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Investigation of polymer-shelled microbubble motions in acoustophoresis

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ABSTRACT

The objective of this paper is to explore the trajectory motion of microsize (typically smaller than a red blood cell) encapsulated polymer-shelled gas bubbles propelled by radiation force in an acoustic standing-wave field and to compare the corresponding movements of solid polymer microbeads. The experimental setup consists of a microfluidic chip coupled to a piezoelectric crystal (PZT) with a resonance frequency of about 2.8 MHz. The microfluidic channel consists of a rectangular chamber with a width, w, corresponding to one wavelength of the ultrasound standing wave. It creates one full wave ultrasound of a standing-wave pattern with two pressure nodes at $w/4$ and $3w/4$ and three antinodes at 0, $w/2$, and w. The peak-to-peak amplitude of the electrical potential over the PZT was varied between 1 and 10 V. The study is limited to no-flow condition. From Gor'kov's potential equation, the acoustic contrast factor, Φ, for the polymer-shelled microbubbles was calculated to about −60.7. Experimental results demonstrate that the polymer-shelled microbubbles are translated and accumulated at the pressure antinode planes. This trajectory motion of polymer-shelled microbubbles toward the pressure antinode plane is similar to what has been described for other acoustic contrast particles with a negative Φ. First, primary radiation forces dragged the polymer-shelled microbubbles into proximity with each other at the pressure antinode planes. Then, primary and secondary radiation forces caused them to quickly aggregate at different spots along the channel. The relocation time for polymer-shelled microbubbles was 40 times shorter than that for polymer microbeads, and in contrast to polymer microbeads, the polymer-shelled microbubbles were actuated even at driving voltages (proportional to radiation forces) as low as 1 V. In short, the polymer-shelled microbubbles demonstrate the behavior attributed to the negative acoustic contrast factor particles and thus can be trapped at the antinode plane and thereby separated from particles having a positive acoustic contrast factor, such as for example solid particles and cells. This phenomenon could be utilized in exploring future applications, such as bioassay, bioaffinity, and cell interaction studies *in vitro* in a well-controlled environment.

1. Introduction

In a current clinical practice a suspension of micro-size gas core bubbles is used as and efficient ultrasound contrast agent (UCA) [1]. As early as 1968 Gramiak and Shah [2] demonstrated the enhancement of the image contrast in echocardiographic study of aorta following the injection of the suspension of agitated saline solution containing free gas bubbles. In order to increase stability of the free gas bubbles, otherwise dissolving in a surrounding media in fractions of a second, the gas core were encapsulated within the solid shell. The solid encapsulating shell is typically made of albumin protein [3], phospholipids [4], or polymers [5]. Moreover, the outermost surface of the encapsulating shell can be further modified to accommodate ligands, pharmacological molecules or genes for molecular imaging and localized drug delivery [6–8]. In these applications the acoustic radiation force which propels and accumulates the

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free-circulating in blood stream bubbles on the target is of particular interest [9,10].

For more than a century, researchers have been exploring the effects of acoustic radiation force on particles suspended in liquid media and the particles' motion with the acoustic waves [11]. In the last two decades, several medical innovations have been based on this acoustic radiation-force phenomenon, including vibro-acoustography [12], shear wave elastography [13], acoustic radiation-force impulse imaging [14], magnetic resonance acoustic radiation-force imaging [15], the assessment of the viscoelastic properties of tissue [16], and the precise manipulation of cells or particles in a standing wave [17]. This study focuses on the application of acoustic radiation forces acting on microbubbles in standing-wave (stationary sound fields) acoustic fields. Blake [18] reported that millimeter-size gas bubbles (resonance frequency was well below the excitation frequency) suspended in a liquid were drawn to the pressure antinodes by the radiation force in a standing wave. The bubbles coalesced owing to the secondary radiation effect (also known as Bjerknes force) when they moved close to each other and merged into a larger bubble with a lower resonance frequency. Once the resonance frequency of a resulting bubble was comparable to the driving frequency, then it propelled away from the pressure antinode to the pressure node [19]. Apart from the frequency effect, acoustic pressure also plays significant roles in bubble motion. At a high enough acoustic pressure, even the driving frequency is well below the bubbles' resonance frequency, resulting in bubble translation and precision around the pressure node [20]. On the other hand, Kundt and Lehman demonstrated that when radiation force was exerted on solid Styrofoam chips, particles accumulated at the pressure nodes in acoustic standing-wave fields [21]. The motion of particles and their directions in a standing-wave acoustic field depend on several factors, such as driving frequency, size, density, and compressibility of the particle [22,23]. If the particle possesses greater compressibility and lower density than the surrounding liquid media, it tends to move toward the pressure antinode; otherwise, the particle moves toward the pressure node [22,23]. Note that this phenomenon is applicable only when the resonance frequency and size of the particle are well below the driving frequency and the wavelength of the incidental acoustic wave.

Based on particle responses upon the radiation force in acoustic standing-wave fields, the field of *acoustophoresis* (acoustic manipulation) has emerged. Acoustophoresis is an emerging clinical tool, especially in noncontact cell handling, useful for concentrating, sedimenting, sorting, and purifying [24–26]. Laurell et al. [27] successfully separated lipid particles from blood cells by using the acoustophoresis separation method. Analogously, the successful separation of platelets and serum from blood [28], the separation of lipid particles from milk [29], the separation of crude oil droplets from environmental water samples [30], and the separation of targeted cells with biofunctional elastomeric particles from nontargeted cells [31,32] have all been reported. Recently, tunable glass-shelled core (core: air, water, and steel) particles were also shown as negative acoustic contrast particles [33]. In that later study, the authors mentioned that the glass-shelled core-particle scenario can extend to UCAs but did not take this into account. Moreover, the authors did not mention the particular type of UCA that is, whether thin- or thick-shelled air-filled gas bubbles were used—nor did they report the type of shell material, such as lipids or polymers.

Thick- or thin-shelled microbubbles are defined based on the ratio between shell thicknesses and the total microbubble radius, where bubbles with a ratio below 5% are considered thin [34]. In the interest of studying sonoporation or cell lysis, Khanna et al. [35] introduced thin Albunex-shelled (human-protein-shelled) microbubbles (Optison®) and erythrocytes (red blood cells) simultaneously into ultrasound standing-wave fields at a frequency of about 1.5 MHz. The authors reported that in the presence of Optison®, the erythrocytes moved more vigorously and randomly in the acoustic field and released significant amounts of hemoglobin. However, the Optison® microbubbles also disappeared within the first frame—that is, within a few milliseconds. In the current study, we utilized thick-shelled polymer microbubbles with the ratio of shell thickness to microbubble radius above 5% [36]. Compared to the shell of lipid- or protein-shelled microbubbles [37,38], the thick encapsulated polymer shell offers increased mechanical stability resulting not only in shelf-life of several months but also in extended circulation time during in vivo tests. Thick shell also offers larger volume for incorporation of therapeutical gas [39] or pharmacological relevant molecules [39] to be delivered locally following ultrasound excitation.

The aim of the study was to investigate the movements of polymer-shelled air-core microbubbles induced by radiation force in acoustic standing-wave fields and to compare these with the corresponding movements of solid polymer beads, currently used as a blood mimicking phantom.

Prior to the experiments, we estimated the acoustic contrast factor, Φ, value of our polymer-shelled microbubbles suspended in water following the Gor'kov potential theory [22]. The sign Φ predicts the particle trajectory motion, which is extensively described in the theory section. Furthermore, the response of the polymer-shelled microbubbles at different driving voltages across the PZT is explored so as to identify the relation between acoustic pressure and the trajectory motion of the microbubbles. Our experimental results are compared with those presented in well-established studies of polymer microbeads [40,41]. Finally, the paper concludes with a thorough discussion of the fundamental physical principles behind the observed phenomena and notes potential applications for polymer-shelled microbubbles.

2. Theory

2.1. Radiation force

Suspended particles in a liquid experience both axial and transverse acoustic radiation forces when they are subjected to standing wave acoustic fields. The axial radiation force acts toward the direction of the wave propagation, which is responsible for a driving particle to either pressure node (velocity antinode) or pressure anti-node (velocity node). The transverse radiation force is acting perpendicular to the wave propagation, that is accountable for grouping the particles to clusters. The mathematical representation of the primary axial radiation force [22] is given in Eq. (1),

$$F^{rad} = -V\left[\frac{f_1}{2}\beta_s\nabla\langle p_1^2\rangle - \frac{3f_2}{4}\rho_s\nabla\langle v_1^2\rangle\right], \tag{1}$$

where V is the volume of the particle; $f_1 = 1 - \frac{\beta_p}{\beta_s}$ and $f_2 = \frac{2(\rho_p-\rho_s)}{2\rho_p+\rho_s}$ are the monopole and dipole scattering coefficients; ρ_p and ρ_s and β_p and β_s are the densities and compressibility of the particle and the surrounding media, respectively; p_1 is the incidental pressure; and v_1 is the velocity of the particle. In 2-D acoustophoresis, microparticles move in a horizontal direction, $x(t)$, and in a transversal direction, $y(t)$. The acoustic pressure field in transverse motion is $p_1 = p_a\cos(ky)$, and when this is substituted in Eq. (1), the radiation force acting on the transverse field becomes [42]

$$F_y^{rad} = \frac{4\pi}{3}k_y a^3 E_{ac}\Phi(\beta,\rho)\sin(2k_y y), \tag{2}$$

where $k(=2\pi/\lambda)$ is wavenumber; λ is the wavelength equal to channel width, w; a is the radius of the single spherical particle; E_{ac} is the