20 Years of Ultrasound Contrast Agent Modeling

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Abstract—The merits of ultrasound contrast agents (UCAs) were already known in the 1960s. It was, however, not until the 1990s that UCAs were clinically approved and marketed. In these years, it was realized that the UCAs are not just efficient ultrasound scatterers, but that their main constituent, the coated gas microbubble, acts as a nonlinear resonator and, as such, is capable of generating harmonic energy. Subharmonic, ultraharmonic, and higher harmonic frequencies of the transmitted ultrasound frequency have been reported. This opened up new prospects for their use and several detection strategies have been developed to exploit this harmonic energy to discriminate the contrast bubbles from surrounding tissue. This insight created a need for tools to study coated bubble behavior in an ultrasound field and the first models were developed. Since then, 20 years have elapsed, in which a broad range of UCAs and UCA models have been developed. Although the models have helped in understanding the responses of coated bubbles, the influence of the coating has not been fully elucidated to date and UCA models are still being improved. The aim of this review paper is to offer an overview in these developments and indicate future directions for research.

I. INTRODUCTION

IN 1968, it was reported that the injection of agitated saline in the aortic root resulted in "a cloud of echoes between the undulating margins of the aortic root" [1]. It appeared that gas mini bubbles in the agitated saline acted as great contrast enhancers. Normally, blood is a poor ultrasound scatterer and it remains dark in an echo image. The addition of gas bubbles to the blood pool by Gramiak and Shah [1] greatly increased the backscattered ultrasound and resulted in an enhanced contrast between the aortic root wall and the blood. Agitated saline is still used for the detection of right-to-left shunts in the heart [2].

Before gas bubbles could be widely applied as UCAs, some improvements were necessary. Bubbles produced by agitation are both large and unstable. A gas bubble is unstable because of the surface tension between the gas core and the surrounding liquid, which forces the bubble to decrease in size. The rate can be calculated using the

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equation by Epstein and Plesset [3] and the parameter values given by Chen *et al.* [4] that an air bubble with a diameter of 5 μ m in air-saturated water disappears in approximately 125 ms and an air bubble with a diameter of 3 μ m disappears in approximately 32 ms at room temperature and ambient pressure.

Moreover, the gas bubbles are effectively removed by the lungs. Unless administered by intracoronary or aortic root injection, the bubbles are unable to traverse the pulmonary circulation to opacify the left cardiac chambers. It takes at least 12 s for a contrast agent to pass from a peripheral vein (i.e., the site of injection) to the end-organ [5]. Thus, to be useful in an echography study, the bubbles should persist in solution for several minutes and have a size of less than 10 μ m in diameter to be able to enter into the systemic circulation after an intravenous injection.

A. Coated Microbubbles

It was found empirically that a small admixture of the patient's blood to the saline improves the stability and effectiveness of the agitated saline as a contrast agent [6]. Surfactants from the blood form a coating around the gas core and promote the lifetime of the microbubble by greatly reducing the surface tension at the interface. Although this was known for many years, it was not before the end of 1980s that sufficiently stable microbubbles were marketed. In 1994, the first commercially available contrast agent approved for human use in the United States, Albunex (Molecular Biosystems, San Diego, CA), was available for sale. Albunex has a coating made of human serum albumin. The albumin coating forms an elastic solid shell around the gas core and is relatively stiff. It enhances the bubble's stability by supporting a strain to counter the effect of the surface tension, which is different than the now more commonly used lipid coatings, which act as surfactants.

The second commercial contrast agent, Levovist, was available soon after Albunex in Europe and Japan in 1996. Levovist (Bayer Schering Pharma AG, Berlin, Germany) consists of galactose microcrystals whose surfaces provide absorption sites on which air bubbles form when suspended in water. A trace amount of palmitic acid further stabilizes Levovist microbubbles. Since 1997, contrast agents have been further stabilized by replacing the air core with high-molecular-weight inert gases such as perfluorocarbons, which have a lower solubility and diffusivity in aqueous liquids compared with air [5]. The perfluorocarbons are exhaled after several passes through the lungs. Ex-

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Fig. 1. Estimated sales figures and availability of ultrasound contrast agents in 2010.

amples of this kind of agent are Optison (GE Healthcare, Chalfont St Giles, UK), which contains octafluoropropane and an albumin shell, and SonoVue (Bracco, Milan, Italy), which has a sulfur hexafluoride core and a phospholipid coating. In SonoVue, the bubbles are not only efficient scatterers because of the stable gas core [7], but also because the relative flexibility of the lipid coating allows large bubble vibrations at low acoustic pressures without immediate bubble destruction [8], [9]. Currently clinically available contrast agents are summarized in Table I and Fig. 1.

B. Function of a Contrast Bubble

The contrast agent microbubbles owe their functionality to their gas core, which is highly compressible in comparison with the surrounding liquid and tissue. This results in a high backscattering of the ultrasound wave. Moreover, the microbubbles can act as resonant systems with resonant frequencies within the same range as medical ultrasound frequencies. A resonant microbubble has a strong increase in scattering cross-section compared with nonresonant microbubbles, e.g., a free gas microbubble has a resonant scattering cross-section that is of an order of a thousand times larger than its geometrical crosssection [10].

For a long time, the backscattered ultrasound intensity from the microbubbles was considered to have small amplitudes, meaning linear oscillations only [11]. In the search for methods to detect emboli in the blood circulation as a result of decompression sickness, researchers had already investigated nonlinear bubble oscillations [12], but it was only realized in the mid-nineties that contrast agent microbubbles also produce harmonic energy, which can be used for imaging [13]–[15].

Name	Manufacturer	Year	Gas	Coating	Approved	Available
Echovist	Bayer Schering Pharma AG	1991	air	galactose	EU, Japan, Canada	_
Albunex	Molecular Biosystems	1994	air	human albumin	EU, USA, Canada	_
Levovist	Bayer Schering Pharma AG	1996	air	galactose, trace palmitin	Worldwide ¹	2
Optison	GE Healthcare AS	1997	C_3F_8	human albumin	EU, USA	EU, USA^3
Definity	Lantheus Medical Imaging	2001	C_3F_8	phospholipids	$Worldwide^4$	Worldwide
SonoVue	Bracco SpA	2001	SF_6	phospholipids	Europe, China, S Korea,	Europe, China, S
					India, Hong Kong, Singapore	Korea, India, Hong Kong, Singapore
Imagent	Alliance Pharmaceutical Corp.	2002	C_6F_{14}	phospholipids	USA	_
Sonazoid	Amersham Health	2006	C_4F_{10}	phospholipids	Japan	Japan
BR38 ⁵	Bracco SpA		$\mathrm{C_4F_{10}/N_2}$	phospholipids		

TABLE I. COMMERCIALLY AVAILABLE ULTRASOUND CONTRAST AGENTS.

¹Approved in 65 countries, but not in the United States.

 $^2\mathrm{Expected}$ to finish in 2010.

³Temporarily unavailable 2006–2010.

⁴Approved in United States, Canada, Mexico, Israel, Europe, India, Australia, Koria, Singapore, UAE, and New Zealand. ⁵In clinical development. The backscattered harmonic energy from the microbubbles may contain higher harmonic, subharmonic, or even ultraharmonic energies [15]–[17], whereas backscattered energy from tissue contains less or no harmonics. Therefore, harmonics can be used to differentiate the microbubbles from the tissue and further enhance the contrast between bubbles (in the blood vessels) and the surrounding tissue in an echo image. A few examples of imaging techniques that are based on the nonlinear response of the microbubbles are second harmonic imaging [18], pulse inversion imaging [19], power modulation imaging [20], and combinations of these imaging techniques. These techniques have now been implemented in commercial ultrasound systems and are widely used in the clinic.

The vast majority of contrast examinations worldwide are used for endocardial border delineation [21]. In addition, contrast enhanced ultrasound aids visualization of perfusion defects in the myocardium [22], [23] and it increases the intensity of Doppler signals for the detection of blood flow. Radiology applications focus on cancer and peripheral vascular disease, for which the estimation of microvascular density and flow rate is particularly important [24].

C. Molecular Imaging and Therapeutic Applications

Apart from the diagnostic application of coated microbubbles, in the past years, there has been great interest on the molecular imaging and therapeutic application of these bubbles [25]–[28]. The first therapeutic applications were based on fast microbubble collapses, which generated strong flows that were applied to induce cell damage, vascular injury [29], and the lysis of thrombus [30]. Oscillating microbubbles can also be used to locally trigger a transient increase in endothelial cell membrane permeability or opening of tight junctions between endothelial cells to allow delivery of therapeutics such as drugs or genes that normally cannot enter these cells or the underlying tissue [31]–[35]. In addition, microbubbles can act as a drug delivery system and carry therapeutics to the affected location in the human body. Therapeutics can be attached to or incorporated into microbubbles and ultrasound can then be used to locally trigger their release [26], [36]. Although the drugs may enter the endothelial cells or the underlying tissue, the microbubbles themselves will stay in the blood vessel.

Molecular imaging is a new discipline that unites molecular changes associated with diseases and *in vivo* imaging. For this purpose, microbubbles are composed such that they carry ligands on their surface [37], [38]. These ligands bind to specific sites on the vessel wall, such as receptors that are upregulated on endothelial cells within tumors or atherosclerotic plaques. The microbubbles indicate these sites by reflecting the ultrasound wave. The ligands are connected to the shell via a lipid, polymer or protein anchor. The strongest effective association and also the most widely used non-covalent specific interaction in biotechnology is (strept)avidin binding to biotin. Biotin is easily anchored to the microbubble shell components and there is a wide range of biotinylated ligands available. Subsequently, (strept)avidin acts as a bridge between the biotinylated bubble and the biotinylated ligand. This procedure has resulted in a large variety of available targeted bubbles. The disadvantage of this method is, however, that streptavidin is a foreign protein for the human body and that these agents will not be applicable in a clinical setting [38]. Covalent coupling methods to attach ligands to the microbubble shell are available for human use [39].

MicroMarker (Bracco and VisualSonics, Toronto, Canada) is a hybrid form with a covalent bond between streptavidin and a lipid, and a non-covalent bond between streptavidin and the antibody. Visistar Integrin (Targeson, Inc., San Diego, CA, USA) is the first commercially available covalent targeted bubble. It is intended for preclinical use and consists of a standard lipid-perfluorocarbon microbubble with a peptide ligand bound to PEGlipid. The principle target for this bubble is $\alpha_{\rm v}\beta_3$ integrin, expressed in angiogenesis. BR55 (Bracco, Geneva, Switzerland) is the first targeted contrast agent tested in the clinic [39]. This agent is functionalized with a heterodimer peptide targeting the vascular endothelial growth factor receptor 2 (VEGFR2) [40], which is upregulated in tumor vessels. The group of Lindner (Oregon Health & Science University, Portland, OR) will develop their lipid-coated decafluorobutane bubble (YSPSL-MB) for commercial use [41], [42]. This agent bears a recombinant dimeric human PSGL-1 binding moiety on its surface. It will bind to both P- and E-selectin to monitor ischemic injury over time, as well as possible transplant rejection.

A special case of a targeted agent is Sonazoid. It is developed as a UCA to detect blood perfusion, but it can also be used as a targeted bubble. The phosphatidylserine in the coating functions as a marker of apoptosis on cell membranes. Macrophages and Kupffer cells will phagocytose cells expressing this marker. Hence, Sonazoid is taken up by the macrophages and Kupffer cells and can be used to detect foci that lack active phagocytic capability, such as tumor nodes in the liver [43]. Table II presents a summary of available UCAs for molecular imaging.

D. Outline Bubble Models

Over the past few years, a handful of reviews have been published with the aim of summarizing the physical principles and engineering of contrast agent microbubbles as well as their progressing applications in imaging and therapy [23], [44]–[46]. Recently, Doinikov and Bouakaz have presented an exhaustive study on the existing contrast agent models. They compared the principle behind the derivation of each model and, specifically, the different formulations introduced for the shell parameters of the microbubbles [47].

The purpose of this review is to give an overview of contrast agent modeling developments over the past 20 years, based on their applicability to the various developed contrast agents. For more insight into the mathematical

TABLE II.	Commercially	AVAILABLE	TARGETED	Contrast	Agents.
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Name	Manufacturer	Linker	Ligand	Target	Possible application
Sonazoid	Amersham Health	NA ¹	NA ¹	Kupffer cells and macrophages	Liver tumors, ischemia/ reperfusion injury
Micromarker	Bracco SpA	streptavidin	Biotinylated ligand of choice	biomarker of choice	depends on ligand
Targestar	Targeson Inc.	streptavidin	Biotinylated ligand of choice	biomarker of choice	depends on ligand
Visistar Integrin	Targeson Inc.	covalent to PEG-lipid	cyclic RGD peptide	$\alpha_{\nu}\beta_3$ integrin	angiogenesis
Visistar VEGFR2 ²	Targeson Inc.	covalent to PEG-lipid		VEGF-like protein	angiogenesis
YSPSL-MB ²	Oregon Health & Science University	covalent to PEG-lipid	PSGL-1	P and E selectin	ischemia/reperfusion injury transplant rejection
$BR55^3$	Bracco SpA	covalent to PEG-lipid	Heterodimer peptide	VEGFR2	angiogenesis
Selectin agent ²	Bracco SpA	streptavidin	biotinilated PSGL- 1 analog	P and E selectin	Inflammatory disease

¹Passive targeting, phagocytic uptake of the bubbles by cells.

²In development.

³In clinical development.

derivation of each model, the readers are referred to the complimentary work in [47]. The contrast agent models discussed in this paper are based on the Rayleigh–Plesset equation, which describes the motion of a gas bubble in a pressure field. We present the Rayleigh-Plesset equation in a fundamental form and briefly explain the role of damping in this equation. Furthermore, we describe how, in the limit of small-amplitude oscillations, the Rayleigh-Plesset equation can be linearized to a form equivalent to that of a forced linear oscillator, which is often used to map out the parameter space from analytical modeling. The next step in the derivation of a contrast agent model is the implementation of the bubble coating. Although theory concerning the uncoated bubble is considered to be known, the influence of the coating is not fully understood yet. Over the last 20 years, different models have been deduced and tested. These developments are driven by both the introduction of new UCAs and insight in bubble behavior; see also the timeline in Fig. 2. To show the steps in the development of the coated bubble models, we arranged them according to the coating material and oscillation amplitude of the bubble.

II. FREE GAS BUBBLE MODEL

A. The Rayleigh–Plesset Equation

Lord Rayleigh pioneered research on the motion of bubbles by studying inertial cavitation, nearly 60 years after the earliest studies done by Besant on the collapse and growth of a spherical cavity within a continuous liquid medium [48]. Inertial cavitation intrigued many when it became apparent that during the collapse of a gas cavity significant amounts of energy are released, high enough to seriously damage screw-propellers of ships. In 1917, Rayleigh considered the collapse of an empty cavity in a liquid [49]. This cavity remained spherical at all times and was located in an incompressible liquid.

The main step from cavitation toward bubble dynamics was the introduction of a variable external driving pres-



Fig. 2. Timeline displaying contrast agent modeling developments. (top) Approval date for clinical use of contrast agents. (bottom) Publication date of model indicated by first author.

sure and the influence of surface tension by Plesset [50]. Plesset described the dynamics of vapor-filled bubbles and Noltingk and Neppiras [51] did the same for gas-filled bubbles. The effect of viscosity on the equation of motion of a bubble in an incompressible liquid was considered by Poritsky. These contributions led together to the Rayleigh–Plesset–Noltingk–Neppiras–Poritsky (RPNNP) equation [52]. Starting from the Navier–Stokes equations assuming liquid incompressibility and including viscous effects in the boundary conditions, we arrive at this equation, which is now known as the Rayleigh–Plesset (RP) equation [53]:

$$\rho_l \left(R\ddot{R} + \frac{3}{2}\dot{R}^2 \right) = \left(p_0 + \frac{2\sigma}{R_0} - p_v \right) \left(\frac{R_0}{R} \right)^{3\kappa} + p_v - \frac{2\sigma}{R} - \frac{4\mu\dot{R}}{R} - p_0 - P(t).$$
(1)

B. The Modified Rayleigh–Plesset Equations

In the first coated-bubble models, the liquid surrounding the bubble was considered incompressible and the standard RP equation (1) was applied. More recent models include liquid compressibility and they are based on modified versions of the RP equation. In an incompressible liquid, the speed of sound would be infinite. When the bubble wall has a speed much lower than the speed of sound $\dot{R} \ll c$, this is a valid assumption. Therefore, for large bubble oscillations $(R_{\text{max}}/R_0 > 2, \dot{R} > 0.01 \cdot c)$, an extended version of the RP equation should be used, such as formulations from the Herring–Keller/Keller–Miksis or Gilmore–Akulichev families [54]. These equations incorporate radiation damping. For low acoustic pressures, it is not necessary to include radiation damping to describe the motion of the bubble accurately, but for higher acoustic pressures, bubble vibrations with larger amplitudes are expected and a more robust and accurate equation may serve as a basis for the coated bubble model, such as the following modified version of the RP equation, which is popular in the field of sonoluminescence [55]:

$$\rho_l \left(R\ddot{R} + \frac{3}{2}\dot{R}^2 \right) = \left(p_0 + \frac{2\sigma}{R} \right) \left(\frac{R_0}{R} \right)^{3\kappa} \left(1 - \frac{3\kappa}{c}\dot{R} \right)$$

$$- \frac{2\sigma}{R} - \frac{4\mu_l \dot{R}}{R} - p_0 - P(t).$$
(2)

C. Harmonic Oscillator

Assuming that the bubble oscillates in the small-amplitude limit, it is possible to linearize the RP equation using $R = R_0(1 + x(t))$ and arrive to the equation for a forced harmonic oscillator [53], [56]:

$$\ddot{x} + \omega_0 \delta \dot{x} + \omega_0^2 x = F(t), \tag{3}$$

where x is the relative radial excursion, $F(t) = F_0 \sin(\omega t)$ is the acoustic forcing term, $\omega_0 = 2\pi f_0$, δ is the dimensionless damping coefficient, and f_0 is the resonance frequency.

D. Damping in the Equation of Motion

Damping reduces the amplitude of oscillations of the bubble. For an uncoated gas bubble, energy losses by damping occur through three mechanisms [53]:

- Radiation damping: Energy is radiated away from the bubble as acoustic waves.
- Viscous damping: Work is done against viscous forces.
- Thermal damping: Energy is lost through thermal conduction between the gas and the surrounding liquid.

Viscous damping is independent of the insonation frequency in a Newtonian fluid. It is the dominant source of damping for microbubbles with a resting diameter of 2 μ m, but its influence diminishes as the size of the bubble increases. Typically, radiation damping is neglected at low frequencies, whereas thermal damping is neglected at high frequencies. The physical background of the transition from low to high frequency is related to resonance and greatly depends on the bubble size. It is in the order of 1 MHz for bubbles with a resting diameter of 10 μ m and 10 MHz for bubbles of 1 μ m diameter [53].

Thermal damping is often neglected in the RP equation [57]. For the time-dependent nonlinear oscillations of bubbles, thermal damping requires rigorous treatments that are not easily incorporated (e.g., [58]–[60]), which is one of the reasons it is often neglected or replaced by a thermal viscosity (e.g., [61]). However, it should be noted that this might not always be valid. Moreover, all else being equal, thermal damping is generally less for a bubble of perfluorocarbon or other fluorine-containing gas than for an air bubble because of the differences in the thermal properties of the gases. For gas bubbles with a diameter of 10 μ m that are insonified with frequencies below 0.6 MHz, thermal damping is the dominant source of damping at almost 100% of the total damping [53].

For a coated bubble, energy is lost through a fourth source of damping, which is viscous energy dissipation in the viscoelastic coating material. It has been shown that the coating is the major source of damping for coated bubbles. Based on optical observations, van der Meer *et al.* [61] reported a 70% contribution of the shell to the total damping of SonoVue lipid-coated bubbles. Where the damping mechanisms of an uncoated bubble are fully understood, the influence of the coating is still an open subject of study and coated-bubble modeling in this direction is still evolving.

Damping reduces the amplitude of the bubble oscillations and it widens the resonance curve of the bubble. The linear analytical solution for the resonance frequency (or resonance curve) can therefore be used to compare the experimental resonance curve of the coated bubbles with simulated data of the free gas bubble as to estimate the values for the viscous damping parameters [61]. In the frequency domain, the contribution of damping is relatively small and the resonance frequency is very close to the undriven natural frequency of the bubble.

III. SOFT-SHELLED BUBBLE MODELS

UCA models can generally be divided into two categories: UCA models for bubbles with soft coating materials, such as albumin or phospholipids, and those for hard coatings, such as polymers. UCAs available in the early 1990s were mainly composed of materials such as galactose and albumin, which are less flexible than the phospholipid coatings that are currently more frequently used (see Table I) and softer than polymers.

An albumin shell consists of denatured albumin, approximately 15 nm thick [9], [62]. The albumin forms a relatively stiff structure around the gas core. The coating of phospholipid bubbles consists of a monolayer of phospholipids and is much thinner (1 to 2 nm) than the albumin shells. The flexible nature of a lipid monolayer and the smaller shell thickness allow larger oscillation amplitudes than does an albumin coating. When hit by a relatively high acoustic pressure (>150 kPa), the lipid-coated bubble may lose gas and lipids because of gas diffusion and lipid shedding, but the bubble may persist, albeit with a smaller diameter [63]. Guidi et al. [64] show that deflation of lipid-coated bubbles is an interesting phenomenon, which can be useful for bubble characterization. Acousticdriven deflation of lipid-coated bubbles is different from the destruction mechanism of albumin-coated bubbles. The albumin coating cracks when the bubble is insonified with a significant acoustic pressure. The cracked bubble exhibits static diffusion with a dissolution rate comparable to that of an uncoated bubble [65].

Early models considered the albumin coating as a solid elastic layer. Therefore, they all modeled the UCA coating with constant properties. To current standards, this seems only valid when the contrast agent bubble oscillates with very small amplitude; however, in those early years of UCA modeling, not much was known about the oscillation of individual contrast bubbles. Available data consisted mainly of acoustic measurements such as scatter and attenuation measurements [66]. The application of high-speed imaging provided more insight into the oscillations of individual bubbles and initiated the development of models suitable to predict larger oscillations of soft-shelled (phospholipid-coated) bubbles, which will be further discussed in the following sections. Fig. 3 shows a few examples of optical techniques from which input for modeling was obtained.

A. Viscoelastic Shell Models

With the arrival of the first commercially available contrast agent Albunex, a need for tools to study UCA behavior was created and the first models for coated bubbles were formulated. In these early days, the starting point for modeling was not the bubble as a harmonic oscillator; on the contrary, the bubbles were supposed to be detected by transient cavitation. Roy *et al.* [70] defined in 1990 a model that predicted transient cavitation thresholds for Albunex. A generalized form of the RP equation was obtained, including a surface layer and boundary conditions for the interfaces between the gas, the surface layer, and the liquid. The surface layer was considered to be a simple viscous liquid.



Fig. 3. Experimental techniques from which input is obtained for modeling. (a) Light scattering; radius-time curve of a Definity microbubble driven at 1 MHz with an acoustic pressure of 308 kPa, reproduced with permission from [67]. (b) Streak imaging of a 2.6-µm-radius bubble excited at 2.4 MHz with a seven-cycle pulse at an acoustic pressure of 360 kPa, reproduced with permission from [68] and (c) Optical images of a 3-µm-radius BR14 microbubble recorded by ultra high-speed imaging, driven at 2.4 MHz and an acoustic pressure of 40 kPa. The two plots below indicate (left) the radius-time curve and (right) the corresponding power spectrum of a 3.8-µm-radius bubble, reproduced with permission from [69].

In the early 1990s, the first publications describing the generation of second harmonic energy by the bubbles also appeared [13]–[15]. It was realized that at low acoustic pressures the contrast bubbles are also capable of stable cavitation. The bubbles are able to radiate ultrasound over a prolonged time. Moreover, it appeared that the backscattered ultrasound contained higher harmonics in addition to the fundamental frequency, which was soon found extremely useful for contrast agent detection.

In search of methods to detect emboli in the blood circulation as a result of decompression sickness, Eatock et al. [12] already used a RP model in 1985. They used this model to study the magnitude of the nonlinear effect in the scattering of ultrasound by nitrogen bubbles in water in the medical diagnostic frequency and acoustic pressure range. In 1994, de Jong et al. [71] extended the RP equation with pressure terms modeling the coating of an Albunex contrast bubble and demonstrated that the nonlinear behavior of the contrast bubbles can be used to discriminate them from surrounding water or tissue. Two shell parameters were implemented in the equation of motion to capture the influence of the relatively stiff Albunex coating. It was assumed to behave as a viscoelastic solid and was described by the shell elasticity $S_{\rm p}$ and shell friction $S_{\rm f}$. Their values were determined under linear conditions for Albunex microbubbles by fitting calculated acoustic transmission and scattering values to measurements [66], [72].

De Jong and coworkers added the coating in an *ad hoc* way to the RP equation. Church was already involved in the formulation of the model by Roy *et al.* [70] and extended this model with the Kelvin–Voigt constitutive law to describe the Albunex coating in 1995 [62]. He accounted for the thickness and viscoelastic properties of the shell by applying essentially Hooke's law for an incompressible material predicting the stresses developing on the shell for small displacements. The model became one of the most frequently used models, especially after Hoff reformulated it in the limit of small shell thickness in comparison with the bubble radius [73]. This approach has a more rigorous theoretical basis compared with the model by de Jong etal.; however, both models assume that the bubble is surrounded by an incompressible Newtonian liquid and the encapsulation is a viscoelastic solid.

Khismatullin and Nadim [74] derived a model applying the Kelvin–Voigt constitutive equation to investigate the effect of a compressible and viscoelastic liquid to the coated-bubble dynamics. The Newtonian liquid was simulated using a 4-constant Oldroyd model. They found that the effect of the compressibility and viscosity of the liquid was outweighed by the influence of the coating. More recently developed models, such as the ones by Morgan *et al.* [68], Marmottant *et al.* [75], and Tsiglifis and Pelekasis [76] include a slightly compressible liquid, which is less complicated than the 4-constant Oldroyd model. However, these studies do not quantify how much the assumption of slightly compressible liquid contributes to the accuracy of the prediction of the bubble response.

B. Surfactant-Coated Models

1) Small Amplitude Oscillations: More insight into the nature of the albumin shell and the development of phospholipid-coated bubbles resulted in the next generation of UCA models. Whereas the first UCA models treated the albumin coating as a solid elastic layer, these models consider the coating, both albumin and phospholipid, as a surfactant.

Morgan *et al.* [68] investigated the experimental contrast agent MP1950 (Mallinckrodt,Inc., St. Louis, MO) composed of a decafluorobutane core coated with a flexible monolayer of phospholipid molecules. To account for the higher bubble wall speeds as a result of the higher flexibility of the phospholipid coating, they used the modified Herring equation [54], which includes radiation damping. The coating is described as a surfactant, which influences the implementation of the coating elasticity. For this term, Morgan *et al.* [68] applied the derivation by Glazman [77]. The coating viscosity was implemented following the formulation of Church [62].

Morgan *et al.* [68] were the first to use optical data of individual bubbles to determine the values of the shell parameters, see Fig. 3(b). Theoretical predictions were fitted to radius-time curves derived from images acquired with a streak camera. The accuracy of the shell parameters determined from individual bubble experiments is higher than parameters acquired in the more common bulk measurements [66]. On the other hand, studies have shown that equally sized bubbles can respond very differently to the same ultrasound field [78], [79]. This indicates that there is a large variation in the properties of individual bubbles. Kooiman et al. [35] have recently investigated the distribution of lipids over the coating surface in fluorescence confocal microscopy studies. For each contrast bubble, different lipid distributions were observed which may be the cause of individual coating properties.

The study by Morgan *et al.* [68] pioneered the use of high-speed imaging to investigate coating properties of individual bubbles and modeling phospholipid-coated bubbles treated as surfactants. However, after a few years, it was demonstrated by Marmottant *et al.* [75] that the pressure contribution derived for the elasticity in Morgan *et al.*'s model leads to unrealistically high surface tension values and a modification was necessary.

Although an albumin coating has a larger thickness than a phospholipid coating, Chatterjee and Sarkar [80] argue that also the albumin shell should not be considered as a solid layer because this coating is only a few molecules thick. They apply a Newtonian interfacial rheological model to simulate the behavior of Optison, which is the successor of Albunex. The coatings of both agents are similar, but the gas core has been replaced by C_3F_8 . In this model, only viscous interfacial stresses are taken into account. As a result, this model also predicted unrealistic values for the surface tension of the albumin coating.

To solve this problem, Sarkar *et al.* [81] extended the model by Chatterjee and Sarkar [80] with an elasticity

term. They compared the outcome of the model by Chatterjee and Sarkar [80], the Newtonian interface model, their new model, the viscoelastic interface model, and the model by Hoff *et al.* [73] with the experimental data on their merit of predicting subharmonics. It was found that the Newtonian model, predicting the unrealistically high surface tension values, was superior to the other two models. They concluded that a new model predicting subharmonics without the unrealistic surface tension values should contain a softening of the encapsulation by assuming a surface dilatational elasticity constant that decreases with an increasing fractional area. More recent models dedicated to large amplitude oscillations of surfactantcoated agents follow this trend and include coating parameters that vary with the bubble surface area [82].

The last model assuming small bubble oscillations described here is the model by Doinikov and Dayton [83]. They developed a theoretical description of lipid-coated microbubbles in which, instead of the Kelvin–Voigt constitutive law, the linear Maxwell constitutive law was applied. For a finite-thickness shell, this model contains six parameters (relaxation time λ , shear viscosity η_s , surface tension coefficients σ_1 and σ_2 , density ρ_s , and thickness of the shell $R_2 - R_1$) to describe the coating. For a zerothickness shell model, these parameters reduce to viscosity and relaxation time instead of shell elasticity and viscosity, the two shell parameters used in the Kelvin–Voigt equation. This Maxwell model was used to investigate the influence of these different shell properties on the resonant behavior of coated bubbles.

2) Large Amplitude Oscillations: The contrast agent bubble models described so far all include the coating as a material with constant properties. The responses of different types of agents are simulated using the same model. The values of the coating parameters are simply adapted for the different shell compositions. However, with the expanding clinical use of phospholipid-coated bubbles, it became clear that the previous models simulating small amplitude oscillations do not suffice. To predict the responses of lipid-coated agents, more sophisticated models are necessary.

Phospholipid UCA detection strategies are mainly based on imaging at low acoustic pressures. At these pressures the backscattered ultrasound by these bubbles is already significant because of the flexibility of the phospholipid coating. Although a low acoustic pressure suggests that the bubbles oscillate with small amplitude and thus exhibit linear responses, recent studies have shown that this assumption cannot be sustained. The term *linear* response refers to a response that relates to the transmit frequency only and has an amplitude that is linearly proportional to the amplitude of the acoustic pressure. As a consequence of the latter condition, any acoustic pressure should lead to a bubble vibration. All small amplitude models fulfill this condition; however, experimental data shows differently. Optical high-speed recordings of individual bubbles revealed that the onset of bubble vibration

of some lipid bubbles is suppressed by what is termed as thresholding behavior [84]. No bubble oscillations were observed below a certain acoustic pressure threshold (of the order of 10 to 100 kPa). It was not excluded that these bubbles did respond at acoustic pressures below the threshold value, but it was apparent that these responses are very small and even though at first sight it can be argued whether they are relevant for clinical use, the fact that these bubbles have a strong response at a slightly higher pressure makes them extremely useful for clinical use, e.g., for power modulation imaging [20].

In addition to thresholding behavior, high-speed recordings displayed compression-only behavior of the lipid-coated bubbles [85]. Bubbles exhibiting this behavior showed no or little expansion phase, but only compression in response to a symmetric ultrasound pressure field. This type of response is highly nonlinear and the backscattered ultrasound by the bubble contains significant amounts of harmonic energy including subharmonic energy [69], see Fig. 3(c). To predict thresholding and compression-only behavior, models with constant coating properties developed for small amplitude (i.e., linear) oscillations do not suffice.

The first model dedicated to the nonlinear behavior of lipid-coated bubbles was proposed by Marmottant et al. [75]. Surface tension measurements on phospholipid monolayers in Langmuir-Blodgett balances showed the dependence of the surface tension on the surface concentration of the phospholipid molecules. Inspired by this effect, Marmottant et al. incorporated in their model an ad hoc effective surface tension, which accounts for the coating elasticity. Depending on the bubble radius (and thus the concentration of phospholipid molecules) the coating can be in three different regimes: buckled, elastic, and ruptured. When the bubble is compressed, the coating material is condensed which leads to buckling, and with the bubble coating in such a tensionless state, the resulting surface tension is zero. When the bubble is expanded, the coating may be ruptured and the gas core will be exposed to the surrounding liquid, leading to a surface tension of that of the gas-liquid interface. In the intermediate elastic regime, the coating is assumed to behave elastically and the model is similar to that of de Jong *et al.* [71].

Whereas the papers by Emmer *et al.* [84] and de Jong *et al.* [85] had already speculated on the influence of the shell on the thresholding and compression-only behavior, the effective surface tension in the Marmottant model appeared to be the key to simulate the observed phenomena. More importantly, Overvelde *et al.* [86] showed that the surface tension of the bubble at rest, which is directly related to the ambient phospholipids concentration, is crucial in determining the bubble response. Typically, the bubble starts off in the elastic regime, where it is relatively stiff. Its resonance frequency is high and it will not easily oscillate when driven below resonance. An increasing acoustic pressure can modify the concentration of lipid molecules on the bubble's surface, forcing the bubble into the buckling regime, which suddenly reduces its stiffness,

resulting in a much lower frequency of maximum response. Consequently, its oscillation amplitude will sharply rise, as the bubble is tuned more into resonance, in very good agreement with the thresholding behavior observed previously. Sijl and coworkers showed through a weakly nonlinear analysis that the rapid change of the bubble stiffness leads to a boost of the nonlinear behavior of the bubbles. Bubbles with a resting surface tension near the buckling regime show compression-only behavior [87] and were observed to oscillate in subharmonic modes at acoustic pressures down to 5 kPa. The change of shell elasticity upon insonation reduces the subharmonic threshold pressure to values far below those of the free gas bubbles (5 kPa versus 50 to 80 kPa) [69]. It should also be noted that the shell has its main influence when the bubble is insonified at driving frequencies below its resonance frequency [88]; above resonance, inertia dominates.

Marmottant *et al.* [75] did not investigate the influence of the coating viscosity on the bubble responses. Recent studies have revealed that the shell viscosity is dilatationrate-dependent and it shows shear thinning behavior [61], [68], [79]. Doinikov *et al.* [88] showed that by using the Church model [62] as a basis and expanding the coating viscosity from a constant to $\kappa(\dot{R}/R) = \kappa_0 + \kappa_1 \dot{R}/R$, one can also predict compression-only behavior. This may serve as a beginning for further investigation on the influence of shell viscosity on bubble behavior.

Recent studies focused on replacing the *ad hoc* surface tension law in the Marmottant model by a more accurate definition to avoid unphysical transitions from one regime into the other [87]. Paul *et al.* [82] argue that the boundaries of the different regimes of the surface tension are not easily established and propose extensions to the linear Hooke law instead. They test a quadratic elasticity model (interfacial elasticity varying linearly with area fraction) and an exponential elasticity model (elasticity varying exponentially) on their merit of predicting subharmonic responses. They found that these models predict lower subharmonic threshold values and therefore match better with their experimental values than does the Marmottant model.

Tsiglifis and Pelekasis [76] chose a more extensive and rigorous approach to model nonlinear lipid-coated bubble behavior such as threshold behavior. In their paper, the Keller–Miksis equation was chosen to model the bubble dynamics. The coating is regarded as a continuum and is described by an elasticity G and viscosity μ_s . The elasticity is however not a constant, but varies with bubble deformation, which is defined by a strain-softening Mooney– Rivlin or a strain-hardening Skalak law. In case of strain hardening, this means that the stress-strain relationship of the coating exhibits a larger slope when the bubble deformations increase. This essentially amounts to an increased apparent elasticity modulus in the bubble model. The opposite holds for the strain-softening law. In their model, the amount of strain softening or hardening is controlled with a control parameter. Tsiglifis and Pelekasis [76] explain that the strain-softening of the shell coating may lead to the threshold behavior. A bubble driven below its resonance frequency responds with relatively low amplitude to the ultrasound field. Increasing the acoustic pressure may drive the bubble's resonance frequency because of the strain-softening nature of the shell toward the ultrasound frequency, which results in a nonlinear increase of the bubble's response. The sudden increase in bubble response, or thresholding behavior, were calculated for acoustic pressures much higher (>400 kPa) than the values observed by Overvelde *et al.* [86].

Doinikov and Bouakaz [89] model threshold behavior in a different way. They propose a criterion in which the microbubble oscillation starts when the acoustic pressure amplitude exceeds a certain magnitude. They found a radius-dependent threshold amplitude, which fits to the experimental data by Emmer *et al.* [84]. The authors indicate that further research is required to understand the specific rheological laws that can be applied to describe this threshold amplitude.

Stride [90] defined a model that was not specifically meant for large deformations, but both the coating elasticity and viscosity are dependent on the instantaneous radius. The coating viscosity and elasticity do not follow a constitutive law, but a description of interfacial tension for insoluble films. For the surface tension, a power law is applied with these parameters: σ_0 is the surface tension of the resting bubble, K is the proportionality constant, and x is the exponent of the power law. The viscosity is described by an exponential law including η_{s0} and Z, which are constants for a specific surfactant and R_x , which is the buckling radius, comparable to the buckling radius defined by Marmottant *et al.* [75].

IV. HARD-SHELLED BUBBLE MODELS

UCAs with polymer shells were introduced in 1990 by Wheatley et al. [91]. The polymer shell is typically very stiff and does not vibrate significantly when insonified at low acoustic pressures (< 200 kPa). The polymer UCA is activated once the acoustic pressure is high enough to crack the shell and thereby releases the gas content of the bubble [92]. A gas bubble temporarily generates a high backscatter of the ultrasound wave. In 1990s, different imaging strategies had been developed based on UCA destruction, such as transient response imaging [93], or using the decorrelation of echoes from successive pulses in Doppler modes, such as harmonic power Doppler [94] or pulse inversion imaging [19]. The SNRs of polymer UCAs used in these imaging methods are competitive with those of phospholipid-coated UCAs. Less favorable is that these SNRs are obtained at higher acoustic pressures which destroy the agent and make real-time imaging impossible.

None of the polymer UCAs available were clinically approved. In 1996, Point Biomedical Corp. (San Carlos, CA) was established, which marketed several polymer UCAs, such as PB127. PB127 (CARDIOsphere) was tested in different clinical trials [95], [96]. However, no approval by the

FDA followed and in 2008, Point Biomedical had to cease its activities. Currently, there is a renewed interest in using polymer UCAs [97] for high-frequency (HF) imaging [98] and its potential as a drug or gene carrier [36], [99].

The still-popular model by Hoff *et al.* [73] was originally developed to model an experimental UCA from Nycomed (Nycomed Amersham, Oslo, Norway), composed of air bubbles encapsulated in a polymer shell. This model, as described previously, considers a viscoelastic behavior for the polymer shell and is thereby only valid for small amplitude oscillations of the polymer bubbles. Taking into account that imaging of polymer UCAs occurs mainly at higher acoustic pressures, this model seems to have limited value to predict polymer-encapsulated bubble responses. In 2004, Allen and Rashid [100] defined a model to predict large amplitude oscillations of polymer bubbles. They did not consider UCAs specifically, but treated polymer spheres in general. The polymer shell is relatively stiff and was therefore assumed to have a neo-Hookean elastic response. It was modeled having certain elasticity, but no shell viscosity was defined. This model does not incorporate the destruction of the polymer coating necessary for many imaging methods.

Marmottant *et al.* [101] formulated a model for solid-shelled bubbles that does include shell rupture. This model is based on their model for soft lipid-coated bubbles [75]. The model for solid shells incorporates an effective membrane tension, which allows the bubble to be in three states. Upon compression, a negative tension builds up. As soon as the tension is negative enough, the coating starts to buckle and the membrane tension vanishes. The elastic state is recovered when the volume returns to its resting value. When the membrane tension exceeds a certain threshold value for coating rupture, the bubble ruptures and the membrane tension saturates to the surface tension of the gas-water interface. This state is irreversible, which is in contrast to the ruptured state for lipidcoated bubbles. This model has been successfully tested in an experimental study on biodegradable polymeric microcapsules for selective ultrasound-triggered drug release by Lensen *et al.* [102].

V. DISCUSSION

The introduction of new UCAs is the primary driving force for the development of new contrast agent bubble models, see also Fig. 2. The first contrast agent bubbles had relatively stiff shells and, as a consequence, a simple Kelvin–Voigt linear elastic relationship sufficed to predict their dynamic behavior when driven by an acoustic pressure. Currently, contrast agents with lipid coatings are used in the clinic, and it has become clear that models that include linear coating properties fail to predict experimentally observed radial bubble responses. The development of high-speed imaging of contrast bubbles has contributed to this insight. Optical recordings of lipidcoated bubbles revealed threshold and compression-only behavior, which cannot be predicted without the use of a nonlinear material law for the contrast bubble coating.

The assumption of a viscoelastic behavior following the Kelvin–Voigt material law, results in a parameterization of the coating into three parameters; the coating thickness, its elasticity, and its viscosity. The advantage of using such a relatively simple law is that this law is also frequently applied in other areas and reasonable values for the coating parameters are known or can be measured in separate tensile tests. For other parameters such as the surface tension, the same advantage holds—its physical limits are known. For more complicated material laws with an increasing number of parameters, the accuracy of its application and resulting parameter values are much more difficult to verify. Material laws are often developed for different research areas such as cell membranes or pulmonary surfactants with different requirements for the studied material. A major difference is that the contrast bubble vibrates in the megahertz frequency range, whereas material properties are, in most cases, tested at hertz frequencies. In addition, we note that sophisticated material laws can incorporate multiple parameters to describe the influence of the coating. An increasing number of parameters often results in an improved fit between simulation and experiment, but has the drawback that the merit of the applied material law and the resulting parameterization cannot be judged easily.

In the past, bulk acoustic measurements on the whole population of contrast bubbles were employed to determine the values of the shell elasticity and viscosity constants. Using these kinds of measurements assumes that the coating parameters are independent of bubble size and equal for each contrast bubble in the population. There is increasing evidence based on high-speed recordings of individual contrast bubbles that this is not the case in reality. Coating properties may differ based on the bubble size. In addition, it has been observed that individual responses of similar-sized lipid-coated bubbles can vary widely, indicating variations in the lipid distribution and concentration of the individual coatings. This should be accounted for when studying the responses of individual bubbles. Besides studying the responses of individual contrast agent bubbles, high-speed recordings are useful to study the influence of additional ligands and bubble adherence on the bubble dynamics, which is essential for future use of bubbles in the apeutic and molecular imaging applications. Initial results have shown changes in bubble resonance frequencies resulting from bubble attachment [103], which may be a way to characterize bound versus unbound bubbles (i.e., to recognize bubbles targeted to the affected site) in the human body.

While discussions concerning the best approach of coated bubble modeling are ongoing, it is important to mention that the basis of all bubble models itself, the Rayleigh–Plesset equation, can also be debated as the best choice to investigate bubble behavior. Applying the Rayleigh–Plesset equation assumes, for example, that the bubble remains spherical and is located in free space. However



Fig. 4. A few cases for which additional contrast modeling work is needed. (a) Non-spherical oscillations and bubble/wall interactions, reproduced with permission from [108], (b) bubble/bubble interactions, reproduced with permission from [109], (c) rupture of soft-shelled agent, reproduced with permission from [110], and (d) rupture of hard-shelled agent, reproduced with permission from [111].

high-speed recordings have revealed that bubble oscillations in the presence of a wall are far from spherical [104]. Moreover, it can be expected that a significant amount of bubbles in the blood circulation are situated near a (vascular) wall, which additionally affects oscillation amplitudes [103], [105]–[107]. Fig. 4 illustrates a few cases for which additional contrast modeling work is needed.

To model the interaction between bubble and wall, a finite element modeling (FEM) approach may be considered; see, for example, Pauzin *et al.* [112]. An additional reason to consider this method for bubble modeling is the increasing use of coated bubbles for therapeutic and molecular imaging. These applications profit from the interaction between bubbles and the vascular wall, which may be modeled using a FEM approach.

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