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● *Original Contribution*

SECONDARY BJERKNES FORCES DEFORM TARGETED MICROBUBBLES

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Abstract—In this study, we investigated the effect of secondary Bjerknes forces on targeted microbubbles using high-speed optical imaging. We observed that targeted microbubbles attached to an underlying surface and subject to secondary Bjerknes forces deform in the direction of their neighboring bubble, thereby tending toward a prolate shape. The deformation induces an elastic restoring force, causing the bubbles to recoil back to their equilibrium position; typically within 100 μs after low-intensity ultrasound application. The temporal dynamics of the recoil was modeled as a simple mass-spring system, from which a value for the effective spring constant k of the order 10^{-3} Nm^{-1} was obtained. Moreover, the translational dynamics of interacting targeted microbubbles was predicted by a hydrodynamic point particle model, including a value of the spring stiffness k of the very same order as derived experimentally from the recoiling curves. For higher acoustic pressures, secondary Bjerknes forces rupture the molecular adhesion of the bubbles to the surface. We used this mutual attraction to quantify the binding force between a single biotinylated microbubble and an avidin-coated surface, which was found to be between 0.9 and 2 nanonewtons (nN). The observation of patches of lipids left at the initial binding site suggests that lipid anchors are pulled out of the microbubble shell, rather than biotin molecules unbinding from avidin. Understanding the effect of ultrasound application on targeted microbubbles is crucial for further advances in the realm of molecular imaging. (E-mail: t.kokhuis@erasmusmc.nl) © **2013 World Federation for Ultrasound in Medicine & Biology.**

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INTRODUCTION

Microbubbles are the most popular ultrasound contrast agent (UCA) used clinically for diagnostic ultrasound imaging. UCA microbubbles are typically 1–10 μm in size and consist of a gas core stabilized by a lipid, protein or polymer shell, which prevents coalescence with other bubbles and reduces dissolution. The microbubbles are contained in the circulatory system following intravenous administration until they are cleared by the reticuloendothelial system (Straub et al. 2007). Because of the compressibility of the gas core inside, the microbubbles undergo volumetric oscillations during ultrasound application, giving them superior echogenicity compared with the surrounding tissue and fluid. The higher echogenicity results in a better contrast-to-tissue ratio and is used in contrast-enhanced ultrasound imaging for enhanced tissue delineation, for perfusion studies or for left ventricle opacification (Dijkmans et al. 2004). Moreover, microbubbles have been shown to behave as non-linear ultrasound scatterers, causing their backscattered echo to contain higher harmonics (Burns et al. 1992; de Jong et al. 1994) or even subharmonics (Lotsberg et al. 1996; Sijl et al. 2010) of the driving frequency. The non-linear characteristics of

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microbubbles are exploited in various imaging modalities, such as amplitude modulation (Mor-Avi et al. 2001) and pulse inversion (Simpson et al. 1999) to improve the contrast-to-tissue ratio.

In the mid 1990s, fabrication of the first so-called targeted microbubbles was reported, where ligands to specific molecular markers were added to the shell (Fritzsch et al. 1994; Klibanov et al. 1997). Ligands can be selected to make targeted microbubbles adhere to regions of the vascular endothelium expressing specific proteins, such as inflammatory markers. Imaging methods involving high-power destructive pulses can be used to discriminate between echoes originating from targeted and freely circulating bubbles (Willmann et al. 2008). More recently, it was shown that targeted microbubbles exhibit a pronounced shift (*i.e.*, a 50% decrease) in their frequency of maximum response compared with free bubbles (Overvelde et al. 2011). Although these experiments were performed in well-controlled model systems, their outcome suggests that acoustic discrimination between targeted and freely flowing bubbles is feasible.

The selective imaging of targeted microbubbles, in combination with their capability to recognize molecular events, facilitates targeted contrast enhancement during ultrasound application, also called *molecular ultrasound* (Deshpande et al. 2010). Molecular ultrasound has great potential to diagnose diseases in an earlier stage, such as in asymptomatic patients, and to assess treatment efficacy of drugs even before morphologic changes occur (Pysz et al. 2010). Recently, a new milestone was achieved when the first study of targeted microbubble imaging in humans was performed (Wijkstra et al. 2012). The Vascular Endothelial Growth Factor Receptor 2 (VEGF-R2) targeted microbubble BR55 (Bracco Research, Geneva, Switzerland) was shown to bind to VEGF-R2 receptors in the prostate of patients scheduled for prostatectomy, making molecular ultrasound potentially the first diagnostic imaging technique for prostate cancer detection and localization in the near future.

For molecular ultrasound to evolve to a robust diagnostic tool, more fundamental knowledge about the effects of ultrasound application on the behavior of targeted microbubbles is needed. This need is emphasized by the observations of Schmidt et al. (2008), who observed detachment and clustering of biotinylated microbubbles targeted to a avidin-coated surface during low intensity ultrasound application. Microbubble detachment reduces the amount of targeted microbubbles at the site of interest, decreasing the echo intensity and therefore complicating the interpretation of the signal. Clustering of microbubbles may change the echogenicity (Dayton et al. 1999; Doinikov et al. 2009). A correct interpretation of the molecular ultrasound signal therefore demands a thorough understanding of the interaction between ultrasound and targeted microbubbles.

The effects observed by (Schmidt et al. 2008) were ascribed to a mutual interaction between the oscillating microbubbles known as *secondary acoustic radiation force*. Because the direction of the pressure gradient (∇P) associated with a sound field emitted by a neighboring bubble oscillates in time, the secondary acoustic radiation force exerted on a bubble has alternating direction in time. However, as the volume (V) of the bubble also oscillates in time, the average of the instantaneous force over one period results in a net force, whose direction depends on the phase difference between the bubble oscillations and the oscillating pressure gradient (Leighton, 1994). This averaged net force is called *secondary Bjerknes force* (Bjerknes, 1906).

More recently, it was observed that targeted microbubbles, which had moved several hundred nanometers under the influence of attractive secondary Bjerknes forces, had moved back to their initial position by the start of a next experiment 80 ms after the ultrasound was turned off (Garbin et al. 2011). However, microbubbles that were in contact with (but not adherent to) the surface were reported to equilibrate at a new position, closer to each other. It was therefore hypothesized that the presence of an elastic restoring force brings the targeted microbubbles back to their equilibrium position after the ultrasound is turned off. The physical mechanism of this restoring force remained elusive: the extension associated with the stretching of molecular bonds is a few orders of magnitude smaller than the observed bubble translations. Moreover, bubbles remained spherical throughout the experiments. However, the authors did not totally rule out that bubble deformation might be involved because the induced deformation could have been below the optical resolution in top view. Furthermore, the formation of elastic wrinkles and folds of excess lipid material (Rychak et al. 2006), similar to what has been observed in neutrophil rolling in shear flow (Park et al. 2002), was proposed as a second possible mechanism of the restoring force.

In this study, we therefore investigated the phenomena associated with the translational dynamics of mutually interacting targeted microbubbles in more detail. We first repeated the experiments as performed by Garbin et al. (2011) for the different microbubbles and experimental configuration used in this study. Next, we investigated the time scale of the microbubble recoil after the ultrasound was turned off. To elucidate the mechanism of the elastic restoring force, simultaneous top and side-view high-speed imaging (Vos et al. 2011) of interacting targeted microbubbles was performed. The article concludes with a comparison between the experimental observations and theoretical predictions of the translational dynamics of interacting targeted bubbles