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● *Original Contribution*

CHARACTERIZING THE SUBHARMONIC RESPONSE OF PHOSPHOLIPID-COATED MICROBUBBLES FOR CAROTID IMAGING

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Abstract—The subharmonic vibration of BR14 (Bracco Research S.A., Geneva, Switzerland) contrast agent microbubbles is investigated within the preferable frequency range for carotid ultrasound imaging (8–12 MHz). The response of the bubbles was recorded optically with an ultra-fast recording camera (Brandaris 128) at three acoustic pressures (50, 100 and 120 kPa). The vibration of the microbubbles was measured as a function of the excitation frequency and its frequency content was determined. Among 390 recordings, 40% showed subharmonic oscillations. It was observed that for smaller microbubbles (diameter $< 3\ \mu$m) the frequency of the maximum subharmonic response increases for increasing pressures (shell hardening) opposite to what has been reported for larger microbubbles (3 μm $<$ diameter $<$ 15 μm). These findings are well predicted by the model proposed by Marmottant et al. (2005) after including the dilatational shell viscosity of the microbubbles measured by Van der Meer et al. (2007), which indicates a marked shear-thinning behavior of the phospholipid shell. (E-mail: t.faez@erasmusmc.nl) © 2011 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Subharmonics, High frequency ultrasound, Ultrasound contrast agent, Microbubbles.**

INTRODUCTION

Clinical background

Atherosclerosis, a chronic, inflammatory disease involving the development of atherosclerotic lesions in the major arteries of the vasculature, is the uppermost underlying cause of cardiovascular disease (Yach et al. 2004) and a major cause of stroke (Golledge et al. 2000). Cardiac events and stroke are often caused by atherosclerotic plaque rupture. Plaques prone to rupture are called "vulnerable" (Virmani et al. 2000; Schaar et al. 2004). It has been shown that *vasa vasorum* plays an important role in atherosclerotic plaque pathogenesis and stability (Zamir et al. 1985; Barger et al. 1984). *Vasa vasorum* consists of a branching network of microvessels feeding the artery wall with nutrients and oxygen (Ritman et al. 2007; Gössl et al. 2003). Proliferation of the *vasa vasorum* into the plaque is suspected to lead to plaque growth and internal hemorrhage (Mause et al. 2009; Sluimer et al. 2009).

Ultrasound is an established tool to measure carotid atherosclerosis for the diagnosis and monitoring of patients at risk of stroke (Eliasziw et al. 1995; Baldassarre et al. 2000). The carotid artery is a relatively superficial artery that is easily accessible for ultrasound imaging. Recent advances in contrast-enhanced ultrasound have shown that this technique can characterize the carotid *vasa vasorum* and intra-plaque angiogenesis and, thus, it is potentially a new diagnostic tool to detect plaque vulnerability (Feinstein 2006; Shah et al. 2007; Vicenzini et al. 2007; Coli et al. 2008; Staub et al. 2009; Xiong et al. 2009; Shalhoub et al. 2010).

Contrast-enhanced ultrasound utilizes an ultrasound contrast agent containing gas-filled microbubbles. Due to their high compressibility, the microbubbles have a very high echogenicity in comparison with surrounding tissue (expressed as the contrast to tissue ratio, CTR). In the early days, only the linear vibration of the bubbles was utilized. The CTR can be further improved by exploiting the nonlinear properties of the contrast bubbles *e.g.*, in harmonic imaging: pulse inversion (Burns et al. 2000)

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and power modulation (Brock-Fisher et al. 1996). However, for the transmit pressures used in these methods (mechanical index (MI): 0.2–1.3), the CTR is lowered as a result of nonlinear propagation of the ultrasound wave through the tissue, which causes the tissue scattered signal to also contain energy at the second harmonic. Propagating ultrasound waves, however, do not contain energy at the subharmonic frequency, which revives a strong interest in subharmonic emissions (backscattered energy at half the transmit frequency) from contrast agents (Shi et al. 1999; Frinking et al. 2001; Chomas et al. 2002). Subharmonic imaging has potentially a larger CTR compared with other imaging methods (Shankar et al. 1998). In recent studies, the subharmonic response of Definity (Lantheus Medical Imaging, North Billerica, MA, USA) has been used to detect microvascularity in mice using an ultrasound biomicroscopy (UBM) system (Goertz et al. 2005) and in rabbit aortas using an intravascular ultrasound scanning system (IVUS) (Goertz et al. 2007; Needles et al. 2010).

Physical background

Several experimental (de Santis et al. 1967; Neppiras 1969) and theoretical (Eller 1969; Prosperetti 1975; Lauterborn 1976) studies have been performed to investigate and to describe the subharmonic resonance of bubbles. This nonlinear effect originates from a parametric instability in the equations describing the bubble dynamics (Plesset 1949). Prosperetti (1974) showed analytically that subharmonic oscillations occur for the amplitude of acoustic pressure above a certain threshold value. This threshold value was found to be minimal when the bubble oscillates at a frequency of twice its resonance frequency. It was also shown that damping increases the threshold pressure for the occurrence of subharmonic for free gas bubbles (Eller 1969; Prosperetti 1974, 1977).

Ultrasound contrast bubbles are coated to prolong their *in vivo* lifetime. The coating not only prevents a quick dissolution of the bubble gas but also has an increased shell viscous damping effect on the bubble oscillations in an ultrasound field. Since the viscoelastic shell of contrast agent microbubbles is known to increase the damping considerably (de Jong 1993; Van der Meer et al. 2007), it is expected that the subharmonic threshold pressure increases. This hypothesis was supported by Shankar et al. (1999). In that study, a purely viscoelastic shell model (de Jong et al. 1993; Church 1995; Hoff et al. 2000) was used to analyze the subharmonic behavior of coated bubbles. They confirmed the increase of the threshold pressure for the generation of the subharmonic by an increased damping. However, experimental studies on both albumin-coated (Optison, GE Healthcare, Chalfont St Giles, UK, and Albunex, Molecular Biosystems, San Diego, CA, USA) and phospholipid-coated (SonoVue, Bracco, Milan, Italy) contrast agents have shown that the threshold pressure to generate a subharmonic echo is lower than what was found for the uncoated bubbles (Chang et al. 1995; Lotsberg et al. 1996; Shankar et al. 1998, 1999; Krishna et al. 1999; Biagi et al. 2007; Frinking et al. 2010).

Recently, Frinking et al. (2009) and Sijl et al. (2010) have shown that the coating of lipid bubbles decreases the subharmonic threshold pressure. During buckling of the lipid coated shell, the bubbles show a highly nonlinear behavior at low acoustic pressures, such as asymmetric oscillation amplitudes whereby the compression amplitude outweighs expansion. This type of behavior is called "compression-only" behavior (de Jong et al. 2007). Marmottant et al. (2005) explained that this behavior is caused by a variable surface tension of lipid coated bubbles. Sijl et al. (2010) show that the initiation of subharmonic in phospholipid-coated microbubbles at lower threshold pressure can be explained with the Marmottant et al. (2005) model. They employ a weak nonlinear analysis of the Rayleigh-Plesset equation and, instead of using a pure elastic model, they describe the shell elasticity of coated bubble as an effective surface tension that varies strongly with the amplitude of oscillation, as proposed by Marmottant et al. (2005). Sijl et al. showed that this mechanism is responsible for the generation of subharmonic oscillations at low acoustic pressures. They report subharmonic threshold values down to 5 kPa for individual lipid-coated BR14 bubbles at driving frequencies between 1 and 4 MHz.

At present, studies on microbubbles subharmonic response have been performed at frequencies relevant for precordial imaging (1–4 MHz). However, the preferred ultrasound frequency for carotid imaging is between 5 and 15 MHz, involving microbubbles that are substantially smaller and may have different behavior. It is, however, unclear at what driving frequency the subharmonic response is maximal and how the response changes with varying driving pressure. The aim of this study is to investigate the dependence of the subharmonic response of a phospholipid-coated microbubble to the acoustic pressures applied at frequencies near 10 MHz.

For uncoated bubbles, it is well known that the fundamental resonance curve becomes asymmetrical and that the frequency of maximum response decreases with increasing acoustic pressure (Prosperetti 1975; Lauterborn 1976). Van der Meer et al. (2007) studied experimentally the fundamental resonance curve of phospholipid-coated microbubbles at constant acoustic pressure of 40 kPa and found an increase of the resonance frequency with respect to uncoated bubbles as a result of increased stiffness of the system. Later, Overvelde et al. (2010) investigated the fundamental resonance curves of the same microbubbles as a function of the acoustic