Doctoral Thesis in Technology and Health

Exploring Polymer-Shelled Microbubbles: Detection Modeling and Application

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Stockholm, Sweden 2020
Exploring Polymer-Shelled Microbubbles: Detection Modeling and Application

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Academic Dissertation which, with due permission of the KTH Royal Institute of Technology, is submitted for public defence for the Degree of Doctor of Philosophy on Friday, December 11th, 2020, at 9:00 a.m. at Room 7327, Hälsovägen 11C, Huddinge, Sweden via Webinar.
献给

我的父亲，母亲和妻子
Abstract

Ultrasound imaging (US) is widely used in clinical practice. Given the low cost and easy access to the ultrasound machine, US has a great potential to improve the health care condition for the majority of the population in the world. The US could be significantly improved by injecting ultrasound contrast agents to opacify the bloodstream. The polymer-shelled microbubbles (MB) are promising candidates for the next generation ultrasound contrast agent. In the current doctoral work, one of the polymer-shelled MBs, the polyvinyl alcohol (PVA) MB was investigated.

In Study I and Study II, I developed a novel contrast pulse sequence, CPS4, to efficiently detect the PVA MBs. The CPS4 is a combination of the sub-harmonic (SH), ultra-harmonic, and pulse inversion techniques. The comparison of the performance of each individual technique and CPS4 was carried out in a tissue-mimicking phantom. The CPS4 demonstrated the highest contrast-to-tissue ratio among all four imaging techniques. However, the SH response of the CPS4 was not fully excited. The high SH pressure threshold, above which the SH response is generated, was suspected to be the reason for the weak SH signal. Therefore, I wanted to optimize the performance of the CPS4 for the PVA MBs detection by boosting the SH signal. The optimization strategy was to lower the frequency-dependent SH threshold by setting the SH excitation frequency, which is the frequency of the ultrasound wave that excites the SH response, at the damped resonance frequency of the PVA MBs. To estimate the damped resonance frequency, a mathematical model based on the Church’s model with frequency-dependent material properties was proposed. The mechanical parameters of the new model were estimated by fitting the measured attenuation coefficient of the PVA MBs suspension with the simulated one. The calibrated model was employed to predict the damped resonance frequency of the PVA MBs, i.e., the optimized SH excitation frequency for the CPS4. The performance of the CPS4 was evaluated in-vitro, driving the system at four SH excitation frequencies in the proximity of the damped resonance frequency of the PVA MBs suspension. The best performance was observed at the SH excitation frequency of 11.25 MHz, which is in line with the simulated damped resonance frequency of 10.85 MHz. The in vitro experiment also revealed that the small particles constituting the artificial blood solution might interact with the PVA MBs and decreased the response echoes in a nonlinear and frequency-dependent fashion. Thus, more efforts are needed to move our model-guided optimization methods for the CPS4 towards clinical application.
In Study III, I modified the PVA MBs to support the dual-modal imaging of CT and US. The main idea of the modification is to incorporate the gold nanoparticles with the PVA MBs. The success of the modification is dependent on the amount of the gold nanoparticles carried by the modified PVA MBs. Two routes were proposed to fabricate candidates that support dual-modal imaging. In the first route, the gold nanoparticles were added during the fabrication of PVA MBs. Thus, the gold nanoparticles were embedded in the PVA shell during its formation (candidate named AuNP-S-MB). In the second route, the gold nanoparticles were loaded into the core of the PVA MBs, substituting air by increasing the permeability (candidate named AuNP-Capsule). The CT revealed an insignificant amount of gold nanoparticles was embedded in the shell of AuNP-S-MB, while detectable gold nanoparticles were loaded into AuNP-Capsule. Moreover, the CT-number of the surrounding liquid of AuNP-Capsule is low, i.e., the gold nanoparticles were locked in the AuNP-Capsule, making the second route a promising step towards the further development of the dual-modal contrast agent for CT and US.

In Study IV, I studied the effect of PVA MBs on the cavitation flows in microscale. The cavitation in clinical practices generates great pressure, which might be harmful and damage cells or beneficial and facilitate the treatment. A better understanding of cavitation generation mechanisms could avoid harmful cavitation, increase the safety of the clinical protocol, and increase the therapeutic cavitation, empower the treatments. Therefore, the effect of PVA MBs on cavitation is of great interest. More specifically, the effect of PVA MBs on the hydrodynamic cavitation was studied. Three microfluidic devices with different wall roughness and structure were fabricated. Two working fluids, PVA MBs suspension and water, were driven with controlled pressure through different microfluidic devices. The high-speed visualization revealed that the PVA MBs trigger the inception of hydrodynamic cavitation at a lower upstream pressure and enhance the cavitation flow in all three microfluidic devices. Furthermore, it takes a longer time for the cavitation bubbles to disappear in the PVA MB suspension.

To conclude the doctoral work, I developed a novel detection sequence, CPS4, optimized it for PVA MBs with a model-guided method, modified the PVA MB to extend its application, and studied the effect of PVA MB on hydrodynamic cavitation. The work promotes the PVA MBs for pre-clinical study, as well as provides an insight into the studies of other clinically approved ultrasound contrast agents. The methodology developed and presented within the thesis can be transferred to other clinically approved ultrasound contrast agents. For instance, the CPS4 and model-guided optimization method could be employed to improve CPS4 to other ultrasound contrast agents.

**Keywords**
Ultrasound contrast agent, polymer-shelled microbubble, ultrasound imaging, contrast pulse sequence, microbubble model, hydrodynamic cavitation
Sammanfattning


För att sammanfatta mitt doktorandarbete har jag utvecklat en ny detektionssekvens, CPS4, optimerat den för PVA MB med en modellstyrad metod, modifierat PVA MB för att utvidga dess tillämpning, och studerat effekten av PVA MB på hydrodynamisk kavitation. Arbetet främjar PVA MB för prekliniska studier, samt ger en inblick i studierna av de andra kliniskt godkända ultraljudskontrastmedlen. Metoderna som utvecklats och presenterats inom avhandlingen kan tillämpas på andra kliniskt godkända ultraljudskontrastmedel. Till exempel kan CPS4 och modellstyrad optimeringsmetod användas för att detektera andra ultraljudskontrastmedel med hög effektivitet, vilket erbjuder en överlägsen upplösning.

Nyckelord

Ultraljud Kontrastmedel, polymer mikrobubblor, ultraljudsavbildning, kontrast sequence, mikrobubbel modellering, hydrodynamisk kavitation
List of Papers

The current doctoral thesis consists of the following peer-reviewed papers. They are referred as Study I, Study II, Study III, and Study IV.

Study I

Study II
Chen, H., Löffler, W., Grishenkov, D. Model-guided customization of a contrast pulse sequence for polyvinyl alcohol microbubbles in manuscript form.

Study III

Study IV
Division of Work

For Study I, H. Chen developed the code to perform the CPS4. D. Evangelou participated in the development of the initial version of the code. H. Chen performed the in vitro experiment and wrote the manuscript. D. Grishenkov contributed to the writing and discussion in the manuscript.

For Study II, H. Chen adapted the model to the PVA MB and performed simulation work. W. Löffler measured the attenuation coefficient of the PVA MBs. H. Chen developed the code to perform the in vitro experiment and wrote the manuscript. D. Grishenkov contributed to the writing and discussion in the manuscript.

For Study III, H. Chen and D. Grishenkov developed the fabrication protocol for the dual-model imaging contrast candidates. H. Chen and Y. Zhao performed the characterization experiments. H. Chen wrote the manuscript. D. Grishenkov and Y. Zhao contributed to the manuscript.

For Study IV, H. Chen and M. Ghorbani performed the experiment. The manuscript was prepared by H. Chen and M. Ghorbani, under the supervision of D. Grishenkov and A. Koşar.
Other Scientific Contributions

Peer-reviewed paper:


Conference abstract:


Acknowledgment

This doctoral work was carried out in the Department of Biomedical Engineering and Health Systems, KTH Royal Institute of Technology and supported by China Scholarship Council. I would like to thank our department and China Scholarship Council for the opportunity to explore the interesting field of the ultrasound contrast agent.

Many thanks to my main supervisor Dmitry Grishenkov for his enthusiasm, valuable suggestions, and immense knowledge. You are not only a good supervisor but also a helpful friend who helps me move my apartment and sends comfort when I am ill. This long journey is much easier with him.

I would also like to thank my co-supervisor Birgitta Janerot-Sjöberg for her input to my study and the helpful discussion.

Many thanks must be dedicated to the members of our research group, Ksenia and Morteza. Thank you for the suggestions, help, and valuable input in my research.

I would like to extend my appreciation to my colleagues, Daniel, David, Tim, Mamo, Chunliang, Rodrigo, Xiaogai, Annaclaudia, Elira, Shiyang, Madelen, Pooya, Reza, Qingling, Qingyang, Lyun, Abdolamir, Fredrik, Ke Lu, Fabian, Irene, Mehdi. I enjoyed the time with you in Flemingsberg.

Warmest gratitude to my friends. Zhou, you provided useful advice from your enormous knowledge as well as your company to the café machine and in the office. You made my days more fun. Fangyuan, thank you for the many suggestions with art. Teng, thank you for the help with the simulation. Xin and Yue, thank you for hosting the BBQ. Xueying, Yang, and Yixing, thank you for being good friends and introducing many nice restaurants. Zhichao and Jingwen, thank you for hosting me during the TIs, although LGD never won any of them. Beien, thank you for hosting me every January in the Netherlands. I will see you soon in China. Yuefeng, thank you for hosting me in Amsterdam. Bo, thank you for hosting me in Ghent. Tailang, I really enjoyed the time with you in the summer school in French.

I am also thankful to my cousin for helpful conversations, which made me less stressed.
I want to express my appreciation to my parents for their supports, understanding, sacrifices, and love. Last, my deepest thanks to my beloved Mingzhu, who became my wife during my Ph.D. studies. She is special and made my life different.

Huddinge, November 2020

Hongjian
### Abbreviations

<table>
<thead>
<tr>
<th>AB</th>
<th>Artificial blood</th>
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<tr>
<td>CE</td>
<td>Contrast-enhanced</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>MB</td>
<td>Microbubble</td>
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<tr>
<td>PI</td>
<td>Pulse inversion</td>
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<tr>
<td>PNP</td>
<td>Peak negative pressure</td>
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<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>SH</td>
<td>Sub-harmonic</td>
</tr>
<tr>
<td>UCA</td>
<td>Ultrasound contrast agent</td>
</tr>
<tr>
<td>UH</td>
<td>Ultra-harmonic</td>
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<td>US</td>
<td>Ultrasound imaging</td>
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1 Introduction

A microbubble (MB) is a hollow sphere in the micrometer range, typically 1 to 10 µm. Over the past decades, MBs are used as ultrasound contrast agents (UCAs) in preclinical studies and clinical diagnosis to enhance the contrast between blood pool and tissue. MBs with different materials of the shell and encapsulated gas were developed to improve their stability and meet the needs of the clinical diagnosis. The subject of the current work is a research-grade, thick-shelled, polymer-based UCA, polyvinyl alcohol (PVA) MB.

Contrast-enhanced (CE) ultrasound imaging (US) increases the sensitivity of US in conjunction with the UCAs. The UCAs produce large echo even at an extremal low volume fraction due to their oscillation in a sound field.

At low peak negative pressure (PNP), typically below 100 kPa [1, 2], the UCAs oscillation produces linear responses and creates a much larger scattering cross-section than the tissue. This allows the detection of the UCAs under conventional B-mode imaging. However, the concentration of the UCAs required for B-mode imaging is relatively high, which results in a considerable attenuation, i.e., shadow effects [3]. At low PNP, the MBs oscillate near their equilibrium radius and remain the spherical shape. However, it is worth noting that the nonspherical oscillations could also occur at low PNP when the MBs are driven by an asymmetric ultrasound field, e.g., the MBs are in the proximity of a wall [4].

At high PNP, typically above 100 kPa [1, 2], the UCAs start to oscillate nonlinearly, leading to nonlinear responses. It is worth mentioning that the tissue also generates nonlinear responses with a weaker amplitude. The nonlinear responses contain different frequency components to the incident ultrasound, which provide unique acoustic ‘fingerprint’. Thereby a relatively low concentration of UCAs is required to be detected.

The MBs rupture as the PNP further increases. The encapsulated gas escapes from the disrupted MBs and generates transient, high-amplitude, broadband bursts [5].

Many imaging techniques based on nonlinear behavior were developed. In general, they could be classified as nondestructive techniques and destructive techniques. Pulse inversion (PI), amplitude modulation, and power modulation are typical nondestructive techniques. The nondestructive techniques transmit multiple pulses and process echoes to cancel the
linear responses and extract the specific nonlinear responses. For instance, PI transmits two ultrasound pulses with the same frequency but with phase inverted. The sum of the two received echoes cancels the linear component of the echoes and highlights the nonlinear component of the received echoes [6]. The destructive techniques could provide a transient high acoustic emission by forcing UCAs to release their gas. The high-amplitude bursts are employed in high mechanical index (MI) imaging techniques, making the high MI techniques a more sensitive indicator of the MBs presence [3]. Moreover, perfusion of the organs can be observed by using destructive techniques. High MI pulses are transmitted to destroy and clear the UCAs in the organs of interest, then low MI imaging techniques are employed to observe the perfusion of the organs [3].

A contrast pulse sequence, which more efficiently catches the unique acoustic “fingerprint” of the PVA MB, could significantly increase the value of the PVA MB. Thus, Study I and Study II focused on the improvement of the nondestructive techniques. A novel contrast pulse sequence, CPS4, combining PI, sub-harmonic (SH) and ultra-harmonic (UH) was proposed and evaluated. Subsequently, the CPS4 was customized to PVA MB to optimize its detection performance.

In the current thesis, efforts were also dedicated to extending the application of the PVA MB. MBs were developed to increase the contrast in CEUS. With substantially improved understanding of the MBs, innovative means were proposed to extend their applications to the fields other than the established practice. In the well-accepted and established practice, MBs are injected intravenously and circulated freely in the blood pool. As a result, only a small fracture of MBs is delivered to the target organ or tissue, which limits the contrast of CEUS. Increasing the contrast, while remaining the injection dose of MBs requires the accumulation of the MBs at the target regions. The persistence of microbubbles occurs naturally in certain special scenarios. A controllable accumulation of the MBs involves the attachment of ligands to the MBs. The ligands, e.g., monoclonal antibodies, peptides, are attached to the MB shell directly or with an extra layer, e.g., avidin-biotin pair and spacer arm [7]. The MBs with ligands circulate in the bloodstream and concentrate at the target regions. For instance, Willmann et al. [8] combined anti-VEGFR2 and anti-β3 integrin with MBs. The dual-targeted MBs accumulate at the tumor region and provide significantly higher echoes than the non-targeted MBs at the tumor region [8]. Moreover, therapeutic components were loaded to the MBs to localize the drug delivery [9]. The drug could be incorporated inside the MBs [10, 11], embedded with the MBs shell [12, 13], or attached to the shell surface via chemical binding [14] and electrostatic force [15]. The therapeutic components are released in the targeted regions, followed by ultrasound exposure.

Besides the therapeutic loading, modifications were implemented to enable MBs to support dual-modality imaging. For instance, Liu et al. [16] fabricated iron oxide nanoparticle-embedded poly(butyl cyanoacrylate) MBs to support both MRI and US. Nevertheless, the MBs were utilized to interact physically with the tissue. For instance, the physical interaction between MBs and the cell, also known as ultrasound-mediated sonoporation, could generate
cell membrane perforation [17, 18], trigger endocytosis [19], and open the cell-cell junctions [20]. The ultrasound-mediated sonoporation increases the permeability of the cell membrane [17-19] and the vessel [20], which facilitates the drug uptake. Moreover, MBs improve the sonothrombolysis (a method of destroying thrombi in blood vessels by ultrasound [21]) by increasing the microvascular patency [22].

The PVA MB has a PVA shell with rich reactive aldehydic groups at the surface. The reactive aldehydic groups are easily modified, making the PVA MB a promising candidate for multifunction devices [23]. Cavalieri et al. [23] demonstrated the synthetic routes for decorating the PVA shell with ligands to functionalize the PVA MB. Furthermore, the thick shell of the PVA MB is capable of carrying payload by embedding the functional species in the shell. For instance, Brismar et al. [24] incorporated the PVA MB with iron oxide nanoparticles to support MRI and US. The iron oxide nanoparticles are not only covalently attached to the shell surface but also embedded physically in the shell [24].

Inspired by the studies mentioned above, Study III focused on the modification of the PVA MB towards a dual-modal contrast agent for CT and US.

In Study I and Study II, the stable cavitation, i.e., the oscillation of the MB around its equilibrium size due to the external sound field, was discussed. The stable cavitation is rather gentle and could last for a relatively long period of time [25], while the transient cavitation could exist for less than one cycle. The transient cavities expand in large-amplitude, typically above double the size, followed by a sudden and violent bubble collapse [25]. Great pressures are generated during the collapse phase of the cavitation, which could harm the human body [26]. For instance, the cavitation could develop on the artificial heart valve [26, 27] and the energy released due to the collapse of the cavitation could damage the artificial heart valve and may cause sudden failure of the valve in extreme scenarios [28, 29]. Moreover, the cavitation even ruptures the blood cells, which leads to the release of tissue factor and thus increases the risk of thromboembolic complications [27]. On the other hand, cavitation could be used as a treatment [26]. Ghorbani et al. [30] proposed using bubbly, cavitating flows to perform urinary stone therapy and presented a medical device prototype to generate the cavitating flows based on small-scale hydrodynamic cavitation.

A better understanding of the cavitation generation could facilitate the development of novel medical practices and devices, which might need to avoid or enhance cavitation. For instance, delivering a large amount of dissolved oxygen to the bloodstream during heart attacks or strokes could reduce cell damage and death. However, the undesired cavitation could cause damage to the patients. Brereton and Creech [31, 32] extend the classical theory of nucleation to interpret heterogeneous nucleation in capillary tubes and modified the protocol of oxygen delivery by minimizes the nucleation sites and limiting flow velocity based on their theory. The new protocol delivers oxygen while avoiding significant cavitation, which facilitates the clinical translation of the treatment.
The MBs are widely used in clinical practices and could provide external cavitation nucleation sites. Thus, the effect of the MBs on the cavitation is of interest. In Study IV, the effect of the PVA MBs on hydrodynamic cavitation was investigated.

To summarize, the current thesis attempted to answer the following research questions:

- Does the novel contrast pulse sequence, CPS4, increase the efficiency to detect MBs? More specifically, PVA MBs.
- Is it feasible to optimize CPS4 to the PVA MBs with the guidance of the PVA MB model?
- Is it feasible to modify PVA MBs to support the dual-modal contrast imaging of CT and US?
- Does the presence of PVA MBs influence the generation and development of hydrodynamic cavitation?
2 Objectives

The aims of the four studies are listed as follows:

Study I  The current study introduced a novel contrast pulse sequence, CPS4. The performance of CPS4 with the in-house UCA, PVA MB, was evaluated in an in vitro experiment against conventional US techniques: SH, UH, and PI alone.

Study II  The current study aimed at optimizing the CPS4 to the PVA MBs. A PVA MB model was adapted from Church’s model and further used to predict the optimal SH excitation frequency of CPS4 for the PVA MBs.

Study III  The current study explored the feasibility of modifying the PVA MBs to support both CT and US as a dual-modal contrast agent.

Study IV  The current study investigated the effects of the PVA MBs on hydrodynamic cavitation.
3 Background

The subject studied in this thesis is PVA MB, a research-grade, polymer-shelled UCA. In this chapter, the development of the UCAs is described. Subsequently, previous studies are summarized to define state of the art in the PVA MB. Last, mathematical models of the UCA and, more specifically, the PVA MB model are elaborated.

3.1 History of ultrasound contrast agents

The UCAs are exogenous substances, which are injected into the blood pool and work as blood pool tracers. The contrast enhancement of US was first reported as early as the 1960s. In 1969, Gramiak et al. [33] reported a contrast effect introduced by the injection of indocyanine green dye. The later studies advocated the contrast effect is produced by the bubbles in the injected liquid [34, 35]. Free gas bubbles were found in the injections of a variety of liquids, e.g., saline, autologous blood, renografin, ether, and similar gas saturated media. Bubbles in the liquids with low surface tension were found to have a higher persistence. Gramiak concluded that the free gas bubbles were produced by cavitation during the injection [33]. A subsequent study revealed that the gas bubbles were caused by hand agitation [35]. The nature of the generation process of the gas bubbles results in their highly polydisperse size distribution, with extreme cases being able to obstruct arterioles. Moreover, the free gas bubbles are not stable and have a short life span. A theoretical calculation suggests that an 8 µm gas bubble will dissolve in between 190 to 550 ms [36]. Those disadvantages limit the diagnostic value and applicability of the free gas bubbles as UCA. Since the gas bubble could not survive from the pulmonary circulation and reach the left heart, the initial applications of the contrast echocardiography focused on the right heart investigations. To assess the left heart, carbon dioxide was injected directly into the pulmonary artery via a balloon-tipped catheter [37]. Other efforts focused on slowing the dissolution time of bubbles. The encapsulated gas bubbles were developed to prevent gas from fast dissolution. Carroll et al. [38] trapped nitrogen gas in gelatin capsules with a diameter of 80 µm. The capsules improve the stability of the gas. However, the large size disables their ability to pass through the capillaries. It was until the Albunex and Levovist were developed, the left heart contrast became feasible after the intravenous injection of UCAs.
Different types of UCAs were also explored over the years. Ophir et al. [39] produced collagen microspheres by emulsifying the mixture of collagen solution and cottonseed oil. Colloidal suspensions contain small solid particles as ultrasound scatterers. The backscatter measurements suggest that the collagen microspheres are more efficient scatterers than red blood cells [39].

Inspired by the enhanced backscattering from the fat in hepatocytes, three lipid emulsions, with particle size range from 0.4 to 4.7 µm and different combinations of oil source and emulsifier, were evaluated in animal experiments. Only poor backscatter was observed [40]. Fink concluded that sufficient backscatter might require an extremely high amount of lipid [40].

Aqueous solutions with acoustic impedance mismatch to the tissue were proposed to enhance the backscatter. Ophir et al. [41] found that the introduction of the sodium citrate aqueous solution in a canine kidney could increase the echo enhancement in the renal cortex.

The UCAs mentioned above did not fully meet the needs of the clinical diagnosis. Extensive studies were performed to develop UCAs that better meet clinical needs. The criteria for the desired UCAs are identified as follow:

- UCAs should be safe for injection
- UCA should be stable during the imaging examination
- UCA should have a size distribution, which allows the UCA to pass the capillaries while not leak from the blood pool.
- UCA should have a strong and specific acoustic response that can be detected by the medical ultrasound imaging system.

Gaseous MB is the most promising type of UCA that have the potential to meet the listed criteria. Considerable attention was turned to the development of the encapsulated gas bubbles. Protein, polymer, and phospholipid shells were used to prevent the gas core from rapid dissolution. Feinstein [42] employed a sonicator to generate agitation and cavitation in a solution of 5% human serum albumin. The air was encapsulated by the serum albumin and form MBs during the process. The resultant MBs were found to be able to pass the pulmonary circulation and be observed in the left ventricle [43]. At this stage, the first generation UCAs were more stable than the free MBs but still have a half-life less than 5 mins [44]. A longer lifespan was achieved for the second generation UCAs by replacing the air gas with low soluble gas, e.g., perfluoropropane, perfluorobutane. The high compressibility of the gas core of the MBs allows them to generate a strong and nonlinear acoustic response. Moreover, the resonance frequencies of the MBs with the size between 1 to 8 µm, i.e., below the diameter of the smallest pulmonary arteriole of approximately 10 µm, fail in the conventional medical ultrasound frequency range, i.e., 1 to 15 MHz. The merits of MBs and their great potential in different clinical scenarios encouraged the development of MBs as UCAs. During the 1990s,
MBs experienced their golden years. Fifteen UCAs candidates were under the development stage [45].

However, the development of UCAs witnessed their downturn after a “black box” warning announced by the U.S. Food and Drug Administration (FDA) in October 2007 [46]. The black box warning was announced consequentially to the reports of four patients’ deaths following the UCA injection. Since then, the urges to remove the warning label are continuously raised by the medical community. Large scale and multi-center studies were performed to demonstrate the safety of UCAs in an extensive variety of clinical scenarios [47, 48]. Those efforts might motivate the FDA to revise the warning to remove the disease-state contraindications and soften the requirement of the post-administration monitoring. Four out of the fifteen UCAs survived through the downturn, reached the market, and became commercially available. The current commercially available UCAs, namely Sonazoid, Definity, SonoVue, and Optison, are listed in Table 3.1.

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Shell material</th>
<th>Core</th>
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<tr>
<td>Sonazoid</td>
<td>Phosphatidylserine</td>
<td>Perfluorobutane</td>
</tr>
<tr>
<td>Definity</td>
<td>Phospholipid</td>
<td>Sulphur hexafluoride</td>
</tr>
<tr>
<td>SonoVue</td>
<td>Phospholipid</td>
<td>Octafluoropropane</td>
</tr>
<tr>
<td>Optison</td>
<td>Serum albumin</td>
<td>Octafluoropropane</td>
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### 3.2 The next generation of UCA

The next-generation UCA should meet the aforementioned criteria for conventional UCAs. Moreover, it should improve the diagnostic outcome and even extend the clinical benefits of the US system. The following criteria should be considered in the development of next-generation UCAs:

- UCA should be stable during storage and easy to use.
- UCA should be stable during the imaging examination.
- UCA should have a monodisperse size distribution.
- UCA should have a strong and unique acoustic response in the clinical diagnosis frequency.
- The acoustic response of UCA should be significantly different from the tissue.
- UCA should have the potential to be modified for extra features such as drug loading or targeting specific tissue.
- UCA should be biocompatible and safe.
3.3 Polyvinyl alcohol microbubble

Polyvinyl alcohol (PVA) is a biocompatible, non-toxic, non-carcinogenic, and bioadhesive polymer material. Moreover, PVA allows for easy modification via simple chemical reactions [49, 50]. Those features make PVA a widely used biomaterial in multiple medical applications. For instance, PVA is employed in contact lenses, eye wetting drops, catheters, leads, vascular embolic agents, tissue adhesion barriers, cartilage replacements, and other areas where the biocompatible surface coating is required [49, 50].

The PVA MB is a promising candidate for the next-generation UCA. The fabrication protocol of the PVA MBs has been developed by Cavalieri et al. [51]. Firstly, the PVAs are oxidated into telechelic PVAs. In the next step, the telechelic PVAs are cross-linked by acetalization and form the PVA shell under a strong shearing force.

The freeze-fracture electron microscopy revealed that the PVA shell is heterogeneous [52]. The density of the polymeric shell gradually decreases from the inner part (i.e., close to the gas core) towards the outer part [52]. Tortora et al. hypothesized that the PVA shell is compact near the gas core and becomes loosely forming a “hairy” structure at the surface with the surrounding liquid [52]. Indirect evidence of Tortora’s hypothesis could be found in other studies. For instance, it was reported that the mean shell thickness of PVA MBs is approximately 200 nm in the solution and 150 nm in the dried-state [53, 54]. Between the cross-linked PVA, Domenici [55] suggests that there are uncross-linked PVA chains entangled with cross-linked PVA and are released with the evolution of time. Fig. 3.1 is an illustration of the PVA shell.

![Illustration of the PVA MB](image)

Fig. 3.1 Illustration of the PVA MB (structures not to scale).

The PVA MB is stable and has a long lifespan, up to months [56]. It has a narrow size distribution with a mean diameter of approximately 3.4 μm. Its size allows the PVA MBs to pass the capillary lumen while not leak from the vessels. The size and yield of the PVA MBs
are dependent on the temperature and shear force during the fabrication process. Those effect factors were studied and reported by Zheng [57].

Efforts have been made to study the acoustic and mechanical properties of PVA MBs [53, 55, 58-62]. In the low-intensity region (typically below 100 kPa), the acoustic properties of PVA MBs were reported [55, 59, 63]. The reported attenuation coefficients of the PVA MB suspension are summarized in Table 3.2 [55, 59].

Table 3.2 attenuation coefficient of different PVA MB suspension

<table>
<thead>
<tr>
<th>Study</th>
<th>Peak attenuation coefficient normalized to the concentration of $10^6$ mL$^{-1}$ (dB·cm$^{-1}$)</th>
<th>Frequency of peak attenuation (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domenici’s [55]</td>
<td>31</td>
<td>11.5</td>
</tr>
<tr>
<td>Grishenkov’s *[59]</td>
<td>21.6</td>
<td>The attenuation increase monotonically</td>
</tr>
</tbody>
</table>

*The attenuation of MB-pH5-RT at room temperature (closest to other studies) is reported.

The inter-study variability could originate from the aging effects [55] and the accuracy of the transducer over a wide frequency range. It is worth mentioning that only one receiving transducer was used in Domenici’s [55] and Grishenkov’s [59] studies. The peak attenuation coefficient in Table 3.2 was normalized with the linear assumption between the attenuation coefficient and concentration.

The phase velocity of MBs suspension was measured in multiple studies. The phase velocity of MBs increase with the frequency of incidence ultrasound [59] and the environment temperature [59]. By contrast, the phase velocity decreases with the volume fraction increasing [55]. The lowest reported phase velocity was approximately 1435 m·s$^{-1}$ for an incidence frequency of 0.5 MHz at room temperature with a volume fraction of $5.5 \times 10^{-5}$ [55]. The highest reported phase velocity was around 1520 m·s$^{-1}$ for the incidence frequency of 13 MHz at 37 °C with an estimated volume fraction of $1.3 \times 10^{-9}$ (the volume fraction was estimated by concentration and mean diameter of the PVA MBs) [59].

The backscattered powers of PVA MBs fabricated in various environments were presented by Grishenkov [59] and Sciallero [63], listed in Table 3.3.
Table 3.3 Average backscattered power of different PVA MB suspensions.

<table>
<thead>
<tr>
<th>MBs type</th>
<th>Mean diameter (µm)</th>
<th>Concentration (mL⁻¹)</th>
<th>Backscattered power (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-pH5-RT*</td>
<td>4.1±0.7</td>
<td>3.7·10⁵</td>
<td>19</td>
</tr>
<tr>
<td>MB-pH5-Cool*</td>
<td>2.7±0.6</td>
<td>3.0·10⁶</td>
<td>21</td>
</tr>
<tr>
<td>MB-pH2-Cool*</td>
<td>2.6±0.5</td>
<td>6.5·10⁶</td>
<td>25</td>
</tr>
<tr>
<td>MBs-chem**</td>
<td>3.8±0.6</td>
<td>8.75·10⁵</td>
<td>18</td>
</tr>
<tr>
<td>MBs-phys**</td>
<td>3.8±0.6</td>
<td>1.0·10⁶</td>
<td>16</td>
</tr>
</tbody>
</table>

*pH5 and pH2 indicate that the pH value of PVA solution used in the PVA MB fabrication. The RT and Cool indicate that the temperatures of the PVA solution are at room temperature and 4°C, respectively.

**Chem indicates the SPIONs were linked to PVA MBs, via modified, by covalent binding. phys indicate the SPIONs were added to the PVA solution before the MBs formation.

The backscattered power changes at a maximum of 3.5 dB in the temperature range from 24 to 37 °C.

It is worth noting that the backscattered power does not increase monotonically with the concentration rising. The saturation and decrease of the backscattered power were observed for the MBs-phys and MBs-chem at the concentration level of 10⁶ mL⁻¹, respectively [63].

Those saturation and decrease, introduced by high attenuation, could cause shadow artifact. To avoid the shadow artifact, a concentration of PVA MBs lower than declining concentration, typically at the level of 10⁶ mL⁻¹, was recommended for further preclinical study [63].

Similarly, a large variation of the phase velocity, introduced by PVA MBs, could lead to an overestimation of velocity in the Doppler tissue imaging [64]. Cautious should be taken when choosing the concentration of PVA MBs in this type of application.

The comparison between PVA MBs and commercially available phospholipid UCA SonoVue (Bracco SpA, Milan, Italy) was reported by Grishenkov et al. [62]. The PVA MBs were found to have comparable or even larger second harmonic and high harmonic responses than SonoVue at the concentration level of 10⁶ mL⁻¹. Some typical values of the acoustic properties of the PVA MBs and SonoVue are reported in Table 3.4.
Table 3.4 Typical values of the acoustic properties of the PVA MBs and SonoVue.

<table>
<thead>
<tr>
<th>UCA name</th>
<th>Mean diameter (µm)</th>
<th>Peak attenuation normalized to concentration of $10^{-6}$ mL$^{-1}$ (dB·cm$^{-1}$)</th>
<th>Resonance frequency (MHz)</th>
<th>Peak backscattered power (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA MBs</td>
<td>3.7</td>
<td>16</td>
<td>12.1</td>
<td>10* [62]</td>
</tr>
<tr>
<td>SonoVue</td>
<td>2.5 [64]</td>
<td>88** [65]</td>
<td>2.5 [65]</td>
<td>6* [62]</td>
</tr>
</tbody>
</table>

*Excited at a frequency of 2.2 MHz with a concentration of $3.5 \times 10^{-6}$ mL$^{-1}$

**Estimated with the assumption that MBs oscillation does not interact [66].

The shell thickness of the PVA MBs was measured. The Cryo-TEM images of the PVA MB revealed that the shell thickness is about 200 nm [54]. Poehlmann et al. [53] measured shell thickness by employing AFM and TEM. The measured shell thickness is 215 and 206 nm for the AFM and TEM, respectively.

In the high-intensity region (typically above 100 kPa), the fatigue of the PVA MBs was investigated. The threshold level of the incident acoustic pressure, $P_{th}$, at which the shell of the MB fractures, was identified to be approximately 1.2 MPa at the frequency of 2.2 MHz [8]. Further investigations were performed by Grishenkov et al. [9] on the dependence of $P_{th}$ on the pulse composition, where both numbers of cycles in the pulse and excitation frequency were varied. It was concluded that below the $P_{th}$, nondestructively imaging, e.g., B-mode and pulse inversion imaging, could be used to detect the PVA MBs. Above the $P_{th}$, destruction/replenishment techniques offer the possibility to study tissue perfusion and delivery the therapeutic payload.

It should be noted that a unique pumping-out fracturing mechanism of the PVA MBs was reported by Kothapalli et al. [67]. Unlike other phospholipid and polymer MBs, no rapid gas release was observed during the fracturing of the PVA MBs. The air escapes from or through the PVA shell gradually via cracks, accumulates, and eventually dissolved in water [67].

### 3.4 Microbubble modeling

In general, models are representations of systems. They are tools for understanding the world. A validated model could reliably interpret observations and repeatably be utilized for prediction with a limited expense. Models were developed to describe the acoustic properties of UCAs. Emmer [68] classified those models into three classes, 1) model with acoustic scatter cross-sections, 2) Rayleigh-Plesset-like models, 3) finite element model. In this thesis, the Rayleigh-Plesset-like models are of particular interest in the current thesis.
3.4.1 The Rayleigh-Plesset equation

The models of the UCAs originate from the study of the cavitation phenomenon. Besant [69] investigated the dynamic of a cavity in an infinite mass of homogeneous incompressible fluid. Inspired by Besant, Rayleigh [5] calculated the pressure generated during the cavitation by assuming the internal pressure is zero or constant. However, Rayleigh acknowledged that under such an assumption, the velocity of the cavity boundary would become indefinite. It was suggested that the indefinite increase could be circumvented by introducing an internal pressure [5] that follows Boyle’s law.

Plesset [70] extended Rayleigh’s study by introducing varying external pressure. Additionally, the effects of surface tension and the vapor pressure of the water were considered for the pressure at the bubble surface. Noltingk and Neppiras expended the discussion to the gas-filled cavitation bubble. All contributions were summarized as the RPNNP (Rayleigh-Plesset-Noltingk-Neppiras-Poritsky) equation, also known as Rayleigh-Plesset equation [68, 71]. In general, the Rayleigh-Plesset equation could be expressed as (3.1). The Rayleigh-Plesset equation describes a pressure balance between the inertia of the liquid around the bubble associated with the bubble oscillation (on the left side) and the pressure difference at the bubble wall and infinity (on the right side).

\[ \rho \frac{3}{2} \dot{R}^2 + R \ddot{R} = P_I - P_\infty \]  

(3.1)

Table 3.5 summarizes all symbols used in the modeling.

The \( P_\infty \) consists of the ambient pressure and the external driven pressure. While the \( P_I \) can be calculated by pressure in the gas core minus the pressure contribution associated with surface tension and liquid viscosity. (3.1) can be expanding to (3.2)

\[ \rho \frac{3}{2} \dot{R}^2 + R \ddot{R} = P_{G,e} \left( \frac{R_0}{R} \right)^{3k} + P_v - \frac{2 \sigma}{R} - \frac{4 \mu \dot{R}}{R} - P_0 - P(t) \]  

(3.2)
Table 3.5 List of symbols used in the modeling

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R$</td>
<td>The radius of MB (with no shell or thin shell)</td>
</tr>
<tr>
<td>$\dot{R}$</td>
<td>The first derivative of the radius of MB with respect to time</td>
</tr>
<tr>
<td>$\ddot{R}$</td>
<td>The second derivative of the radius of MB with respect to time</td>
</tr>
<tr>
<td>$R_{10}$</td>
<td>The initial radius of MB</td>
</tr>
<tr>
<td>$R_1$</td>
<td>The inner radius of MB</td>
</tr>
<tr>
<td>$R_{1,lo}$</td>
<td>The initial inner radius of MB</td>
</tr>
<tr>
<td>$R_{1,e}$</td>
<td>The inner radius at the unstrained equilibrium position</td>
</tr>
<tr>
<td>$R_2$</td>
<td>The outer radius of MB</td>
</tr>
<tr>
<td>$R_{2,lo}$</td>
<td>The initial outer radius of MB</td>
</tr>
<tr>
<td>$U_1$</td>
<td>The speed of the gas-shell interface</td>
</tr>
<tr>
<td>$\dot{U}_1$</td>
<td>The acceleration of the gas-shell interface</td>
</tr>
<tr>
<td>$d$</td>
<td>The shell thickness</td>
</tr>
<tr>
<td>$\rho_L$</td>
<td>Liquid density</td>
</tr>
<tr>
<td>$\rho_s$</td>
<td>Shell density</td>
</tr>
<tr>
<td>$P_{G,e}$</td>
<td>The equilibrium pressure inside the gas core of the MB</td>
</tr>
<tr>
<td>$P_\infty$</td>
<td>The pressure at infinity</td>
</tr>
<tr>
<td>$P_0$</td>
<td>The ambient pressure</td>
</tr>
<tr>
<td>$P_l$</td>
<td>The pressure in the liquid at the bubble wall</td>
</tr>
<tr>
<td>$P(t)$</td>
<td>The external driven pressure as a function of time</td>
</tr>
<tr>
<td>$P_v$</td>
<td>The vapour pressure</td>
</tr>
<tr>
<td>$\mu_l$</td>
<td>Shear viscosity of the surrounding liquid</td>
</tr>
<tr>
<td>$G''(\omega)$</td>
<td>Loss modulus as a function of frequency</td>
</tr>
<tr>
<td>$\mu_s$</td>
<td>Shear viscosity of the shell</td>
</tr>
<tr>
<td>$\mu_{s0}$</td>
<td>Static shear viscosity of the shell</td>
</tr>
<tr>
<td>$\mu_{s1}$</td>
<td>Frequency-dependent shear viscosity of the shell</td>
</tr>
<tr>
<td>$\sigma_l$</td>
<td>Surface tension of the liquid</td>
</tr>
<tr>
<td>$\sigma_1$</td>
<td>Surface tension at the gas-shell interface</td>
</tr>
</tbody>
</table>
\[ \sigma_2 \quad \text{Surface tension at the shell-liquid interface} \]
\[ \kappa \quad \text{Polytropic gas exponent} \]
\[ G_S \quad \text{Shear modulus of the shell} \]
\[ G'(\omega) \quad \text{Storage modulus as a function of frequency} \]
\[ G_{Seq} \quad \text{Static shear modulus of the shell} \]
\[ G_{S1} \quad \text{Frequency-dependent shear modulus of the shell} \]
\[ c_m \quad \text{Speed of sound in the liquid-MB mixed medium} \]
\[ c_i \quad \text{Speed of sound in the liquid} \]
\[ k_m \quad \text{Wavenumber in the liquid-MB mixed medium} \]
\[ k_i \quad \text{Wavenumber in the liquid} \]
\[ \alpha \quad 1 + \left( \frac{\rho_L - \rho_S}{\rho_S} \right) R_{1,0}^{3,0} \]
\[ Z \quad 8 \frac{\sigma_2 R_{z,0}^2}{V_s G_S} \]
\[ V_s \quad R_2^3 - R_1^3 \]
\[ A \quad \text{Attenuation} \]
\[ \gamma(R) \quad \text{Effective surface tension} \]
\[ k^s \quad \text{Interfacial dilatational surface viscosity} \]

### 3.4.2 Ultrasound contrast agent model with coating

To date, the UCAs used in the clinical and research applications are gas-filled microbubbles coated with the shell. The coating of the MBs changes the dynamic of UCAs. Terms associated with shell elasticity and viscosity were introduced to describe the effects of the encapsulation. A generic expression was proposed by Katiyar [72].

\[ \rho \left[ \frac{2}{3} R^2 \dot{R} + R \ddot{R} \right] = P_{eq} \left( \frac{R_{to}}{R} \right)^{3 \kappa} \left( 1 - 3 \frac{3 \kappa R}{c_i} \right) - \frac{2}{R} \gamma(R) - \frac{4 R k^s}{R^2} k^s - \frac{4 \mu R}{R} \dot{R} - \rho_0 - P(t) \quad (3.3) \]

The \( \gamma(R) \) and \( k^s \) are introduced to interpret the effect of the shell on the MB’s dynamic. Worth noting that term \( 1 - \frac{3 \kappa R}{c_i} \) was introduced to incorporate radiation damping, and the vapor pressure was neglected.
In the early stage, the effective surface tension is considered linearly dependent on the MB surface area [71]. Table 3.6 summarizes the linear effective surface tension in the frequently cited models.

<table>
<thead>
<tr>
<th>Model name</th>
<th>Effective surface tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Jong’s model [73]</td>
<td>$\sigma_l + S_p \left( \frac{R}{R_{to}} - 1 \right)$</td>
</tr>
<tr>
<td>Church’s model [74]</td>
<td>$\sigma_1 + \sigma_2 + \frac{2V_x G_S}{R_2} (1 - \frac{R_{1e}}{R_1})$</td>
</tr>
<tr>
<td>Hoff’s model [75]</td>
<td>$6G_S d \frac{R_{to}^2}{R^2} (1 - \frac{R_{to}}{R})$</td>
</tr>
</tbody>
</table>

The ultra-high-speed visualization revealed nonlinear MB oscillation, e.g., buckling [67, 76] and cracks [67]. That nonlinear MB oscillation suggests that the linear shell assumption is invalid at large radius displacement.

Models with nonlinear shell assumptions were proposed. The nonlinear shell assumes that the effective surface tension is an S-shape-like function of the plane strain of the MB surface area. The effective surface tension is limited to zero for the compression caused MB buckling and to the surface tension of the surrounding liquid for the expansion caused MB rupture. For instance, Marmottant’s model [77] expresses the effective surface tension as (3.4)

$$\gamma(R) = \begin{cases} 
0, & R \leq R_b \\
\gamma \left( \frac{R^2}{R_b^2} - 1 \right), & R_b < R < R_r \\
\sigma_{w}, & R_r \leq R 
\end{cases}$$

The effective surface tension in Marmottant’s model [77] has an S-shape with sharp turning points at $R_b$ and $R_r$. Those sharp turning points are simplified expressions for the effective surface tension, which in reality is smooth. Sijl [76] modified the expression of the effective surface tension by smoothing it around the $R_b$ and $R_r$. Similar modifications were made by introducing linearly varying elasticity or elasticity with an exponential decay in Paul’s models [78]. Validation of the nonlinear effective surface tension was performed [79], which finds a good agreement between the measured effective surface tension and the assumed one in Marmottant’s model [77]. However, it is worth clarifying that the measurement of the effective surface tension was indirect. The effective surface tension was recovered by fitting the attenuation curve [79].
The nonlinear surface tension provides a better representation of the MB shell. For instance, the introduction of the nonlinear effective surface provides a lower resistance for the compression phase than the expansion phase, which explains the “compression-only” behavior of the MBs [2].

3.4.3 Subharmonic threshold predicted by models

The UCA models were used to investigate the generation of SH response. Eller and Flynn [80] applied perturbative analysis to a free gas MB model with surface tension and damping neglected. It was found that the pressure threshold, above which the SH response from the free gas MB presents, is dependent on the excitation frequency and is minimal at twice the resonance frequency. Prosperetti [81] applied a similar method to coated MBs to investigate the effect of the encapsulation on the SH threshold. It was found that the nonlinear properties of the shell lower the SH threshold significantly due to the inherently nonlinear feature of SH response [81]. Numerical simulations of MB oscillation based on various models revealed that the nonlinear shell properties, e.g., rupture and buckling, move the minimum threshold away from the twice the resonance frequency to a flatten valley around the resonance frequency of the MB [72].

3.4.4 The PVA MB model

Several models have been adapted for PVA MBs. Grishenkov [59] and Poehlmann [53] adapted the Church’s model [74] to the PVA MBs with frequency-dependent mechanical properties. On the other hand, Löffler [82] and Domenici [55] adapted Hoff’s model [75] to the PVA MBs with a thin shell assumption and frequency-independent mechanical properties. To simplify the calculation, all the models assume either the shell thickness is a constant value for all PVA MBs or the ratio between shell thickness and the PVA MB radius is constant. Table 3.7 summarizes the assumptions of PVA MB models.
Table 3.7 Summary of PVA MB models’ assumptions

<table>
<thead>
<tr>
<th>Author</th>
<th>Shell thickness assumption</th>
<th>Mechanical properties</th>
<th>Density gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grishenkov [59]</td>
<td>Constant ratio</td>
<td>Frequency-dependent</td>
<td>Homogenous</td>
</tr>
<tr>
<td>Poehlmann [53]</td>
<td>Constant thickness</td>
<td>Frequency-dependent</td>
<td>Homogenous</td>
</tr>
<tr>
<td>Löffler [82]</td>
<td>Constant thickness and constant ratio*</td>
<td>Frequency-independent</td>
<td>Homogenous</td>
</tr>
<tr>
<td>Domenici [55]</td>
<td>Constant thickness**</td>
<td>Frequency-independent</td>
<td>Heterogeneous</td>
</tr>
</tbody>
</table>

*Two models, one with constant thickness assumption another with constant ratio assumption, were studied in Löffler’s study.
**In Domenici’s study, the shell thickness is independent of the radius, but a function of time.

The PVA MB has a thick PVA shell with a shell thickness of approximately 200 nm [53, 54]. Thus, the thin shell assumption in Hoff’s model becomes invalid. Furthermore, Poehlmann et al. [53] measured the shell thickness of then PVA MBs and found the shell thickness is independent of the radius. Thus, the constant thickness assumption represents the PVA MB better than the constant ratio assumption. Moreover, the PVA shell has frequency-dependent mechanical properties [59]. Therefore, the frequency-independent mechanical properties assumption leads to a poor fitting of the attenuation coefficient in the low-frequency region in both studies of Löffler and Domenici [55, 82]. The poor fitting could be addressed by introducing frequency-dependent mechanical properties. Last but not least, the PVA shell has a heterogeneous density [55]. The inner shell (close to the gas core) is compact and has a high density. The outer shell is loose and has a low density. The homogenous assumption on the shell density could cause the overestimate of the shell thickness.
4 Method and Theory

This chapter presents the method and theory employed in Studies I to IV. Firstly, the PVA MB model in Study II is introduced. Subsequently, methods used in Studies I to IV are described with details in the appended papers.

4.1 Theory of the PVA MB model

In the current thesis, the Church’s model was adapted for the PVA MBs. The governing equation can be expressed as (4.1)

\[ R_1 \dot{U}_1 \left[ 1 + \left( \frac{\rho_L - \rho_S}{\rho_S} \right) \frac{R_1}{R_2} \right] + U_1^2 \left[ \frac{3}{2} + \left( \frac{\rho_L - \rho_S}{\rho_S} \right) \left( \frac{4R_2^3 - R_1^3}{R_2} \right) \right] \]

\[ = \frac{1}{\rho_S} \left( \frac{P_{g,a}}{R_1^3} \right) - \frac{2\sigma_1}{R_1} - \frac{2\sigma_2}{R_2} - 4 \frac{U_1}{R_1} \left( \frac{V_S \mu_S + R_1^3 \mu_L}{R_2^3} \right) \]  

(4.1)

The \( \sigma_1 \) is set to be zero and \( \sigma_2 \) is set to be 0.051 N m\(^{-1}\) following Doinikov [83] and Morgan [84].

At small-amplitude oscillation of MBs, the coefficients are:

\[ \alpha = 1 + \left( \frac{\rho_L - \rho_S}{\rho_S} \right) \frac{R_{1,lo}}{R_{2,lo}} \]  

(4.2a)

\[ \omega_0^2 = \frac{1}{ap_3 R_{1,lo}^2} \left[ 3KP_0 - \left( \frac{\rho_L - \rho_S}{\rho_S} \right) \frac{R_{1,lo}}{R_{2,lo}} - \frac{2\sigma_2 R_{1,lo}^2}{R_{2,lo}^2} \right. \]

\[ + \frac{4}{R_{2,lo}^2} \left( V_S \mu_S(a) + \frac{R_{1,lo}^3 \mu_L}{R_{2,lo}^3} \right) \left[ 1 + Z \left( 1 + \frac{3R_{1,lo}^2}{R_{2,lo}^2} \right) \right] \]  

(4.2b)

\[ \sigma_d = \frac{4}{ap_3 R_{1,lo}^2} \left( \frac{V_S \mu_S(a) + R_{1,lo}^3 \mu_L}{R_{2,lo}^3} \right) \]  

(4.2c)
\[ R_{1,e} = R_{1,t0} \left[ 1 + \frac{\sigma_1 R_{1,t0}^2}{2 V_s G_s} \right] \] (4.2d)

\[ G_S(\omega) = G'(\omega) = G_{seq} + G_{s1} \omega^2 \] (4.2e)

\[ \mu_S(\omega) = \frac{G''(\omega)}{\omega} = \mu_{s0} - \mu_{s1} \omega \] (4.2f)

Letting

\[ \frac{k_m}{c_m} = \frac{c_l}{c_m} = u + iv \] (4.3)

The complex speed of sound \( c_m \) could be expressed as (4.4):

\[ \frac{c_l^2}{c_m^2} = 1 + \frac{4\pi \rho c_l^2}{\alpha \rho_s} \int_0^\infty R_{1,t0} f(R_{1,t0}) dR_{1,t0} \] (4.4)

The attenuation \( A \) could be calculated by (4.5)

\[ A = -20(\log_{10} e) \text{Im}(k_m) = -20(\log_{10} e) \frac{\omega \nu}{c_w} \] (4.5)

### 4.2 Study I PVA MB detection

#### 4.2.1 Fabrication of PVA MBs.

The PVA MBs are fabricated following the protocol from Cavalieri [51]. Firstly, 200 mL PVA solution with a concentration of 0.02 g/mL was prepared by dissolving PVA powder (Sigma Aldrich, MO, USA) in Milli-Q water. The PVA solution was then heated up to 80°C. 380 mg of NaIO₄ (Sigma Aldrich, MO, USA) were introduced to the solution to oxidate PVA into telechelic PVAs. The oxidation reaction lasted for one hour at a temperature of 80°C. Strong shearing force is applied to the resultant solution by a homogenizer (Ultra-turrax, IKA, Königswinter Germany) at the air/water interface for two hours. After the stirring process, the transparent solution becomes a milk-like suspension, which contains rich PVA MBs. The PVA MBs are collected and washed with Milli-Q water ten times with a time interval of 24 hours. The PVA MBs sample used in this study has a mean diameter of 3.4±0.9 µm and a concentration of 2·10⁶ mL⁻¹.

#### 4.2.2 Ultrasound visualization

A tissue-mimicking phantom (Model 524 Peripheral Vascular Doppler Flow Phantom, ATS Laboratories) is employed. The phantom consists of two wall-less channels with diameters...
of 6 and 8 mm, respectively. A peristaltic pump is utilized to circulate the fresh PVA MBs sample through the phantom via the 8 mm channel. A L7-4 transducer (linear array L7-4, Advanced Technology Laboratories, Philips, WA, USA) is placed on the top of the phantom in such a way that the two wall-less channels can be observed in a single ultrasound image. Four ultrasound sequences (listed in Table 4.1) are emitted at various PNP. The ultrasound pulses are transmitted at a single frequency, either 3 or 9 MHz, for PI, SH, and UH imaging techniques. For CPS4, two ultrasound pulse groups are transmitted at the frequency of 3 and 9 MHz, respectively. The pulses in each group have the same frequency but an inverted phase. For all imaging techniques, ultrasound focused at 128 points distributed homogenously on the horizontal line at a depth of 17.2 mm in the phantom.

Table 4.1 The ultrasound sequences considered in study I

<table>
<thead>
<tr>
<th>Sequence technique</th>
<th>Transmission frequency (MHz)</th>
<th>Receive filter bandwidth (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>3</td>
<td>5.4 to 6.6</td>
</tr>
<tr>
<td>SH</td>
<td>9</td>
<td>3.8 to 5.2</td>
</tr>
<tr>
<td>UH</td>
<td>3</td>
<td>3.8 to 5.2</td>
</tr>
<tr>
<td>CPS4</td>
<td>3 and 9</td>
<td>3.8 to 5.2</td>
</tr>
</tbody>
</table>

US images are acquired for the four imaging techniques at four voltages range from 20 to 80 V. The rejection thresholds (pixel intensity) as 6, 13, 20, and 27 were set for the whole images at the transmitting voltage of 20, 40, 60, and 80 V, respectively. The performance of the four imaging techniques was evaluated by calculating the contrast to tissue (CTR). The CTR of each image was determined by the ratio between the mean pixel intensity of the sample and tissue. It was calculated following (4.6)

$$\text{CTR} = 20 \times \log_{10} \frac{\text{mean pixel intensity of sample}}{\text{mean pixel intensity of tissue}}$$

(4.6)

The region of interest (ROI) for the sample is selected in the center of the 8 mm channel at a depth of around 17.4 mm. The ROI for the tissue is selected in the middle of the two channels at a depth of around 17.4. The pixel intensity is proportional to the echo amplitude. Fig. 4.1 is an illustration of the ROI selection.
Fig. 4.1. The ROI for the sample is inside the yellow box on the left. The ROI for tissue is inside the blue box in the middle.

The PNP at the focuses in the phantom was estimated following (4.7)

\[
P_e = \frac{P_v}{10^{-\frac{\alpha \cdot d \cdot f}{20}}}
\]  

(4.7)

Where \( P_v \) is the PNP obtained in water, \( d \) is the depth of focus, \( \alpha \) is the attenuation coefficient of the phantom, and \( f \) is the ultrasound frequency.

The PNPs of the excitation ultrasound waves at the various voltage are presented in Fig 4.2

![Fig. 4.2. Estimated PNPs of excitation ultrasound waves.](image-url)
4.3 Study II Optimization of PVA MB detection

4.3.1 Fabrication of PVA MBs.

The PVA MBs were fabricated following the protocol described in 4.2.1.

4.3.2 PVA MBs isolation

The PVA MBs were processed with isolation to remove the small MBs. The isolation procedure was adapted from Feshitan et al. [85]. 50 mL PVA MB suspension was loaded in a separation funnel of 50 mL. The separation funnel was then held by a retort stand for 110 min. The lower part of the suspension, which contains small MBs, was removed and replaced by Milli-Q water. This procedure was repeated five times in total. Fig. 4.3 is an illustration of the isolation procedure.

![Illustration of the isolation process](image)

Fig. 4.3. Illustration of the isolation process

4.3.3 Attenuation measurement

The attenuation of the PVA MB suspension was measured using the experiment set up illustrated in Fig. 4.4. The PVA MBs suspension with a mean diameter of 3.71±0.75 µm and a concentration of $10^6$ mL$^{-1}$ was loaded into a cell with a thickness of 14 mm. An oscilloscope was employed to trigger and display the transmitted and received ultrasound waves. A pulser/receiver (Panametrics PR 5072, Waltham, Ma, USA), connected to the oscilloscope, excited the transducers and received the signal from the transducers. Five flat transducers (listed in table 4.2) were immersed in a water tank (only one at a time) with degassed water and subsequently used to produce a pressure field below 100 kPa. An aluminium reflector was located at the bottom of the water tank to reflect the ultrasound wave. The reflected ultrasound waves were recorded and transferred into the frequency domain. Using the spectrum of the reflected ultrasound waves, attenuation was calculated following (4.8)
\[ \alpha = -\frac{20}{2L} \left( \log_{10} \left| \frac{s_{MB}(\omega)}{s_{ref}(\omega)} \right| \right) \]  

(4.8)

Where \( s_{MB} \) is the spectrum of reflected ultrasound waves traveling through the PVA MB suspension, \( s_{ref}(\omega) \) is the spectrum of reflected ultrasound waves traveling with absence of PVA MB suspension. \( L \) is the dimension of the cell along the wave propagation direction.

Fig. 4.4. Schematic diagram of the experiment set up for the attenuation measurement

Table 4.2 Transducers used in the attenuation measurement

<table>
<thead>
<tr>
<th>Transducer model</th>
<th>Labeled center frequency (MHz)</th>
<th>-6 dB Bandwidth (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panametrics V306</td>
<td>2.35</td>
<td>1.59 to 3.00</td>
</tr>
<tr>
<td>Panametrics V382</td>
<td>3.5</td>
<td>2.28 to 4.40</td>
</tr>
<tr>
<td>Panametrics V309</td>
<td>5</td>
<td>3.43 to 6.07</td>
</tr>
<tr>
<td>Panametrics V311</td>
<td>10</td>
<td>5.09 to 11.16</td>
</tr>
<tr>
<td>Panametrics V319</td>
<td>15</td>
<td>8.69 to 14.11*</td>
</tr>
</tbody>
</table>

*The Labeled center frequency of Panametrics V319 is not in the measured -6 dB bandwidth.

4.3.4 Model calibration

The mechanic parameters were estimated by fitting the simulated attenuation coefficient to the measured attenuation coefficient.

4.3.5 Ultrasound visualization

The experiment set up used in 4.2.2 was employed. US images were obtained with four sequences listed in Table 4.3. In every test, ten images were obtained for each sequence. All tests were repeated three times. The PNP of the excitation wave for SH and UH were approximately 0.59 and 1.46 MPa, respectively.
### Table 4.3 The excitation and receiving frequency of the CPS4

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>UH excitation frequency (MHz)</th>
<th>SH excitation frequency (MHz)</th>
<th>Receiving frequency (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 1</td>
<td>3</td>
<td>9</td>
<td>4.5</td>
</tr>
<tr>
<td>Sequence 2</td>
<td>3.33</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Sequence 3</td>
<td>3.91</td>
<td>11.25</td>
<td>5.63</td>
</tr>
<tr>
<td>Sequence 4</td>
<td>4.29</td>
<td>12.86</td>
<td>6.43</td>
</tr>
</tbody>
</table>

The PVA MBs used in the imaging experiment experienced a shorter standing time of 60 min. Consequently, the PVA MBs used in the imaging had a larger mean diameter of 3.86 ± 0.79 µm. The concentration of the PVA MB sample was $2 \cdot 10^6$ mL$^{-1}$.

### 4.3.6 Imaging analyzing

The CTR was calculated for all US images following the same method described in 4.2.2.

### 4.4 Study III Towards dual-modal contrast agent

#### 4.4.1 Fabrication of PVA MBs.

The PVA MBs were fabricated following the protocol described in 4.2.1.

#### 4.4.2 Incorporation of the PVA MB with the gold nanoparticles

Two methods were applied to incorporate the PVA MBs with the gold nanoparticles to support CT and US. The resultant candidates are named as AuNP-S-MB and AuNP-Capsule. The AuNP-S-MB was fabricated by adding gold nanoparticles during the shell formation of PVA MB. While the AuNP-Capsule was fabricated by changing the shell permeability of PVA MB and loading gold nanoparticles into its air-core.

#### 4.4.3 Microscopy examinations

The plain PVA MBs, AuNP-S-MB, and AuNP-Capsule were diluted 20 times with MiliQ water and loaded in a counting chamber (Brand GmbH, Wertheim, Germany). An optical microscope (ECLIPSE Ci-S, Nikon, Tokyo, Japan) equipped with a 20x objective, was employed to observe the three samples. Eight images were obtained for each sample. The images were analyzed by ImageJ to determine the size and concentrations of the sample.
4.4.4 Acoustic characterization

A high-power tone burst pulser-receiver (SNAP-Mark4, RITEC Inc, Warwick, USA) and a flat single element transducer (V382, Olympus NDT, Waltham, USA) with the central frequency of 3.5 MHz were connected and utilized to measure the pressure dependence of the acoustic attenuation coefficient of the plain MB suspension with a concentration of $10^7$ mL$^{-1}$. The plain MB suspension was loaded in a cell and immersed in a water tank. The transducer, cell, and aluminum reflector are aligned as Fig 4.5.

![Fig. 4.5. Schematic diagram of the experiment set up for the pressure-dependent attenuation measurement.](image)

4.4.5 X-ray characterization

1.5 mL gold nanoparticles solution, plain PVA MBs, AuNP-S-MB, AuNP-Capsule, and AuNP-Capsule supernatant were loaded in the Eppendorf tubes, respectively. The CT images of the samples were obtained by the quantum FX-CT micro-CT (PerkinElmer Inc, MA, USA) at the voltage of 50 kV, 70 kV and 90 kV with a current of 200 mA, and exposure time of 120 s. The images were processed by MiaLab (Mia-Solution AB, Stockholm, Sweden) to obtain the CT number of the samples.
4.5 Study IV Effect of PVA MB on cavitation

4.5.1 Fabrication of PVA MBs

The PVA MBs were fabricated following the protocol described in 4.2.1.

4.5.2 Cavitation assessment

The experimental set-up to assess the cavitation was built as Fig. 4.6. The flows of water and PVA MB suspension were driven by the high-pressure gas through the microfluidic devices. The pressure at the inlet varies from 1 to 7 MPa. Meanwhile, the pressure at the outlet is constant at 0.1 MPa. Three microfluidic devices were fabricated with the same geometry but different surface roughness and sidewall roughness, see Table 4.4. The three microfluidic devices have a smooth channel, a side wall roughened channel, and a surface roughened channel, respectively. The cavitating flow inside the microfluidic device was observed by a high-speed CMOS camera equipped with a macro-camera lens (type K2 DistaMax).

Fig. 4.6 Schematic diagram of the experiment set up (structures not to scale)
Table 4.4 The working conditions and geometric properties of the microfluidics devices

<table>
<thead>
<tr>
<th></th>
<th>Smooth</th>
<th>Surface roughened</th>
<th>Sidewall roughened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivalent sand-grain roughness</td>
<td>0</td>
<td>5.863 μm</td>
<td>5.863 μm</td>
</tr>
<tr>
<td>Arithmetic surface roughness</td>
<td>0</td>
<td>1 μm</td>
<td>1 μm</td>
</tr>
<tr>
<td>Side wall roughness</td>
<td>N/A</td>
<td>N/A</td>
<td>0.1 D_h</td>
</tr>
<tr>
<td>Microchannel length</td>
<td>1.6 mm</td>
<td>1.6 mm</td>
<td>1.6 mm</td>
</tr>
<tr>
<td>Microchannel hydraulic diameter</td>
<td>86 μm</td>
<td>86 μm</td>
<td>89 μm</td>
</tr>
<tr>
<td>Extended channel width</td>
<td>900 μm</td>
<td>900 μm</td>
<td>900 μm</td>
</tr>
</tbody>
</table>
5 Results

The main results of the four studies included in this thesis are highlighted in this chapter with details in the appended papers.

5.1 Study I PVA MB detection

Fig 5.1 demonstrates the gray-scale images for all imaging techniques at different PNP. In general, the overall pixel intensity increases with the PNP increases. Besides, the PI provides the highest signal from the PVA MBs, while the CPS4 provides the lowest tissue signal.

![Image of US images of PI, CPS4, SH, and UH imaging techniques with estimated PNP at 3 and 9 MHz (MPa)]

Fig. 5.1. US images of the PI, CPS4, SH, and UH imaging techniques. The channel on the left contains PVA MBs, the channel on the right contains degassed water.
The CTRs of all imaging techniques are displayed in Fig. 5.2. Overall, the CTRs increase with the increase of PNP. The CPS4 has the highest CTR among the four imaging techniques considered in Study I at all PNP, followed by PI. It is worth noting that the SH response was not fully developed in Study I.

![Graph showing CTRs for different imaging techniques at various PNP](image)

Fig. 5.2. CTRs of the SH, UH, PI, and CPS4 at different PNP.

### 5.2 Study II Optimization of PVA MB detection

Table 5.1 reports the estimation of the parameters in the PVA MB model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell thickness</td>
<td>125 nm</td>
</tr>
<tr>
<td>Static shear viscosity of the shell</td>
<td>0.9 Pa s</td>
</tr>
<tr>
<td>Frequency-dependent shear viscosity of the shell</td>
<td>$7.43 \times 10^{-9}$ Pa s$^2$ rad$^{-1}$</td>
</tr>
<tr>
<td>Static shear modulus of the shell</td>
<td>11.5 MPa</td>
</tr>
<tr>
<td>Frequency-dependent shear modulus of</td>
<td>7.8 Pa (s$\cdot$rad)$^{3/4}$</td>
</tr>
<tr>
<td>Compensation factor</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The simulated attenuation of the PVA MBs used in calibration is shown in Fig. 5.3. The attenuation coefficient increases from 2 MHz and plateau around 15 to 16 dB/cm in the
excitation frequency between 11 to 12 MHz. The damped resonance frequency, at which the simulated attenuation coefficient is the highest, of the PVA MBs is identified as 12.14 MHz.

![Resonance frequency f = 12.14](image)

Fig. 5.3. Simulated and measured attenuation coefficient of the PVA MBs used in the attenuation measurement.

The simulated attenuation spectrums of the PVA MB used in the imaging and calibration are compared (Fig. 5.4). The damped resonance frequency of the PVA MB used in imaging is estimated at 10.85 MHz. It is lower than that of the PVA MB used in calibration because the size of the PVA MBs in calibration is smaller.

![Simulated attenuation spectrums](image)

Fig. 5.4. The simulated attenuation spectrums of the PVA MB used in the imaging and calibration
Based on the simulation result, the predicted optimal SH excitation frequencies is 10.85 MHz. The corresponding UH excitation frequencies and receiving frequency is 3.62 and 5.43 MHz, respectively.

Fig. 5.5 shows that the CTR of the CPS4 with PVA MBs in water increases from sequence 1 to 3 as the SH excitation frequency increases. The highest CTR of CPS4 with PVA MBs in the water of 12.6 dB is provided by sequence 3, which has the SH excitation frequency (11.25 MHz) in line with the predicated optimal SH excitation frequency (10.85 MHz). Further increasing SH excitation frequency instead decreases the CTR. The introduction of AB reduces the CTR for all sequences. The highest CTR of PVA MB in AB is achieved by sequence 4.

5.3 Study III Towards dual-modal contrast agent

The Microscopy examinations of plain MB, AuNP-S-MB, and AuNP-Capsule revealed their similar narrow size distribution (see Fig. 5.6) with a mean diameter of 3.4±0.8, 3.2±0.7, and 3.5±0.8 µm.
The attenuation coefficients of the plain PVA MBs are shown in Fig. 5.7. The attenuation coefficient at fundamental frequency remains constant at around 4.2 dB/(cm·MHz) below the PNP of 100 kPa. The decrease of the attenuation coefficient at fundamental frequency was observed at the PNP above 100 kPa. The attenuation coefficient at the second harmonic increases from 2.2 to 2.7 dB/(cm·MHz) below the PNP of 100 kPa and plateaus around 3 dB/(cm·MHz) in the PNP range between 100 to 230 kPa before dropping. The attenuation coefficient at the third harmonic increases from 1.1 to 1.9 dB/(cm·MHz) below the PNP of 230 kPa and decreases from 1.9 to 1.55 dB/(cm·MHz) above the PNP of 230 kPa.
The CT-numbers of gold nanoparticles solution, plain PVA MBs, AuNP-S-MB, AuNP-Capsule, and AuNP-Capsule supernatant are shown in Fig. 5.8. The gold nanoparticles solution has the highest CT-number, followed by AuNP-Capsule at all voltages. The CT-number of plain PVA MBs, AuNP-S-MB, and AuNP-Capsule supernatant remains low at all voltages considered in the current study.

![Bar chart showing CT-numbers of different samples](image)

Fig. 5.8 CT-number of gold nanoparticles solution, plain PVA MBs, AuNP-S-MB, AuNP-Capsule, and AuNP-Capsule supernatant

### 5.4 Study IV Effects of PVA MB on cavitation

The results revealed that cavitation inception PVA MBs occurs at a lower upstream pressure than the pure water, especially with the sidewall roughened channel; see Fig. 5.9. Moreover, the cavitation flows are more difficult to suppress with the presence of PVA MBs. Fig. 5.10 shows that at the same upstream pressure, the PVA MBs suspension generates a larger cavitation flow compare to the pure water. Besides, the pressure of the shock wave generated by the cavitation was estimated. The shock wave pressure generated by PVA MBs in the sidewall roughened channel is the highest, while the water in the smooth wall channel generates the lowest shock wave pressure. The shock wave pressure can reach as high as 60 MPa in the case of upstream pressure of 1 MPa, and 180 MPa under upstream pressure of 7 MPa.
Fig. 5.9. The inception of twin cavities inside different microchannels in the cases of PVA MBs [(a)–(c)] and water [(d)–(f)].

Fig. 5.10. The development of cavitating flows inside the side wall roughened microchannel with increasing upstream pressure for the cases of PVA MB suspension (upper row) and water (lower row).
6 Discussion

In Study I, the CPS4 demonstrated a higher efficiency (i.e., higher CTR) to detect the presence of the PVA MBs than the conventional imaging techniques. In Study II, the CPS4 was customized to PVA MBs to optimize its performance by setting the SH excitation frequency at the damped resonance frequency predicted by the PVA MB model.

In Study III, it was found that a sufficient amount of gold nanoparticles could be loaded and locked in the core of the AuNP-Capsule by increasing the permeability of the PVA shell.

In Study IV, it was revealed that the PVA MB suspension employed as working fluid could intensify the cavitation flow.

6.1 The introduction and optimization of the CPS4

The pixel intensity from the PVA MBs increases with the PNP because the nonlinear responses, including SH, UH, and second harmonic, are more pronounced at higher PNP. The pixel intensity from the tissue also increases with the PNP. The growing propagation distortion increases the pixel intensity from the tissue for the PI. It is reported that the tissue does not produce a significant UH and SH response [86, 87]. However, the UH and SH signal from the tissue-mimicking phantom was found ample in the images acquired with the current experimental setup, probably due to the spectral leakage, i.e., the transmitted ultrasound wave contains the frequency component at the UH and SH due to the limited pulse duration. The spectral leakage is more severe at a higher PNP. Therefore, the pixel intensity from the tissue increases with the PNP for the CPS4, UH, and SH. Increasing the transmitted pulse duration could reduce the spectral leakage and improve the CTR of CPS4 UH and SH. However, this would degrade the axial resolution as a cost.

The PI obtained the highest pixel intensity from the PVA MBs. While the CPS4 provided the lowest pixel intensity from the tissue. Moreover, the CPS4 provides the highest CTR because it has the lowest tissue signal and moderate UCAs signal. The alternative method to combine PI, SH, and UH was proposed by Shekhar [88]. In contrast with CPS4, Shekhar’s sequence transmits at the same frequency ($f$) to excite SH and UH signal, while listens at SH ($\frac{1}{2}f$) and
UH \( \frac{3}{2} f \) frequencies [88]. Listening concurrently at SH and UH frequencies requires a broadband transducer, which introduces additional noise to the tissue signal and degrades the CTR. Besides, the production of such a broadband transducer is a huge challenge. The CPS4 receives the SH and UH signals at the same frequency. Thus, only a conventional transducer with a common bandwidth is required for the CPS4. Consequently, the additional noise caused by the broadband transducer is avoided.

The PNP's of the excitation waves were estimated by the linear assumption of the attenuation coefficient. Errors may occur if the assumption fails at the high transmission frequency considered in this section.

It could be expected that similar results would be obtained for CPS4 in conjunction with other UCAs. Nevertheless, validations are recommended before extending the conclusion.

It is worth noting that the SH response in Study I was not fully developed. In Study II, efforts were made to optimize CPS4 to the PVA MBs by boosting the SH response.

As mentioned in 3.4.3, the nonlinearity of the PVA shell shifts the minimum SH pressure threshold away from twice the resonance frequency to the flatten valley around the resonance frequency [72]. The PVA MBs have a narrow size distribution. Therefore, the resonance frequencies of the PVA MBs are in the proximity of the damped resonance frequency of the PVA MBs population. Moreover, at the damped resonance frequency, the total power absorbed by all PVA MBs is the highest, which leads to large oscillation amplitude and thus a high chance for the SH generation. Therefore, the damped resonance frequency of the PVA MBs is considered as the optimal SH excitation frequency.

To predict the damped resonance frequency, the improved PVA MB model was proposed and calibrated. The parametric study showed that the estimated shell thickness of the PVA MB is 125 nm, which is lower than the shell thickness measured in the hydrate-state (around 200 nm) and close to the thickness measured in the dried-state [53]. This suggests that the PVA shell could be roughly divided into two parts 1) high-density part, which is close to the gas core and provides mechanical properties 2) low-density part, which is close to the surrounding liquid, has a ‘hairy’ structure, and provides negligible mechanical properties.

The improved PVA MB model provides a good fit at all frequencies considered in the current study. In contrast, a poor fitting in the low-frequency region was observed in other models with the assumption that the mechanical properties are frequency-independent [55, 82].

To the best of our knowledge, the current PVA MB model is the only model of the PVA MB that considers the frequency-dependent properties and the density gradient of the PVA shell at the same time.

It should be noted that the model employed in Study II assumes the oscillation amplitude to be moderate. The model needs to be modified to describe a large amplitude oscillation.
The predicted damped resonance frequency of the PVA MBs used in the imaging is 10.85 MHz. Sequence 3 has the SH excitation frequency of 11.25 MHz, which is the closest to the damped resonance frequency. Sequence 3 with PVA MBs in water provided the highest CTR of 12.6 dB in the in vitro experiment. That agrees with the optimal SH excitation frequency predicted by the PVA MB model. As mentioned above, it is expected that the SH pressure threshold is low and the oscillation amplitude is large at the damped resonance frequency. Thus, it is likely that the highest number of PVA MBs in water was excited to generate the SH signal with sequence 3.

The introduction of AB degraded the CTR of all sequences. The highest CTR of 10 dB was provided by sequence 4. The reduction of the CTR with the presence of AB was inconsistent at different sequences. A better understanding of the effects of high viscosity liquid and small particles on the MBs oscillation is required when extending the model guided optimization method towards the clinical application.

6.2 Modification of PVA MB to support CT and US

The similar narrow size distribution and mean diameter of the plain MB, AuNP-S-MB, and AuNP-Capsule indicate that the modifications of the PVA MBs do not significantly change the size of the PVA MBs. Similarly, no significantly geometric changes were observed when magnetite nanoparticles were incorporated with PVA MBs [24].

The acoustic characterization revealed the nonlinear ultrasound response of plain MBs occurred already at PNP as low as 100 kPa, indicating the potential of AuNP-S-MB and AuNP-Capsule to work as UCAs.

The radiography test results denoted that only a negligible amount of gold nanoparticles had been embedded into the shell of AuNP-S-MB, because CT-number of AuNP-S-MB was at the same level of plain PVA MBs and water. A considerable amount of gold nanoparticles had been loaded into the core of AuNP-Capsule, substituting the air. Moreover, the CT-number of AuNP-Capsule supernatant was similar to water. This suggests that the gold nanoparticles were locked inside the core of AuNP-Capsule.

It was concluded that loading the gold nanoparticles into the core of the PVA MB is a promising route to develop a dual-modal contrast agent to support CT and US.

6.3 Effects of PVA MBs on cavitation flow

In Study IV, the cavitation inception, developed cavitating flow, and supercavitation were visualized inside the microfluidic devices. The bubbly flow was not observed because of the relatively high surface tension of the PVA MBs suspension and water. The PVA MBs suspension as a working fluid triggers inception at a lower upstream pressure and enhances
the cavitating flow intensity due to the higher possibility of heterogeneous nucleation. The existence of the PVA MBs increases the surface area and provides more nucleation sites inside the microfluidic device. In contrast, homogeneous nucleation is more dominant for the pure water as a working fluid neglecting the existence of the dissolved gas content inside the fluid. Thus, according to our observations, the inception occurred at a relatively higher pressure, and the cavitating flow developed at higher upstream pressures for water compared to the PVA MBs suspension. Moreover, the results revealed that the presence of the PVA MBs decreases the desinent cavitation number, i.e., the disappearance of the cavitation bubbles takes a longer time in the PVA MBs suspension.

This study gives valuable insight into the improvement of the medical protocols and devices, which involve MBs. For instance, the safety of the MBs injection on patients with prosthetic heart valves should be assessed because the MBs might trigger or enhanced the cavitation on prosthetic heart valves and thus causes thromboembolic complications. Moreover, the protocol and the device for the MBs injection might be improved to avoid cavitation, which reduces the concentration of the MBs.
7 Conclusion

In the current thesis, the modification of the PVA MBs was investigated. The detecting technique for PVA MBs was introduced and optimized. The effect of PVA MBs on the intensification of the hydrodynamic cavitating flow was demonstrated. The conclusions related to each of the papers are presented as follows.

**Study I** and **Study II** introduced a novel contrast pulse sequence, CPS4, and optimized the CPS4 for the detection of the PVA MBs. In **Study I**, the CPS4, a combination of SH, UH, and PI, was first introduced. The *in vitro* experiment revealed that the CPS4 provides a higher CTR than the conventional SH, UH, and PI imaging techniques, i.e., the CPS4 has superior performance than the reference techniques. In **Study II**, model-guided customization of the CPS4 for the PVA MBs was demonstrated. A PVA MB model was adapted from Church’s model to describe the dynamics of the PVA MB. The parametric study recovered the mechanical properties of the PVA MB and indicated that the shell could be divided into two parts, the inner part with a high-density (125 to 150 nm thick) and the outer part with a low-density ‘hairy’ structure (50 to 75 nm). The CPS4 was optimized by setting the SH excitation frequency at the damped resonance frequency of the PVA MBs suspension, which is predicted by the PVA MB model. The performance of the optimized imaging sequence was verified by imaging the PVA MBs in a tissue-mimicking phantom. The result indicated that the best performance of the CPS4 was achieved by setting the SH excitation frequency near the damped resonance frequency.

**Study III** explored the feasibility of modifying PVA MB to support both CT and US imaging. Two candidates of the dual-modal contrast agent, AuNP-S-MB and AuNP-Capsule, were fabricated by embedding gold-nanoparticles in the PVA shell and loading the gold-nanoparticles into the air-core of the PVA MBs, respectively. The modification of the PVA MBs to the AuNP-S-MB and AuNP-Capsule does not change their size significantly. The plain PVA MBs demonstrate nonlinear acoustic response at PNP of the excitation wave as low as 100 kPa, making AuNP-S-MB and AuNP-Capsule a potential UCA. The radiography test showed that an insignificant amount of gold nanoparticles had been embedded into the shell of AuNP-S-MB, while a noticeable amount of gold nanoparticles had been loaded into the core of AuNP-Capsule. The conclusion could be drawn that loading the gold
nanoparticles into the core of the PVA MB is a promising route to develop a dual-modal contrast agent to support CT and US.

Study IV evaluated the effects of PVA MBs on the inception and the development of hydrodynamic cavitation. Three microfluidic devices with the smooth, surface roughened and sidewall roughened microchannels were employed to investigate the cavitating flows. The PVA MB induces the inception of the cavitation at a lower upstream pressure with all microfluidic devices utilized in the current study. Furthermore, at the same upstream pressure and in the same microfluidic devices, the PVA MBs suspension generates a larger cavitating flow than pure water. Additionally, the disappearance of the cavitation bubbles takes a longer time with the presence of the PVA MBs. Thus, a conclusion could be drawn that the introduction of PVA MBs in the water intensifies the hydrodynamic cavitating flow in microscale.
8 Future Work

For **Study I**, the CPS4 was evaluated in the *in vitro* experiment environment. A clinical translation of CPS4 needs further studies in the *in vivo* environment. Furthermore, the performance of CPS4 to detect other clinically approved UCAs is of great interest to the potential clinical translation. Of course, the CPS4 should be customized to the specific UCA of interest before the evaluation following the optimization method demonstrated in **Study II**.

For **Study II**, the PVA MB model was employed to describe the dynamic of the PVA MB. The unexpected oscillation, e.g., nonspherical oscillation, could degrade the representativeness of the model. Other types of models, e.g., the finite element model could potentially address those issues. However, the trade-off between the enormous computational expense and the increasing accuracy of the model should be carefully considered. Moreover, efforts are needed to understand the mechanism of the interaction between the MBs and small particles in the bloodstream (e.g., red blood cell) to push our model-guided optimization methods towards clinical applications.

For **Study III**, the next step is to restore the gas core of the AuNP-Capulse. The freeze-drying is a promising method to restore the gas core.

For **Study IV**, the finding motivates the future studies of the MBs-intensified cavitation under clinically relevant scenarios. For instance, using the CEUS to visualize the hearts with artificial valves could lead to MBs-intensified cavitating flow, which could increase the risk of thromboembolic complications and is dangerous for patients with preexisting heart conditions.
9 References


