity®, Lantheus Medical Imaging N. Billerica, MA, USA) were imaged using an ultra-high speed optical camera system. Microbubbles were isolated in solution at room temperature and allowed to float against the top surface of a 200 μm cellulose capillary tube (Spectrum Laboratories, Rancho Dominguez, CA, USA). Optical images from an upright microscope (BXFM, Olympus, Nederland BV, Zoeterwoude, The Netherlands), with a 60× magnification objective (LUMFPL, Olympus; NA = 1.00, water immersion) and a 4× magnifier (U-CA, Olympus) were captured by the Brandaris-128 camera as described previously in Ref. 17. Each recording contained 128 frames captured at $13 \times 10^6$ frames per second, allowing the radial oscillations from a six cycle 1.6 MHz pulse to be measured. To demonstrate the mechanism of quasi-stable lipid shedding, Fig. 1(a) shows eight non-consecutive frames from a recording of a microbubble during the compression phase of one cycle of a 300 kPa peak negative pressure pulse. The bubble can be seen to fragment in frame 4, and shedding of a lipid bubble is observed. Initial resting radius of the microbubble was $R_0 = 4.8 \, \mu$m, which was reduced to $R_0 = 3.5 \, \mu$m at the end of...

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the oscillation, and the microbubble was observed to remain spherical and stable after the insonation. This figure shows that microbubbles can lose a significant amount of shell material to produce a subsequently stable microbubble. However, as 300 kPa incident pulses are above the fragmentation threshold and oscillations are likely to be non-spherical. In order to characterise the effect of shedding on the oscillation amplitude, subsequent optical measurements are performed at acoustic pressures below the fragmentation threshold.

43 single definity microbubbles were insonated with consecutive pulses of six cycle 100 kPa peak negative pressure every 80 ms. Microbubbles were observed to reduce in radius by up to 15\% in response to a total of 64 insonations over a total of 2.55 s. Following each reduction in radius, it was observed that the subsequent bubbles remained spherical and optically resembled those of a similar size but previously not insonated. The reductions in radius occurred during the insonations, and no significant reduction in radius occurred between insonations. Figures 1(b)–1(d) show examples of optical observations of three individual lipid shelled microbubbles, which demonstrate the stages of microbubble deflation. Incident pulses can be seen to produce stable and spherical oscillations, allowing the component of the radial oscillation corresponding to the fundamental frequency of the pulse, $A_{\text{fund}}$, to be calculated at different time points of the microbubble’s deflation.\(^{18}\) Fig. 1 shows oscillations from microbubbles that are initially above resonance (b), at resonance (c), and below resonance (d), with resonance radius ($R_{\text{res}}$) defined as that which gives maximum amplitude of oscillation. The resonance radius of the first insonation agrees with previous investigations of similar lipid shelled microbubbles.\(^{19,20}\) The amplitudes of subsequent oscillations do not provide a significant departure from the resonance curve as calculated from the microbubble’s first pulse response, and shown in Fig. 1(e). The data from 43 microbubble’s first pulse responses have been fitted with a nonlinear least squares fit, and the 95\% confidence interval is shown in Fig. 1(e). The majority of subsequent responses are contained within this interval. Microbubbles larger than the resonant radius ($R_0 > 2.5 \mu m$) show an increase in amplitude of oscillation in response to subsequent oscillations and tend towards the mean resonance curve with each reduction in size. Microbubbles below the resonance peak ($1 \mu m < R_0 < 2.5 \mu m$) have decreasing amplitude of oscillation with each radius reduction. Microbubbles with $R_0 < 1 \mu m$ size show no observable oscillations above the resolution limit of the system.

A comparison between theory and experiment may help reveal the mechanism associated with the “quasi-stable” state of microbubbles resulting from exposure to consecutive ultrasound pulses. Under the influence of normal diffusion processes, a 10 \mu m uncoated air bubble would dissolve in 1.17 s in a degassed water solution.\(^{21}\) We have shown previously that the lipid shelled Definity\(^{\circ}\) microbubbles used here will take much longer to dissolve due to the less soluble gas and the encapsulating lipid shell.\(^{22}\) Thus changes in the scattered signal due to diffusion would be expected to be
The data shown in Fig. 2 show a significant correlation between the amount of compression lead to an increased likelihood that the bubbles will undergo shedding. Bubble signals are grouped by their relative expansion to compression (E/C) ratio, where relative expansion \( E = (D_{\text{max}} - D_0)/D_0 \), as previously defined by Ref. 23, into three categories: E/C \( \leq 0.5 \), 0.5 < E/C \( \leq 1 \), and E/C > 1. Although bubbles of all three categories are present above compression ratios of C > 0.4, the lack of expansion dominated signals (E/C > 1) with both small amount of compression and large amounts of deflation suggests that compression is the dominant parameter for reduction in area. This data suggests there is a compression threshold below which the lipid shell is able to resist monolayer collapse or else recover from small amounts of collapse without loss of material. This may be related to low amplitude oscillations containing inadequate mechanical force to eject material.

The microbubbles investigated here are floating against the wall of a tube, which has previously been shown to produce asymmetric oscillations. By comparing our settings with Vos et al., we can conclude that the microbubble oscillations here present small asymmetries, and are thus relevant to targeted microbubble applications that are attached to a cell membrane or other surface and exposed to similar ultrasound fields. The experiments here were carried out at room temperature, which is well below the range of temperatures (42–55 °C) at which a phase transition would be expected to take place. However, previous experiments have shown that lipid shelled microbubbles may undergo expansion at body temperature and show increased relative radial excursions upon insonation, both effecting their survival. In-vitro measurements made at room temperature, such as those presented here, therefore require careful interpretation with regard to behaviour in-vivo.

The data here present evidence that suggest that a mechanism of shell material shedding takes place at acoustic pressures below the microbubble fragmentation threshold, and that this mechanism can be reproducibly controlled. This mechanism may be studied further with the use of fluorescence imaging. This “quasi-stability” may be treated as a memory effect and help optimise signal processing algorithms to further improve the sensitivity of ultrasound contrast imaging. In addition, the controlled loss of shell material may be the subject of future drug delivery investigations. In particular where therapeutic materials are bound to the outside of the microbubble, this mechanism of shell degradation requires better understanding to ensure efficacy in delivery to a target site. The potential for formation of new encapsulated structures from shed lipid and the implications for drug uptake will also require further investigation.

FIG. 2. Calculated loss of area from 32 microbubble radial oscillations, which show radius reductions from 1% to 15%. Change in area \( \Delta A \) has been plotted against relative compression ratio \( C = (D_0 - D_{\text{min}})/D_0 \), with signals classified by their expansion to compression ratio (E/C), where E = (D_{\text{max}} - D_0)/D_0.

绝缘层的取向在1 ms的周期中使两种超声波的实验结果相似。类似于气体在声波辐射时的球体周期，在各个周期的响应一致，但观察到在球体周期的出现，在后续周期中持续至一个稳定的周期状态。它被提出在这一研究中这些观察结果都与脂质层相关。

脂质泡是由于声波的压缩而减小的，与相对的球体周期相关的信号时间尺度上已经观察到。单个10μm的气泡，当体积变化小于15%时，脂质泡层的密度保持相对恒定。脂质泡层已被观察到在减小时期后重新出现，因此提出了脂质层的半径。

脂质泡的减小在后续周期中是相关的。当减小的体积满足一定条件时，脂质泡层会失去其稳定性。脂质泡层的直接比例是脂质层的体积或者脂质层的面积密度，脂质层的面积密度在脂质层中大约是恒定的。

脂质泡层的减小在1 ms的周期中显著。脂质泡层的减小在脂质层中可能会直接与脂质层的体积成正比。脂质泡层的减小只有在脂质层能够抵抗单层破裂或恢复时才会发生。在脂质层中，脂质泡层的减小都与脂质层的体积变化有关。

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