An Ultrasound Contrast Agent Microbubble in a Microvessel: A Numerical Approach

by

Nazanin Hosseinkhah

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Medical Biophysics
University of Toronto

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Doctor of Philosophy

Medical Biophysics University of Toronto
University of Toronto

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Abstract

The blood brain barrier (BBB), a selective barrier separating blood from the parenchyma of the central nervous system, restricts more than 98% of neurotherapeutics from traveling into the brain. Focused ultrasound (FUS) exposure combined with circulating microbubbles is an emerging technique capable of safely opening the BBB locally, transiently, and non-invasively, enabling targeted drug delivery in the brain. However, the mechanisms of the microbubble-vessel interactions central to this process are not fully understood.

In this thesis, a comprehensive numerical model of a microbubble within a microvessel was developed aiming to shed light on bubble-vessel interactions, vessel wall mechanical stresses and acoustic emissions during FUS-induced BBB opening. An upward shift in the bubble’s resonance frequency relative to unbound bubbles was calculated, whose magnitude was dependent on the vessel elasticity. The synergistic effects of acoustic frequency and vessel elasticity on wall stresses were investigated. Vessel wall stresses were found to be maximal when the bubble was driven above resonance. The numerical model was validated with ex vivo high-speed optical imaging experiments. Resultant amplitudes of bubble oscillation were within 15% of corresponding experimental measurements. Vessel wall stresses calculated during bubble compression (and vascular invagination) were larger than those during bubble expansion, implying that vascular
damage could occur during this phase. The acoustic emissions from the ultrasound-stimulated microbubble were calculated, and their correlation with the vessel wall stresses was investigated. The normalized second harmonic decreased as a function of pressure until reaching a minimum, "transition point", after which point it was found to increase. The second and fourth harmonics of confined bubbles at this point were larger than those of unbound bubbles. Above the transition point, stresses induced by larger bubbles increased with a steeper slope. The results presented in this thesis could help in enhancing contrast imaging strategies, understanding bubble-vessel interactions, and optimizing ultrasound pulse parameters to maximize vessel wall stresses to improve drug delivery efficacy. Furthermore, the calculated acoustic emissions could provide feedback to online monitoring techniques and enable calibration of in vivo pressures.
Acknowledgment

The completion of this thesis would have not been possible without the help and contribution of many people for several years. Foremost, I would like to thank my supervisor, Dr. Kullervo Hynynen. His leadership approach allowed me to practice innovation. I highly appreciate his mentorship and patience throughout these years. I am also very grateful to my supervisory committee, Dr. Peter Burns and Dr. David Steinman, who continually led me towards the right direction with my project and provided insightful comments. I would like to extend my sincere appreciations to Dr. David Goertz for providing helpful guidance through challenging situations. I also want to thank Dr. Tom Matula and Dr. Hong Chen for their solid collaborations in providing me with essential experimental data. My sincere thanks go to Tam, Alex, Shannon, Meaghan, Chris, Dan, Anna, Kairavi, Alec, Ryan, Nikita and Charles for all their help.

I would like to thank my marvelous husband, Omid, for all his support throughout my PhD program. Words cannot describe how grateful I am to him for all of the sacrifices he made throughout all of my endeavours. I would not have contemplated this road if not for my parents, Nesa and Mohammad. I am forever grateful for their unconditional love and support. Special thanks to my sister, Nasim, for being such a caring and fabulous person in my life. Many thanks to my loving aunt who has always been there for me.

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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ALE</td>
<td>Arbitrary Lagrangian-Eulerian</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>CS</td>
<td>Circumferential Stress</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite Element Method</td>
</tr>
<tr>
<td>FUS</td>
<td>Focused Ultrasound</td>
</tr>
<tr>
<td>HIFU</td>
<td>High Intensity Focused Ultrasound</td>
</tr>
<tr>
<td>MI</td>
<td>Mechanical Index</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RP</td>
<td>Rayleigh–Plesset Equation</td>
</tr>
<tr>
<td>SLS</td>
<td>Standard Linear Solid Model</td>
</tr>
<tr>
<td>TJ</td>
<td>Tight Junction</td>
</tr>
<tr>
<td>UCA</td>
<td>Ultrasound Contrast Agent</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>WSS</td>
<td>Wall Shear Stress</td>
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</tbody>
</table>
# List of Symbols and Acronyms

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition of symbol</th>
</tr>
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<tbody>
<tr>
<td>$r$</td>
<td>Radial direction in cylindrical coordinate system (m)</td>
</tr>
<tr>
<td>$z$</td>
<td>Axial direction in cylindrical coordinate system (m)</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Azimuthal direction in cylindrical coordinate system (°)</td>
</tr>
<tr>
<td>$L$</td>
<td>Half of the vessel length (m)</td>
</tr>
<tr>
<td>$c(t)$</td>
<td>Creep function (Pa$^{-1}$)</td>
</tr>
<tr>
<td>$\varepsilon(t)$</td>
<td>Vascular strain</td>
</tr>
<tr>
<td>$\tau_\sigma$</td>
<td>Relaxation time for constant stress (s)</td>
</tr>
<tr>
<td>$\tau_\varepsilon$</td>
<td>Relaxation time for constant strain (s)</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Relaxation time (s)</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Applied stress on the vessel (Pa)</td>
</tr>
<tr>
<td>$\mu_0$</td>
<td>Spring constant on first branch (Pa)</td>
</tr>
<tr>
<td>$\mu_1$</td>
<td>Spring constant on second branch (Pa)</td>
</tr>
<tr>
<td>$\eta_1$</td>
<td>Viscosity of dashpot (Pa s)</td>
</tr>
<tr>
<td>$E$</td>
<td>Young's modulus (Pa)</td>
</tr>
<tr>
<td>$D_{Max}$</td>
<td>Maximum vessel displacement (m)</td>
</tr>
<tr>
<td>$D(t)$</td>
<td>Vessel displacement (m)</td>
</tr>
<tr>
<td>$v_f$</td>
<td>Fluid velocity (m/s)</td>
</tr>
<tr>
<td>$e$</td>
<td>Diameter strain</td>
</tr>
<tr>
<td>$\delta_{ij}$</td>
<td>Kronecker delta</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Fluid density (kg/m$^3$)</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Poisson’s ratio</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Dynamic viscosity of the fluid (Pa s)</td>
</tr>
<tr>
<td>$G$</td>
<td>Fluid velocity gradient (1/s)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$K$</td>
<td>Gas compressibility (N/m)</td>
</tr>
<tr>
<td>$m$</td>
<td>Inertia associated with the radiation mass (kg)</td>
</tr>
<tr>
<td>$\delta_{th}$</td>
<td>Boundary layer thickness (m)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Damping</td>
</tr>
<tr>
<td>$\delta_{total}$</td>
<td>Total damping</td>
</tr>
<tr>
<td>$\delta_{ac}$</td>
<td>Radiation damping</td>
</tr>
<tr>
<td>$\delta_{visc}$</td>
<td>Fluid viscous damping</td>
</tr>
<tr>
<td>$\delta_{thermal}$</td>
<td>Thermal damping</td>
</tr>
<tr>
<td>$\delta_{shell}$</td>
<td>Shell viscous damping</td>
</tr>
<tr>
<td>$P$</td>
<td>Pressure in the fluid (Pa)</td>
</tr>
<tr>
<td>$P_{f-b}$</td>
<td>The pressure on the fluid just outside the bubble wall minus the Laplace pressure (Pa)</td>
</tr>
<tr>
<td>$P_b$</td>
<td>Pressure on the fluid just outside the bubble wall (Pa)</td>
</tr>
<tr>
<td>$P_g$</td>
<td>Gas pressure (Pa)</td>
</tr>
<tr>
<td>$P_{g0}$</td>
<td>Gas pressure at resting state (Pa)</td>
</tr>
<tr>
<td>$P_0$</td>
<td>Ambient pressure in microvessels (Pa)</td>
</tr>
<tr>
<td>$P_v$</td>
<td>Vapour pressure (Pa)</td>
</tr>
<tr>
<td>$P_a$</td>
<td>Acoustical pressure amplitude (Pa)</td>
</tr>
<tr>
<td>$P_{ac}$</td>
<td>Acoustic pressure wave (Pa)</td>
</tr>
<tr>
<td>$P_s$</td>
<td>Scattered pressure from bubbles (Pa)</td>
</tr>
<tr>
<td>$P(t)$</td>
<td>Ultrasound pressure pulse at the bubble wall (Pa)</td>
</tr>
<tr>
<td>$P_Y$</td>
<td>Laplace pressure (Pa)</td>
</tr>
<tr>
<td>$P_i$</td>
<td>Intravascular pressure (Pa)</td>
</tr>
<tr>
<td>$P_{out}$</td>
<td>Pressure on the outer diameter of vessel wall (Pa)</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>Variation in the pressure (Pa)</td>
</tr>
<tr>
<td>$f$</td>
<td>Acoustic frequency (Hz)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
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<td>-------------</td>
</tr>
<tr>
<td>$R_0$</td>
<td>Initial bubble radius (m)</td>
</tr>
<tr>
<td>$R$</td>
<td>Bubble radius (m)</td>
</tr>
<tr>
<td>$\dot{R}$</td>
<td>Time derivative of bubble radius (m/s)</td>
</tr>
<tr>
<td>$V$</td>
<td>Bubble volume ($m^3$)</td>
</tr>
<tr>
<td>$Q$</td>
<td>Power density (w/$m^3$)</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>Bubble radial variation</td>
</tr>
<tr>
<td>$\sigma_{ij}$</td>
<td>$ij$ th components of the stress tensor (Pa)</td>
</tr>
<tr>
<td>$\varepsilon_{ij}$</td>
<td>$ij$ th components of the strain tensor</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Total curvature ($m^{-1}$)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Surface tension (N/m)</td>
</tr>
<tr>
<td>$\gamma_{\text{water}}$</td>
<td>Surface tension of water (N/m)</td>
</tr>
<tr>
<td>$k$</td>
<td>Polytropic index</td>
</tr>
<tr>
<td>$r_v$</td>
<td>Initial vessel radius (m)</td>
</tr>
<tr>
<td>$r_i$</td>
<td>Inner vessel diameter (m)</td>
</tr>
<tr>
<td>$r_o$</td>
<td>Outer vessel diameter (m)</td>
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<tr>
<td>$\chi$</td>
<td>Shell elastic modulus (N/m)</td>
</tr>
<tr>
<td>$\kappa_s$</td>
<td>Shell viscosity (kg/s)</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>The non-linear elastic correction coefficient of shell (N/m)</td>
</tr>
<tr>
<td>$A$</td>
<td>Bubble area ($m^2$)</td>
</tr>
<tr>
<td>$a_0$</td>
<td>Bubble wall acceleration ($m/s^2$)</td>
</tr>
<tr>
<td>$A_{\text{buckling}}$</td>
<td>Bubble area at shell buckling regime ($m^2$)</td>
</tr>
<tr>
<td>$R_{\text{break–up}}$</td>
<td>Bubble radius at break-up regime (m)</td>
</tr>
<tr>
<td>$A_{\text{break–up}}$</td>
<td>Bubble area at break-up regime ($m^2$)</td>
</tr>
<tr>
<td>$c_0$</td>
<td>Wave speed propagating along a flexible tube (m/s)</td>
</tr>
<tr>
<td>$c$</td>
<td>Speed of sound in the medium (m/s)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
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</tr>
<tr>
<td>$C$</td>
<td>Tube compliance (Pa$^{-1}$)</td>
</tr>
<tr>
<td>$S_0$</td>
<td>Initial vessel area (m$^2$)</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>Variation in the vessel area (m$^2$)</td>
</tr>
<tr>
<td>$R_{eq}$</td>
<td>Spherical bubble's equivalent radius (m)</td>
</tr>
<tr>
<td>$R_{max}$</td>
<td>Maximum bubble radius (m)</td>
</tr>
<tr>
<td>$r_{v max}$</td>
<td>Maximum vessel radius (m)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Ultrasound wavelength in the gas (m)</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Resonance frequency (rad/s)</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Applied angular frequency (rad/s)</td>
</tr>
<tr>
<td>$\omega_M$</td>
<td>Minnaert frequency (rad/s)</td>
</tr>
<tr>
<td>$\sigma_{\theta \theta}$</td>
<td>Circumferential (Hoop) stress (Pa)</td>
</tr>
<tr>
<td>$\tau_{rz}$</td>
<td>Fluid shear stress at the vessel wall (Pa)</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Shear stress (Pa)</td>
</tr>
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</table>
Chapter 1

1. Introduction and Background

1.1 Blood Brain Barrier and Drug Delivery

1.1.1 Blood Brain Barrier

Paul Ehrlich in 1885 provided the first experimental evidence of the blood brain barrier (BBB). He observed that water soluble dyes injected into the circulatory system stained all organs except the brain and spinal cord. Later, Ehrlich's student Goldmann performed an experiment in which trypan blue injected directly into the cerebrospinal fluid (CSF) stained all cell types in the brain but failed to penetrate into the periphery [1]. These observations suggested the presence of a barrier between the central nervous system (CNS) and the peripheral circulation. In 1900, Lewandowsky was the first to use the term “blood-brain barrier” after he and his colleagues had performed experiments to demonstrate the limited permeation of a chemical compound (potassium ferrocyanate) into the brain. However, it was not until 1960s that the arrival of electron microscopy aided in seeing this barrier anatomically [1].

The blood brain barrier (BBB) is a selective barrier separating the blood from the parenchyma of the central nervous system [2]. It is comprised of endothelial cells, neurons, astrocytes, microglia and pericytes. Figure 1-1 demonstrates a schematic cross-sectional presentation of a typical cerebral capillary (note that the tight junctions between two or more cells in larger vessels are not shown in this figure). Endothelial cells of the BBB are quite distinct from those of other regions of the body. The features distinguishing cerebral capillaries are: presence of tight junctions (A belt-like region where adjacent cells fuse, TJ) [3], lower degree of pinocytosis (which is a form of endocytosis that small particles are brought into the cell, forming an invagination, and then suspended within small vesicles) [4], lack of fenestrations [5] and a high numerical density of mitochondria [6].
The BBB has several functions [7]. It plays a role in supplying nutrients to the brain and mediating the efflux of waste products. In order to produce an optimal environment for neuronal functions, the BBB restricts fluid and ionic passage between the blood and the brain [8]. This barrier helps in keeping the neurotransmitters and neuroactive agents in the CNS, separate from those in peripheral tissues and blood. Due to its special structure, drug delivery into the brain faces some challenges.

1.1.2 Challenges with Drug Delivery

Millions of Americans and many more worldwide are affected by disorders associated with the CNS such as neurodegenerative diseases and brain cancer making it one of the main causes of disability [9], [10]. The BBB forms a virtually impermeable barrier for large (>400-500 Da) and hydrophilic molecules [11]. This imposes a challenge for drug delivery into the brain as BBB restricts more than 98% of the therapeutic agents from traveling into the brain tissue [10]. Improved drug therapy could enhance the treatment of most CNS disorders.
1.1.3 Current Approaches to Drug Delivery

An ideal drug delivery method across the BBB should be efficient, safe, non-invasive and localized. There are typically three main approaches for the drug delivery: Drug modifications, bypassing the BBB or techniques to temporarily increase BBB permeability.

Drug modifications (e.g. using carrier molecules) are expensive and normally adequate concentrations are not achieved [12]. This technique is non-localized and high doses of such drugs could cause side effects in other organs [13].

Bypassing the BBB can be done through intranasal delivery (i.e. through the nasal epithelium). However, this technique has low efficiency [14]. Other methods for bypassing the BBB include surgical intervention (intracerebral or intraventricular drug injections) [15]. However this technique is invasive and induces unwanted damage to the healthy tissue.

Temporal and reversible disruption of the BBB can be achieved with intra-arterial injection of chemical compounds such as mannitol or other hyper-osmotic solutions [16], [17]. As a result of this method, tight junctions get disrupted due to the shrinkage of the endothelial cells resulting in a widespread permeabilization. This is a non-targeted method and the entire tissue volume is affected. As a result of global permeabilization, cytotoxic compounds might grant access to the CNS [13].

Since the 1950s and following the exposure of animal brains to ultrasound, the BBB disruption was achieved [18]–[20] making ultrasound an alternative approach for localized drug delivery.

1.1.4 Focused Ultrasound

Focused ultrasound (FUS) is an emerging technique to disrupt the BBB for targeted drug delivery. It has advantage over other techniques as it is localized, transient, non-invasive and image-guided. Furthermore, ultrasound is a non-ionizing, portable, low cost, and fast modality which makes it more accessible.

Early experiments with high intensity focused ultrasound (HIFU) were almost always associated with damage [21], [22]. Thermal effects as well as cavitation (cavitation generally refers to as the formation and activity of bubbles or gas cavities in a medium exposed to an ultrasonic field [23])
were hypothesized to be the potential mechanisms in the BBB disruption following HIFU. Furthermore, in other cases where BBB was disrupted without tissue damage, the experiments could not be reproduced consistently [21]. Although tissue damage may be desirable in some cases (e.g. in cancer), safety and efficacy concerns regarding the BBB disruption with FUS alone limits its potential applicability. However, with the addition of preformed microbubbles to FUS, the BBB opening has been shown feasible and consistent at thresholds below the thermal damage in the tissue [24].

1.1.5 Focused Ultrasound with Microbubbles

The application of small gas bubbles was first introduced by Gramiak and Shah [25]. They noticed enhancement of ultrasound signal in the aortic root when agitated saline was injected during echocardiography. The active agent responsible for sonographic enhancement was free gas bubbles. Since then microbubbles have gone under intensive investigations as a major ultrasound contrast agent (UCA) for imaging as well as therapeutic applications. In medical applications, microbubbles are intravascular agents designed to be a few micrometers in size so that they can pass through the capillary network without causing an embolism. Furthermore, commercially available preformed bubbles have shell coatings for greater stability and are comprised of gases with low water solubility for an extended lifetime [26].

In 2001, Hynynen et al. used low-power focused ultrasound in combination with preformed Optison microbubbles [24]. They reported that the power required to induce BBB disruption was about two orders of magnitude below that required to induce thermal damage to the brain tissue. Since then, many successful experiments of low power ultrasound and microbubble mediated BBB disruption have been reported. Comprehensive reviews on this field are given in [27]–[29]. Magnetic resonance imaging (MRI) is commonly used to monitor and target the FUS-induced BBB disruption. As example of the blood brain barrier opening is shown in Figure 1-2, in which a T1-weighted MR image of a rabbit brain shows gadolinium contrast agent enhancement. This is an indicator for BBB opening due to bubble activity [30].
Figure 1-2 T1-weighted MR image of a rabbit brain with arrows pointing at gadolinium enhancement showing the blood brain barrier opening [30]. Figure re-printed with permission from publisher.

Drug delivery with microbubbles is performed through two mechanisms; 1) co-administration of the microbubble and therapeutic agents and 2) embedding the therapeutic agents into the shell, attaching to the shell, or incorporating within the gas core of the microbubbles [31]. Delivery of antibodies, chemotherapy agents, genes and even stem cells has been shown feasible using FUS combined with microbubbles [9], [13].

With the appropriate exposure parameters, histology assessments following FUS and microbubbles did not show ischemia or apoptosis [24], [32]. Small regions of extravasated red blood cells were observed, which did not have adverse effects [32], [33]. However, since the choice of appropriate parameters are crucial, acoustic monitoring techniques were developed to ensure the BBB disruption safety [34].

1.1.6 Mechanism of Microbubble-Mediated BBB Disruption

The mechanism associated with microbubble-mediated BBB disruption could be divided into physical effects (those applied to the vessel wall) and cellular effects. Transcellular (through the cell) and paracellular (between cells through TJs) are the two cellular mechanisms of passage observed with electron microscopy following BBB disruption with microbubbles. Increased number of vesicles [35], upregulation of caveolae proteins [36], formation of cytoplasmic channels [35] as well as pore formation [37] were observed as evidences of the transcellular passage. As part of the paracellular route of the BBB disruption, opening of the tight junctions (as molecular structure of tight junctions were disassembled) [35], [38] has been shown to occur post FUS and
microbubbles. The entrance of macromolecules through the BBB is speculated to happen through the paracellular pathway [39].

Some of the potential physical mechanisms that results in the BBB disruption include bubble collapse or microjet (which happen at relatively higher pressures and could potentially damage the cells), microthermal mechanisms (which are reported theoretically and yet to be tested experimentally [40]) and bubble stable oscillation (which could stretch the endothelial cells or induce fluid movement and consequently exert vessel wall stresses). These mechanisms could be enhanced by acoustic radiation force, pushing bubbles closer to the vessel wall [41].

Recent studies on the BBB opening experiments with two-photon microscopy revealed that smallest vessels (2.5-12.5 µm in radius) are most susceptible to permeabilization [42], [43]. This could be due to the fact that the bubbles are in a closer interaction within smaller vessels.

1.1.7 Research Objective

Understanding bubble/vessel wall interactions and induced mechanical stresses is significant for elucidating the BBB opening and optimizing the experimental conditions. A model of a confined bubble within a microvessel could shed light on the bubble behaviour, bubble–vessel interactions and vessel wall mechanical stresses. Furthermore, such a model could provide acoustic information from confined bubbles, which has great implications in the development of real-time monitoring techniques or in vivo pressure calibrations.

1.2 Physical Effect of Microbubbles on Neighboring Cells

In general, physical bioeffects of microbubbles on neighbouring cells are mechanical, thermal and chemical. Chemical effects, such as free radical production by hydrolysis inside the bubble become important when bubbles undergo violent collapse [44]. Bubbles made from high atomic number gases (e.g. Optison with octafluorocarbon) have a low probability of free radical production which mitigates the risk of chemical effects [45]. Elevated tissue temperatures induced by activated bubbles (thermal bioeffects) especially in long therapeutic pulses were observed experimentally and simulated numerically [40], [46]. However, this is not the focus of the current thesis. Bubble induced mechanical bioeffects have been reported [47], which include the reversible perforation
of the cell membrane, cell detachment and lysis [48], [49] or vascular rupture, indicated by erythrocyte extravasation [50]. The focus of this thesis is on the mechanical stresses induced by bubble activity. Two of the main mechanical stresses are wall shear stress (WSS) and circumferential stress (CS). Figure 1-3 represents a schematic illustration of shear and circumferential stresses on the vessel wall induced by activated bubbles.

Figure 1-3 Schematic illustration of shear stress ($\tau_{rz}$) and circumferential stress ($\sigma_{\theta\theta}$) on the vessel wall.

1.2.1 Shear Stress and Microstreaming

Nyborg showed that small gas bodies are effective sources of highly localized streaming, or "microstreaming" [51]. The streaming effects from bubbles were elaborated in a recent study by Oh et al. where dye perfusion into a tissue model due to oscillating bubbles was performed [52]. They showed that the mass of dye perfused into a tissue phantom for 30 seconds was increased by about 20% and the surface concentration and penetration length of the drug was increased by 12% and 13%, respectively. The streaming, near pulsating bubbles, induces shear stress on neighboring cells or vessel wall [53], [54]. It is important to calculate these shear stresses because of the bioeffects they can cause.

Since bubbles are confined to the intravascular space the specific cells affected by vibrating bubbles are endothelial cells. In general (not specific to brain vasculature), shear stress induced as a result of microstreaming could cause biological effects ranging from activation of normal shear stress sensors (e.g. ion channels), endothelial surface layer damage, membrane reversible perforation, to cell detachment and lysis [48]. Previously transient pore formation due to oscillating bubbles on endothelial cells [37], [55] or on other cell lines [56], [57] has been reported. Microbubbles and ultrasound also induce the formation of H$_2$O$_2$ and influx of calcium ions [58], which are directly correlated to endocytosis [59], [60]. Furthermore, the activation of endocytosis
pathway may occur as a result of microstreaming and shear stresses induced by microbubble oscillation in the ultrasound field [37].

Micro-streaming established close to a pulsating bubble near a solid boundary has a sharp velocity drop (time independent) across a thin boundary layer [51]. Nyborg derived the boundary layer thickness around vibrating objects with known irrotational oscillatory velocities and small oscillation amplitudes. This boundary thickness is described by:

$$\delta_{th} = \frac{\mu}{\pi \rho f}$$  \hspace{1cm} (1.1)

where \( f \), \( \mu \) and \( \rho \) are the frequency, fluid viscosity and density, respectively. This drop produces the following velocity gradient (from the theory derived by Nyborg) [61]:

$$G = \frac{2 \pi f \varepsilon_0^2}{R_0 \delta_{th}}$$  \hspace{1cm} (1.2)

where \( \varepsilon_0 \) equals to \( R - R_0 \) (\( R \) is the amplitude of the instantaneous radius of a pulsating bubble and \( R_0 \) is the equilibrium radius). Shear stress from Newtonian flow is proportional to the fluid velocity gradient. Therefore, the shear stress (\( \tau \)) experienced due to the velocity gradient is:

$$\tau = \mu G = 2\pi^2 \varepsilon_0^2 \frac{3 \sqrt{\rho f^3 \mu}}{R_0}$$  \hspace{1cm} (1.3)

Equation (1.3) evaluates the shear stress in a linear fashion on a flat line (of cells) in the vicinity of a pulsating bubble. Based on this equation, the shear stress generated around a 2.5 \( \mu \)m bubble in radius oscillating at 160 kPa and 1 MHz is in the order of 10-40 kPa.

Non-spherical bubbles within compliant microvessels generate complex streaming patterns and numerical methods are required to investigate the stresses induced by the complicated bubble behaviour.

1.2.2 Circumferential (Hoop) Stress

Zhong et al. observed rupture along the axial direction of regenerated cellulose hollow fibers at high acoustic pressures for shock-wave lithotripsy applications in combination with Albunex bubbles [62]. This indicated that the failure of the fiber was due to the circumferential stress. Also,
when ultrasonic parameters are fixed, the circumferential stress is higher in smaller vessels [63]. In another study by Neal and Michel, the effects of intravascular pressure was assessed on frog microvascular endothelium [64]. Vessels with their end closed downstream were pressurized. As a result, oval gap openings were observed with their larger axis along the vessel axis indicating that circumferential stress in these microvessels could be responsible for the vascular bioeffects. Therefore, circumferential stress (often referred to as the ‘Hoop Stress’) that is tangent to the vessel wall circumference exerted on the vasculature due to oscillating bubbles could be another important stress to study. Other vascular stresses include radial ($\sigma_{rr}$, along the vessel radius) and longitudinal ($\sigma_{zz}$, along the vessel axis) stresses. However, the vessel wall is more susceptible to the circumferential stress as Rowe et al. discussed; this is because of the multidirectional layered structure of the vessel wall [65]. Throughout this thesis the focus will be on the circumferential stress.

For a thick wall cylinder defined as when the ratio of vessel radius to thickness is less than 10, the circumferential stress ($\sigma_{\theta\theta}$) is calculated using the following equation [66]:

$$
\sigma_{\theta\theta} = \frac{P_i r_i^2 - P_out r_o^2}{r_o^2 - r_i^2} + \frac{r_i^2 r_o^2}{r^2} \left( \frac{P_i - P_out}{r_o^2 - r_i^2} \right) 
$$  \hspace{1cm} (1.4)

where $r_i$ and $r_o$ are inner and outer vessel diameters and $P_i$ and $P_{out}$ are pressure on the inner diameter and outer diameter on the vessel wall respectively. $r$ is a radial point between, $r_i$ and $r_o$.

As the microbubble's behaviour is complicated, understanding bubble characteristics will further help with the model development.

### 1.3 Microbubble Characteristics

The highly compressible nature of gas microbubbles makes them undergo volumetric oscillations in response to ultrasound. Gas-filled bubbles scatter ultrasound on the order of 1000 times more than their geometric cross section [67] making them a good candidate for use as ultrasound contrast agents (UCA). Bubbles are small enough to pass though the capillary bed but still large enough to be confined within the intravascular space.
Generally a bubble’s behaviour can be divided into stable or unstable. Inertial cavitation is a form of unstable cavitation and in that the inertia forces dominate during bubble contraction leading to a violent collapse. Stable cavitation refers to stable bubble oscillations in response to the ultrasound pulse. Fig. 1-4 presents steak images (which are continuous recordings of the oscillation of a single line across the microbubble diameter [68]) of a stable cavitation in response to the driving waveform along with a diagram of the corresponding bubble size [69], [70].

![Diagram of stable cavitation](image)

**Figure 1-4** Microbubble oscillates in response to the driving pressure for a 2.6-µm radius bubble at 310 kPa and 2.4 MHz [68], [69]. Figure re-printed with permission from publisher.

Microbubbles have complicated behaviours when excited by an ultrasound pulse. Bubble oscillations depend on the acoustic parameters (e.g., acoustic pressure and frequency), fluid around the bubble (fluid viscosity and density), the bubble’s surrounding (e.g., whether it is in free field, within a vessel, or close to a wall) and the individual bubble’s characteristics (e.g., size, gas material and encapsulating shell material). When acoustic pressure is increased, a bubble’s oscillation can range from linear to non-linear and eventually to bubble disruption [71].
1.3.1 Unbound Free Bubbles

A spherical bubble’s equation of motion is typically derived using an energy balance between the pulsating bubble and an infinite fluid [72]. The liquid pressure at a point remote from bubble wall can be assume to be \( P_\infty = P_0 + P(t) \). As time progresses and due to the applied pressure \( P(t) \), the bubble radius \( R_0 \) becomes \( R \) and liquid acquires a kinetic energy of

\[
\frac{1}{2} \rho \int_R^{\infty} \dot{r}^2 4\pi r^2 dr
\]

where \( \rho \) is the fluid density, \( r \) is the radial direction in a spherical coordinate and \( \dot{r} \) is its time derivative. If the liquid is assumed incompressible, from the mass conservation law the liquid mass flowing across a surface at some radius outside the bubble equates to the flow at the bubble wall. This gives:

\[
\dot{R} / R = R^2 / r^2 \quad (1.6)
\]

where \( \dot{R} \) indicates the derivative of bubble radius with respect to time. Equation (1.6) can be rearranged for \( \dot{r} \) and substituted in equation (1.5) for integration. The result becomes \( 2\pi \rho R^3 \dot{R}^2 \).

Due to conservation of energy, this kinetic energy (equation (1.5)) is equal to the work done by the difference in the pressures remote from bubble (i.e. \( P_\infty \)) and at the bubble's wall (i.e. \( P \)):

\[
2\pi \rho R^3 \dot{R}^2 = \int_{R_0}^{R} (P - P_\infty) 4\pi R^2 dR \quad (1.7)
\]

The internal pressure within the bubble (the gas \( P_g \) plus vapour pressure \( P_v \)) is greater than the pressure in the liquid \( P \) by the Laplace term \( P_L \), pressure due to surface tension: \( P + P_L = P_g + P_v \). At the equilibrium \( R = R_0 \) and the terms are shown with a subscript e. It is generally assumed that the gas inside microbubbles obeys the ideal gas law \( P_g = P_{ge} \frac{R_0}{R}^{3k} = (P_0 + \frac{2\gamma}{R_0} - P_v) \left( \frac{R_0}{R} \right)^{3k} \), where \( k \) is the Polytropic index, and \( P_{ge} = P_0 + \frac{2\gamma}{R_0} - P_v \) is the gas pressure at equilibrium. The Laplace pressure \( \frac{2\gamma}{R_0} \) is derived from a special case for a spherical bubble. If an imaginary line cuts a spherical bubble to halves, the internal pressure tends to push the hemispheres apart (with the force \( \pi R^2 P_L \)). This force is counteracted by the surface tension acting around the
circumference of the circle (i.e. \(2\pi R = \pi R^2 P_\gamma\), \(P_\gamma = \frac{2\gamma}{R}\)). In chapter 3, the Laplace pressure for a non-spherical bubble will be described.

An integration of equation (1.7) with respect to \(R\) results in \(\frac{P - P_\infty}{\rho} = \frac{3R^2}{2} + R\ddot{R}\). Substituting for \(P_\infty = P_0 + P(t)\) and liquid pressure \((P)\), the following ordinary differential equation is derived:

\[
R\ddot{R} + \frac{3R^2}{2} = \frac{1}{\rho} \left\{ \left( P_0 + \frac{2\gamma}{R_0} - P_v \right) \left( \frac{R_0}{R} \right)^3 + P_v - \frac{2\gamma}{R} - P_0 - P(t) \right\}
\]  
(1.8)

where the second term on the left hand side is associated with the fluid inertia. The pressure terms on the right hand are gas pressure, vapour pressure, Laplace pressure, ambient pressure and applied acoustic field respectively.

Bubbles can be treated as forced harmonic oscillators and could be modeled as the motion of a mass attached to a spring. The resonance frequency of this mass and spring system is:

\[
\omega_0 = \sqrt{\frac{K}{m}}
\]  
(1.9)

where \(K\) represents the compressibility of the gas inside the bubble and effects of the surface tension, and \(m\) is the inertia associated with the radiation mass due to bubble’s oscillation. At resonance the acoustic energy transfer to the bubble is maximized. In a regime where bubbles undergo a small amplitude oscillation (if \(R(t) = R_0 + R_\varepsilon\) and \(P(t) = P_a e^{i\omega t}\), where \(\omega\) is the applied angular frequency, then \(R_\varepsilon \ll R_0\) and assuming vapor pressure and viscosity are negligible, equation (1.8) becomes:

\[
\ddot{R}_\varepsilon + \omega_0^2 R_\varepsilon = \frac{P_a}{\rho R_0} e^{i\omega t}
\]  
(1.10)

where \(\omega_0\), the resonance frequency, becomes the Minnaert frequency \((\omega_M)\) and is given by

\[
\omega_0 = \omega_M = \sqrt{\frac{1}{\rho R_0^2} \left( 3k \left( P_0 + \frac{2\gamma}{R_0} \right) - \frac{2\gamma}{R_0} \right)}
\]  
(1.11)
1.3.2 Encapsulating Shell

In medicine, in order to stabilize the bubbles in the blood circulation, stabilizing shells were designed that lowered the bubbles’ surface tension and prolonged their lifetime [26], [69]. Bubbles are on the order of 1–10 µm in diameter. The gas core usually comprises of perfluorocarbon, nitrogen or sulfur hexafluoride. The shell is made up of lipid, albumin-protein or polymer material and with the thicknesses ranging from 10 to 200 nm. Depending on the shell type, a bubble’s behaviour is different. Polymer shells are relatively rigid and the response of these bubbles to ultrasound is not significant. The focus of this thesis is mainly on phospholipid shelled bubbles with a few nanometer shell thicknesses.

1.3.3 Damping

Damping is a phenomenon that affects the bubble oscillation, resonance frequency and scattering cross section. The equation of motion of a damped linear harmonic oscillator becomes:

\[ \ddot{R}_e + \omega_0^2 R_e + \delta \omega_0 \dot{R}_e = \frac{p_a}{\rho R_0} e^{i \omega t} \]  \hspace{1cm} (1.12)

and the resonance frequency in such a system is:

\[ f_{\text{res}} = f_0 \sqrt{1 - \frac{\delta^2}{2}} \]  \hspace{1cm} (1.13)

where \( \delta \) is the system’s damping. In general, for an encapsulated bubble as a harmonic oscillator with spherical symmetry, the total damping is a sum of radiation damping (\( \delta_{ac} \), sound re-radiated into the fluid), thermal damping (\( \delta_{thermal} \)), fluid viscous damping (\( \delta_{visc} \)) and shell viscous damping (\( \delta_{shell} \)) [72]–[74].

\[ \delta_{\text{total}} = \delta_{ac} + \delta_{visc} + \delta_{thermal} + \delta_{shell} \]  \hspace{1cm} (1.14)

The thermal damping is assumed negligible when MHz frequencies and micron-size bubbles are used [74], [75]. Following sections describe more about the models developed to take into account the fluid viscous, shell viscous and acoustic radiation damping terms.
1.3.4 Rayleigh–Plesset Equation

When the effects of fluid viscosity (which are manifested through the boundary conditions) are added to equation (1.8), the well-known Rayleigh–Plesset (RP) equation is obtained:

\[
R \ddot{R} + \frac{3 \dot{R}^2}{2} = \frac{1}{\rho} \left\{ \left( P_0 + 2\gamma \frac{R_0}{R} - P_v \right) \left( \frac{R_0}{R} \right)^3 + P_v - 2\gamma \frac{R}{R} - \frac{4\mu \dot{R}}{R_0} - P_0 - P(t) \right\}
\] (1.15)

where \( \frac{4\mu \dot{R}}{R_0} \) is the fluid viscosity pressure term. Normally the vapour pressure \( (P_v) \) is assumed trivial in comparison to the gas pressure. Modification to RP equation to account for fluid compressibility and encapsulating shell have resulted in various models which are described in section §1.4.1.

1.3.5 Primary and Secondary Bjerknes Forces

Ultrasound exerts a force on microbubbles which is proportional to the bubbles’ volume and pressure gradient and is called the primary radiation force. In some applications where targeted bubbles need to reach the vessel wall or a thrombus, ultrasound radiation force can be beneficial [76].

In the case of a traveling wave, the radiation force is oscillatory and the temporal average of the pressure gradient yields a net force applied in the direction of acoustic propagation [77]. In case of a standing wave, bubbles will travel to either the nodes or antinodes depending on the phase relation between the oscillations and the external pressure field [77]. From the theory of forced harmonic undamped oscillators it is known that bubbles of less than resonant size oscillate in phase with the external acoustic pressure and those larger than resonance oscillate \( \pi \) out of phase with the external field [77]. Consequently, in a standing wave, bubbles less than resonant size will be forced towards antinodes whereas bubbles greater than resonant size will be driven towards the nodes [77]. Bubbles can also exert radiation forces on one another and are referred to as secondary radiation forces. A local pressure gradient is produced by an oscillating bubble such that neighbouring bubbles within this scattered field will experience a force. The force can be attractive or repulsive depending on the phase relation between the bubble’s oscillations; for example, bubbles oscillating in phase attract, and bubbles oscillating out of phase repel [78], [79].
1.3.6 Presence of a Boundary

Blood vessel flexibility and that of the surrounding tissue vary with the tissue types (fat, muscle, brain) and decreases as a tumour develops. Bubble behaviour depends on the confining surrounding (e.g. the vicinity to the wall or in a tube as well as the vessel wall elasticity/viscosity). A microbubble’s resonance frequency, maximum relative expansion and damping change due to the wall presence. In an experiment by Garbin et al. when a bubble was placed close to a wall, its amplitude of oscillations was suppressed by more than 50% [80]. It was experimentally shown before that the presence of a confining wall significantly changes the flow field around the bubble [81]. Bubbles’s acoustic response was altered as well as an increase of about 10% in the resonance frequency of a 1.5 µm (in radius) MicroMarker™ close to an elastic agarose boundary was observed [82]. This could be relevant in tumour imaging applications (as in tumour vasculature the interstitial pressure throughout the interior of the tumour is remarkably high which alters the vessel elasticity [83]) or targeted microbubble imaging.

1.4 Theoretical Models of Microbubbles

Different models of unbound and confined bubbles have been proposed. Some of the most relevant models are presented in the following sections.

1.4.1 Unbound Bubble Models

An equation of motion for the unbound spherical pulsating bubble can be derived using an energy balance. Additional modifications were made to RP equation to account for the compressibility of the liquid (such as those developed by Keller and Miksis [84], Gilmor [85] and Herring-Trilling [86], [87]). Furthermore, for encapsulated bubbles, RP equation was modified and shell terms were added to the right hand side of the equation (1.15) (such as models developed by de Jong et al. 1994 [88], Church 1995 [75], Hoff et al. 2000 [89], Sarkar et al. 2005 [90], Marmottant et al. 2005 [91] and Chatterjee and Sarkar 2003 [92]). A comprehensive review of the models developed for unbound bubbles is given by Qin et al [68].
1.4.2 Confined Bubble Models

Arterioles and capillaries less than 25 µm in diameter are abundant within the body, and account for a large proportion of the blood volume (e.g., 80% to 90% of the blood in the myocardium is in the capillaries) [93]. The capillary diameter in rat brain varies from 1.7-10 µm in diameters with a mean of 4 µm [94]. Therefore, a microbubble \textit{in vivo} can rarely be considered to be in an infinite space, and will more often be closely surrounded by the vessel wall. Therefore, to predict \textit{in vivo} bubble behaviour a realistic model is required. A summary of various confined bubble models is shown in Table 1-1.

Table 1-1 Confined bubble models

<table>
<thead>
<tr>
<th>Bubble Model Assumptions</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Bubble close to a boundary</td>
<td>Analytical and numerical solutions were developed</td>
<td>Fong et al. 2006 [95]; Krasovitski and Kimmel 2004 [96]; Nyborg 1958 [51]; Sato et al. 1994 [97]; Afanasiev and Grigorieva 2006 [98]; Blake et al. 1999 [99]; Doinikov et al. 2009 [100]; Doinikov et al. 2010 [101]; Doinikov and Bouakaz 2010 [102]; Doinikov et al. 2012 [103]; Wu 2002 [54]</td>
</tr>
<tr>
<td>(b) Confined bubble within a rigid tube/parallel plates</td>
<td>Analytical and numerical solutions were developed</td>
<td>Sassaroli and Hynynen 2004, 2005 [104], [105]; Oguz and Prosperetti 1998 [106]; Cui et al. 2006 [107]; Ye and Bull 2006 [108]; Hay et al. 2013 [109]; Qamar et al. 2013 [110]</td>
</tr>
<tr>
<td>(c) Confined bubble within a compliant tube</td>
<td>Numerical solutions were developed</td>
<td>Ye and Bull 2006 [108]; Miao 2008 [111]; Martynov et al. 2009 [112]; Gao et al. 2007 [113]; Qin and Ferrara 2006, 2007 [114], [115]; Wiedemair et al. 2012 [116]</td>
</tr>
</tbody>
</table>
Various simplifications and assumptions were used in developing most of the confined bubble models (discussed in detail in the following section) which may not reflect the behaviour of encapsulated bubbles within compliant microvasculature and the need for a comprehensive model remains. Some of the challenges are discussed in the following section.

**1.4.3 Challenges with Current Confined Bubble Models**

Different models of confined bubbles have been developed; however, there are some limitations with current models. In most of these studies the wall curvature was ignored, the flow was simplified as a potential flow (assuming irrotational flow) or the fluid was assumed inviscid. The viscous effects play a particularly important role in bubbles smaller than 50 µm [117]. Other limitations include the ignorance of the encapsulating shell, simulating bubbles in rigid vessels or recruitment of an unbound bubble model to describe confined bubble behaviour.

The models developed to describe bubbles in rigid vessels (section (b) in Table 1-1) are far from reality as biological tissues are compliant and bubble behaviour is significantly different in elastic versus rigid vessels [108].

The confined bubble models developed inside a compliant vessel (section (c) in Table 1-1 except [116]) ignored the encapsulating shell. The shell is important as bubble resonance frequency can increase by about 50% and shell viscosity could contribute to 70% of the total damping [74].

Wiedemair *et al.* [116] developed a confined bubble model within elastic microvessels. However, to describe the bubble they recruited a modified RP equation that was developed for bubbles in rigid tubes. Therefore, the impact of vessel elasticity was not incorporated in the bubble behaviour.

Non-spherical bubble oscillations become important to consider when bubbles are oscillating near a wall [68], [118]–[121] and most of the confined bubble models apply a bubble symmetry assumption (e.g. those developed in [105], [104], [106], [109], [110] and [116]). While these models are suitable for low acoustic pressures they may not reflect the reality at higher pressures or bubbles within smaller vessels; furthermore, a bubble in a small blood vessel deviates from its spherical shape and forms an ellipsoid [122].
The models developed for bubbles close to a wall (section (a) in Table 1-1) do not generally represent bubbles in capillaries. Furthermore, as discussed above, bubbles were assumed close to rigid vessel (e.g. [100], [51] and [101]), unshelled (e.g. [95], [96], [97], [98] and [99]), or with a spherical symmetry (e.g. [103]).

Previously, a number of bubble models were developed to calculate the shear stress [54], [96], [102], [111], [113]. Wu used a modified RP equation which describes an unbound bubble to calculate the stress on a rigid wall [54]. In reality, the bubble’s amplitude of oscillation can get suppressed by more than 50% when bubbles are placed close to a boundary [80] and adaptation of a confined bubble is required. Doinikov and Bouakaz [102] calculated the wall shear stress generated from oscillating bubbles. However, a bubbles’ model developed for rigid vessels was used and the influence of vessel elasticity on the bubble behaviour was not incorporated. Krasovitski and Kimmel modelled a two-dimensional computational domain with a bubble oscillating inside a potential flow close to a wall [96]. In their study, the solution for potential flow was obtained numerically using a boundary integral method and the shear stresses on the vessel wall were obtained. Miao et al. coupled the boundary element method and Finite Element Methods to simulate an axisymmetrical two-dimensional model of a microbubble inside a vessel with different elasticities [111] where the fluid was treated as a potential flow. These numerical models ([96] and [111]) assumed unshelled bubbles. As stated earlier, adaptation of the confined model to account for the encapsulating shell is crucial. These limitations create a need for an encapsulated confined bubble model within a compliant vessel.

1.5 Numerical Analysis

Due to the highly non-linear nature of bubble oscillations, finding an analytical solution is extremely difficult unless various approximations are made [123]. Numerical analysis is a more feasible approach for complex problems and could provide the solution to either ordinary or partial differential equations. Finite element, Boundary element, Finite Volume and Finite difference methods are among standard techniques to provide numerical solutions to differential equations. Skalak and Ozkaya suggested Finite Element Methods (FEM) for biofluid problems as biological phenomena often involve non-linear governing equations, moving boundaries, and irregular
boundary geometries [124]. FEM was the numerical method for solving differential equations in this thesis.

1.6 Outline of the Thesis and Specific Aims

In this thesis, models of a confined bubble within a microvessel were developed numerically. As a result of such models, more insight is gained on the bubble behaviour, vascular mechanical stresses and acoustic emissions to further aid in BBB opening experiments and monitoring. Specific aims of the thesis breaks into three parts which are described in chapters 2, 3 and 4 respectively.

1- Developing a three dimensional confined bubble model within elastic vessels (lower pressures ~ kPa):

1) To elaborate on resonance frequency shift and microstreaming phenomena
2) To calculate vessel wall stresses
3) To perform sensitivity analysis on bubble oscillation and wall stresses when frequency, pressure, bubble size or vessel size are subject to change
4) To elaborate on non-spherical oscillations of off-centre bubbles and associated stresses

2- Developing a non-spherical bubble model (higher pressures ~ MPa), and comparing simulations with ex-vivo experimental data:

1) To estimate the vascular viscoelastic properties of experimental ex-vivo rat mesenteric microvessels
2) To calculate vessel wall stresses induced by a forced bubble during its expansion/contraction phase
3) To develop a non-spherical, confined bubble model within viscoelastic vessels (higher pressures) while accounting for surface tension and encapsulating shell
4) To validate the numerical model with the experimental data
5) To predict conditions based on bubble/vessel distance where vessel wall stresses dominate during bubble compression
3- Recruiting the confined bubble model with appropriate parameters specific to the BBB opening experiments:

1) To elaborate on the encapsulated confined bubble behaviour
2) To predict acoustic emissions from confined bubbles
3) To calculate vessel wall stresses

Chapter 5 covers the conclusion, summary and future directions.
Chapter 2
A 3D Model of a Confined Microbubble and its Mechanical Wall Stresses (Sensitivity Analysis)¹

2.1 Introduction

As mentioned in chapter 1, confined bubbles oscillate and behave differently from unbound bubbles. Caskey et al. experimentally studied the oscillation of microbubbles in 12, 25 and 195 μm microvessel phantoms and found that the bubble's amplitude of oscillation is substantially decreased when compared to the Raleigh–Plesset model of an unbound bubble [125]. Resonance frequency and damping are other factors that are affected by the surrounding media.

Shear stresses produced by bubble oscillations may generate bioeffects if cells are present in the microstreaming field. Circumferential stress exerted along the vasculature circumference is another important stress, and could be responsible for vascular rupture. In order to safely use microbubbles, it is important to understand the mechanisms involved, and estimate the mechanical stresses that microbubbles induce on the vasculature.

In section §1.4.3, challenges in confined bubble modeling were discussed. Here, a three-dimensional model of a confined encapsulated bubble was developed inside an elastic or rigid microvessel. The focus was towards using the most general form of fluid equations, and incorporating fewer assumptions to address some of the bubble modelling challenges. A finite element method was used. To increase the model's accuracy, we implemented a two-way coupling between the bubble's wall and the surrounding fluid, as well as a two-way coupling between the fluid and the vessel wall. Symmetrical and asymmetrical bubble oscillations were studied for bubbles in the centre of the vessel or off-centre respectively. The three dimensional geometry used in this work accounts for wall curvature in small vessels. This parameter is not included in previous two dimensional models [96]. Using this model, a parametric study was performed to investigate the influence of acoustical and vascular properties, vessel/bubble size and off-centre bubbles on the bubble oscillation and wall stresses.

2.2 Methods

In our model, a microbubble was placed in the centre of a microvessel, along the z axis, as seen in Figure 2-1. All simulated vessels were 5 μm in radius, 2 μm in thickness and 204 μm in length unless otherwise mentioned. Figure 2-1 illustrates the three dimensional geometry of the bubble, blood and microvessel that was used for the numerical simulation. Due to symmetry only half of the domain was calculated to improve computation time (Figure 2-1a). The vessel was free (not fixed to the surrounding tissue) and it was assumed as a "tube in liquid" where the capillary walls themselves bear the distending stress, and the elasticity values were chosen according to this assumption [126].

![Diagram](image)

Figure 2-1 A schematic illustration of the 3 dimensional geometry of bubble, blood and microvessel used for the numerical simulation. This is in a cylindrical coordinate where $\theta$ is in the vessel circumferential direction ($r$ (radial), $\theta$ (azimuthal), $z$ (axial)). (a) Bubble in the middle of the vessel, (b) an off-centre microbubble, (c) side view of an off-centre bubble.

2.2.1. Fluid Domain

Blood was assumed to be incompressible and Newtonian. Navier-Stokes equations were used [127]:

![Diagram](image)
\[
\rho \left( \frac{\partial \mathbf{v}_f}{\partial t} + \mathbf{v}_f \cdot \nabla \mathbf{v}_f \right) = \nabla \cdot \left[ -P I + \mu (\nabla \mathbf{v}_f + (\nabla \mathbf{v}_f)^T) \right]
\]  

(2.1)

where \( \mathbf{v}_f \) is the fluid velocity and \( T \) depicts matrix transpose. \( \rho \), the fluid density, was set to 1000 kg/m\(^3\). Due to the inverse Fahraeus–Lindqvist effect, in which blood viscosity increases with decreasing microvessel size, the blood viscosity used in this work was set to be 0.005 Pa s [128], [129]. A comparison of unbound and confined bubbles in viscous fluids with different viscosities is presented in section §5.2.

2.2.2. Microbubble

The bubble radius was set to 2 \( \mu \text{m} \) unless otherwise mentioned. Using the gas equation, the bubble's oscillation should satisfy the following equation:

\[
P_b = \left( P_0 + \frac{2Y}{R_0} - P_v \right) \left( \frac{R_0}{R} \right)^3 + P_v - \frac{2Y}{R} - \frac{4\mu \dot{R}}{R} - S(R) - P(t)
\]

(2.2)

where \( S(R) \) depicts the shell model. \( P(t) \), the acoustic pressure at the bubble wall, is \( P_a \sin(2\pi ft) \) and \( P_a \) and \( f \) are the acoustical pressure amplitude and frequency respectively and \( t \) is time. The acoustical properties, such as ultrasound frequency and pressure, were controlled from \( P(t) \) in equation (2.2). The ideal gas law [72] was used to model the bubble oscillation (first term on the right hand side). \( P_v \), the vapour pressure, was assumed to be zero [71]. The third and fourth terms on the right hand side in equation (2.2) represent the surface tension, and the fluid viscosity effect on the bubble wall respectively. Note that even though the fluid is assumed to be viscous, the inclusion of a fluid viscous term in equation (2.2) is due to the fact that the fluid viscous effects on the bubble wall manifest themselves through the boundary condition (not through the Navier Stokes equation) [72]. Poiseuille flow and Couette flow are special solutions of Navier–Stokes equations assuming a laminar flow. Here, a general form of Navier–Stokes equation was applied and those special cases of flow were not used.

Various encapsulating shell models have been developed in recent years [130]. However, in a linear regime they reduce down to the de Jong shell model [88] which is an analog to Hooke’s elastic and Newton’s viscous laws. The de Jong shell model, which is suitable for thin-shelled agents (e.g. lipid coated bubbles), was utilized by van der Meer et al. [74]. The shell used here has
the form given in [74]: \( S(R) = \frac{4k_s \dot{R}}{R^2} + 4\chi \left( \frac{1}{R_0} - \frac{1}{R} \right) \) with shell viscosity and elasticity terms. The bubble's shell viscosity \( (\kappa_s) \) was set to \( 10^{-8} \) [kg/s] and the shell elasticity \( (\chi) \) was set to 0.54 [N/m]. These are the shell properties reported for BR-14 (Bracco SA, Geneva) microbubbles with phospholipid shell and perfluorobutane gas [74].

Some of the constants used included the ambient pressure inside capillaries \( P_0 = 104.6 \) kPa and the surface tension = 0.072 N/m [131]. The Polytropic index of filling gas range from isothermal condition at \( k = 1.0 \) [132] to adiabatic at \( k = 1.4 \) [114]. While small gas bubbles at low ultrasound frequencies behave isothermally, larger bubbles (4 µm) at above 1 MHz frequencies behave between isothermal and adiabatic conditions [104]. A gas Polytropic index of 1.07 was chosen following van der Meer et al. [74].

In this work, it was assumed that the incoming acoustic wave is spherical and acting on the bubble wall. Due to the large acoustic wavelength (i.e. a few millimetres) compared to the bubble size, we could safely assume that the acoustic pressure is the same around the bubble wall. The frequency was varied over the range 0.1 to 5 MHz in this work and pressure varied from 52 to 400 kPa.

### 2.2.2.1 Spherical Symmetry

A spherical symmetry on the bubble wall was assumed using equation (2.2). The liquid pressure everywhere on the bubble surface was integrated, and normalized to the bubble surface area. The resultant pressure gave \( P_b \) in equation (2.2) and then from this equation the equivalent bubble radius was calculated at each time. In reality at high pressures the bubble in a confined vessel oscillates forming an ellipsoid shape (Fig. 3a of reference [133]). The resultant spherical radius in this section is analogous to the equivalent radius of an ellipsoid bubble, by equating the ellipsoid bubble surface area with a sphere area.

The bubble wall velocity \( (\dot{R}) \) was set equal to the fluid velocity. This condition allowed the coupling of bubble to fluid such that any changes in the bubble size caused the fluid to move. The fluid pressure at the bubble wall was fed back to equation (2.2) to control the bubble oscillation as a result of the fluid pressure change. In most of the simulations, a spherical model was used to save computational cost.
2.2.2.2 Asymmetrical bubble

A model of asymmetrical bubble oscillation was developed where the bubble surface (hemisphere) was divided into 48 sections, each section with the same area (shown in Fig 2-1b). The local pressure at each section was integrated and normalized to the area of that section. Using equation (2.2), the radius of each local section is calculated and the integration of these local radii forms the overall bubble shape. We used a finer mesh to accurately account for all the sections and simultaneously solve for all 24 (due to symmetry) equations. This simulation was computationally expensive and only the off-centre bubbles were incorporated. In section §2.4 the results from a spherical symmetric and asymmetrical bubble model are compared in more detail.

2.2.3. Blood Vessel Wall Deformation

The fluid and vessel were coupled in two ways. The continuity equation was satisfied \( \rho \nabla \cdot \mathbf{v}_f = 0 \), where \( \rho \) was the fluid density, \( \mathbf{v}_f \) was the fluid velocity field. The Navier–Stokes equations for a viscous incompressible Newtonian liquid and equations for an elastic vessel were solved simultaneously:

\[
 \rho \frac{\partial \mathbf{v}_f}{\partial t} + \rho (\mathbf{v}_f \cdot \nabla) \mathbf{v}_f = \nabla \cdot \left[ -P \mathbf{I} + \mu (\nabla \mathbf{v}_f + (\nabla \mathbf{v}_f)^T) \right] = \nabla \cdot \mathbf{\sigma} \quad (2.3)
\]

where \( P \) was the pressure in the fluid, and \( \mathbf{\sigma} \) was the stress tensor.

The elastic vessel wall was modeled using a linear elastic equation (Hooke's law), assuming a homogeneous and isotropic medium. The Young's modulus was chosen between 1-10 MPa to fit the physiological range [134]. Also, a rigid vessel with 100 MPa Young's modulus was simulated. Although a Young's modulus of 100 MPa is not in the physiological range, it can still mimic microvessels in bone. It was simulated to represent the effects of a rigid vessel on the resonance frequency. The linear elastic constitutive equation is given as

\[
 \sigma_{ij} = \frac{E}{1+\nu} \left( \epsilon_{ij} + \frac{\nu}{1-2\nu} \epsilon_{kk} \delta_{ij} \right) \quad (2.4)
\]

where \( \delta_{ij} \) is the Kronecker delta (if \( i = j, \delta_{ij} = 1 \), and if \( i \neq j, \delta_{ij} = 0 \)) and \( \sigma_{ij} \) and \( \epsilon_{ij} \) are the \( ij \)th components of the stress and strain tensors, respectively. Young’s modulus (\( E \)) and Poisson’s ratio (\( \nu \)) are the material parameters. Other vascular properties were chosen from [134], in which the
vessel density was 1055 kg/m$^3$, Poisson's ratio was 0.49 and the vessel elasticity was in the mega Pascal range.

Figure 2-2 shows the coupling framework between bubble, fluid and vessel in a numerical stable scheme. As the fluid pressure on the bubble wall changes, the bubble radius gets updated using equation (2.2), then the bubble wall velocity couples back to the fluid. On the fluid-vessel interface as a result of the fluid pressure vessel wall undergoes a displacement, the vessel wall velocity is then coupled back to the fluid. The information in the fluid at the bubble and vessel interfaces gets updated at each time step.

2.2.4 Boundary Conditions and Method of Solution

At the vessel wall, fluid velocity was set equal to the vessel wall velocity as the boundary condition. The initial fluid pressure, as well as the pressure at vessel ends, were set to the ambient pressure in the capillaries, $P_0$.

The vessel length (which was set to 204 μm (i.e., 2×L)) is long enough to make the results independent of the length, thus the microbubble has little impact from the vessel ends. When the vessel ends were fixed or free, the difference in bubble oscillation was 0.03%, and the variation in maximum shear and circumferential stress were less than 2%.
The numerical part was solved with the finite element method using Comsol Multiphysics 3.5a (COMSOL AB. Burlington, MA) along with a structural mechanics module. In the finite element method, each computational geometry is divided into small units to produce element meshes. In this three dimensional model, tetrahedral meshes were used. Different mesh configurations from coarse to fine were tested and optimized values were chosen. The coarse mesh consisted of 8 mesh elements on the bubble circumference (mesh size of 1.6 µm for a 2 µm bubble) to fine mesh consisting of 30 mesh elements (mesh size of 0.4 µm for a 2 µm bubble). Distal to the bubble wall, on the vessel end boundary, the mesh size was varied from 4 µm (coarse mesh) to 2 µm (fine mesh). The results were insensitive to the mesh configuration. Typically more than 10,000 mesh elements were used for this bubble-blood-vessel geometry. The time step of this time dependent model was set to 0.01 µs. At 1 MHz frequencies, this gives 100 sampling points for each oscillation period.

Since the bubble and vessel wall boundaries of our computational domain were moving in time, the simulation was performed using a Moving Mesh (Arbitrary Lagrangian-Eulerian) method [135], [136]. This method was used because new mesh does not need to be generated for each configuration of the boundaries. Instead, the mesh nodes are perturbed so they conform to the moved boundaries. When the material motion is more complicated, like in a fluid flow model, the Lagrangian method is not appropriate. For such models an Eulerian method, where the mesh is fixed, is often used. However, this method cannot account for moving boundaries. The Arbitrary Lagrangian-Eulerian (ALE) method is an intermediate between the Lagrangian, where the mesh movement follows the movement of the physical material and Eulerian methods, where the mesh is fixed. ALE allows moving boundaries without the need for the mesh movement to follow the material.

After solving the model, the shear stress was evaluated at the vessel wall using the shear stress equation e.g. in Cartesian coordinates [127]:

\[
\tau_{xz} = \mu \left[ \frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right] \tag{2.5}
\]

where coordinates are (x, y, z) and fluid velocities are (u, v, w).
The circumferential stress was calculated using thick wall cylinder equations [134] and equation (1.4). Bubble oscillation, fluid shear stress and circumferential stress were calculated as a function of acoustic frequency, acoustic pressure, vessel rigidity, vessel/bubble size and off-centre bubbles.

2.2.5 Validation

In order to compare the oscillation of an unbound bubble with the solution of the Rayleigh-Plesset equation [72], a simulation was performed in which a bubble was placed inside a free fluid without any confinement. Figure 2-3 illustrates the result of this simulation along with the solution to the Rayleigh-Plesset equation. The numerical simulation is very similar to the Rayleigh-Plesset equation except in the compression phase. The discrepancy in the bubble compression phase between the Rayleigh-Plesset equation and numerical simulations could arise from different ordinary differential equation solvers used by Matlab and Comsol. This difference is resolved in a later version of Comsol (as shown in figure 3-5 when the Rayleigh-Plesset equation is compared with the numerical solutions).

![Graph showing comparison between FEM simulation and Rayleigh-Plesset equation](image)

**Figure 2-3** Comparison between the FEM simulation on an unbound bubble and the solution of Rayleigh-Plesset equation. $P_a = 2.5 P_0$, $f = 1 \text{ MHz}$, $R_0 = 2 \mu m$.

For a confined bubble, this model was validated using experimental data from references [80] in which a microbubble was placed close to a wall. Figure 2-4 shows the comparison between an unbound bubble and a microbubble kept at a wall with an optical tweezers system. Parameters in simulation were set so that the bubble size and shell materials match those performed in the
experiment ($R_0 = 2.45 \mu m$, $f = 2.25$ MHz, Pa = 200 kPa, $\chi = 0.8$ Nm$^{-1}$, $\kappa_s = 2\times10^{-8}$ kg s$^{-1}$ [137]). An unbound bubble oscillation from the Rayleigh-Plesset equation was also plotted for comparison. The bubble oscillation from the numerical simulation followed a similar trend as the experimental data. In the numerical data, there was a deviation of 40% and 160% from the experimental results in the first and second expansion phase cycles, respectively (The relative expansion was defined as the maximum peak value minus the initial bubble size. The relative expansion of the numerical curve was subtracted from the relative expansion of the experimental curve and normalized to the relative expansion of the experimental curve. The resultant multiplied by 100 gave the percentage of deviation). The Rayleigh-Plesset equation has a 500% deviation from the experimental results and therefore the numerical result is much closer to experimental results than the solution of the Rayleigh-Plesset equation. The deviation in the numerical result could be due to a slight distance between the bubble and wall in our numerical work or from the spherical symmetric bubble assumption. In the above experiment, when the bubble was kept away from the wall, bubble oscillation was similar to the Rayleigh-Plesset solution (Figure 2 in reference [80]) and numerical simulations from our work obtained similar results for the unbound bubble.

**Figure 2-4** Numerical bubble oscillation comparison with experimental data of optical tweezer [80]. The dotted line shows the Rayleigh-Plesset solution of an unbound bubble. Solid line is the experimental result of the bubble oscillation close to a wall [80] and solid line with dot represents the bubble radius from numerical simulation of this model. Pa = 200 kPa, $f = 2.25$ MHz, $R_0 = 2.45 \mu m$. 

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For further validations, several aspects were considered. It was ensured that fluid mass was conserved; \( \text{div}(\rho \mathbf{v}_f) = 0 \). With an incompressible fluid, \( \rho \) was constant and therefore \( \text{div}(\mathbf{v}_f) = 0 \). The propagating wave speed along a flexible tube wall was estimated with a simplified equation which assumes a linear relationship between area and pressure. This wave speed is \( c_0 = \frac{1}{\sqrt{\rho C}} \) where \( \rho \) is the blood density and \( C \) is the compliance; \( C = \frac{1}{S_0 \Delta P} \) where \( S_0 \) is the initial vessel area, \( \Delta S \) is the variation in the area and \( \Delta P \) is the variation in the pressure. We calculated the wave speed from the above equation and also obtained the wave speed from the vessel wall movement in our numerical model. For a 5 MPa elastic vessel, the propagating wave speed was 23.7 m/s and there was a good agreement between the above equation and the model, with less that 3% error.

Since the asymmetrical bubble simulation was computationally expensive, symmetrical bubble oscillation was performed for most of the results. The bubble radius of the symmetrical model (in which the fluid pressure is integrated over the bubble surface) is analogous to an equivalent radius. This equivalent radius might be larger than asymmetrical bubble expansion radius in \( r \) direction (semi minor length of an ellipse) due to damping. Therefore, the results might have been overestimated, especially in the regimes with larger bubbles compared to the vessel size, higher acoustic pressure and stiffer vessels. A comparison between a spherical symmetric and asymmetrical bubble model inside a rigid vessel resulted in a 0.3% and 1% deviation in shear and circumferential stresses respectively (\( E=100 \text{ MPa}, P_0=261 \text{ kPa}, f = 1 \text{ MHz}, R_0 = 2 \mu m, r_v = 5 \mu m \)). For a large bubble within a vessel, the stress variations were 3% and 12% in shear and circumferential stress respectively (\( E=5 \text{ MPa}, P_0 =261 \text{ kPa}, f = 3.25 \text{ MHz}, R_0 = 4 \mu m, r_v = 5 \mu m \)). Therefore, the spherical symmetric bubble model holds for the values investigated in this work with higher sensitivity to vessel/bubble ratio and less sensitivity to the vessel rigidity. However, when all the parameters are chosen in such a way to present an extreme case (\( i.e. \) for stiffer vessels, smaller vessel to bubble ratios and higher pressures), the bubble deviates from the spherical shape and the results are over estimated. A test simulation was performed to represent an extreme case that jeopardizes the spherical symmetry assumption. In this simulation, a 4 \( \mu m \) bubble inside a rigid vessel (\( E=100 \text{ MPa} \)) excited at 3.25 MHz and 680 kPa was considered with both spherical symmetry bubble model and asymmetrical model. As a result, the shear and circumferential stresses in asymmetrical bubble model were 31% and 27% less than those stresses calculated in
the spherical symmetric model. Thus, the stress values of a large spherical bubble inside a rigid vessel at high acoustic pressure is overestimated but still in the same order of magnitude as the asymmetrical bubble. Here, most of our results were obtained at 2.5 $P_0$ (261 kPa) acoustic pressure. When the spherical symmetric versus asymmetrical bubble models were compared at this pressure (261 kPa), the difference of shear and circumferential stresses between the two models were 0.4% and 4% respectively ($E=5$ MPa, $P_0=261$ kPa, $f = 1$ MHz, $R_0 = 2 \mu$m, $r_v = 5 \mu$m).

Bubbles smaller than the resonant size oscillated in phase with the external acoustic pressure. Those larger than resonance oscillated $\pi$ out of phase as inertia dominates. At 3.25 MHz, and inside a 5 $\mu$m vessel (elasticity of 5 MPa), a 2 $\mu$m bubble is at resonance. Radial excursion ($\frac{R-R_0}{R_0}$) of 1 $\mu$m (below resonance) and 4.5 $\mu$m (above resonance) bubbles within 5 $\mu$m vessels sonicated at 3.25 MHz are shown in Fig 2-5. As expected the results show that the two bubbles oscillated out of phase from each other.

![Figure 2-5](image)

**Figure 2-5** Radial excursion of a (a) 1 $\mu$m, and (b) 4.5 $\mu$m bubble shown in a black solid line. The driving acoustic pressure in red dashed line is shown for comparison. $E = 5$MPa, $P_a = 2.5$ $P_0$, $f = 3.25$ MHz, $r_v = 5 \mu$m.

### 2.3 Numerical Results

In this section the bubble/vessel behaviour and streamline patterns are illustrated first. Then bubble excursion and wall mechanical stresses subjected to a sensitivity analysis are evaluated. Acoustic frequency and pressure, vessel rigidity and vessel/bubble sizes and the bubble position in the middle of the vessel were the parameters that varied here. Bubble’s radial oscillation ($\varepsilon_0$ which is $R - R_0$) and microvessel wall ($z = 0, r = r_v$) are shown in Fig. 2-6.
Figure 2-6 Radial variation of the bubble wall and vessel wall versus time for $7 \mu$s. $E = 5\text{ MPa}$, $P_a = 2.5 P_0$, $f = 1 \text{ MHz}$, $r_v = 5 \mu\text{m}$, $R_0 = 2 \mu\text{m}$. The solid line shows bubble radius oscillation and the dashed line represents the vessel wall movement above the bubble.

Figure 2-7 illustrates the radial expansion of the vessel wall versus the axial direction ($z$) at different snapshots in time. The bubble is oscillating at the origin of the axial direction. As time progresses, waves form from the origin and travel towards the end of the vessel.

Figure 2-7 Wave propagation along the vessel wall. Radial expansion of the vessel wall versus normalized axial direction ($z$) from the middle of the vessel to its end, showing a wave traveling inside an elastic vessel at different snapshots in time, $E = 5 \text{ MPa}$, $P_a = 2.5 P_0$, $f = 1 \text{ MHz}$, $R_0 = 2 \mu\text{m}$ and $r_v = 5 \mu\text{m}$. The axial direction is normalized to the length of the vessel ($L=102 \mu\text{m}$).
The fluid streamlines for a rigid and an elastic vessel are illustrated in Figs. 2-8a and 2-8b respectively. The addition of wall flexibility resulted in a much more complicated flow field than in the rigid wall. Due to the collision of two opposing flows stagnation points, where the local velocity of the fluid is zero, are evident in Fig. 2-8b.

![Streamlines and stagnation points](image)

Figure 2-8 Streamlines and stagnation points of (a) a rigid vessel, (b) an elastic vessel at time 4 vs. $E = 5\text{MPa}$, $P_a = 2.5\ P_0$, $f = 1\ \text{MHz}$, $r_v = 5\ \mu\text{m}$, $R_0 = 2\ \mu\text{m}$.

Both shear and circumferential stressed oscillated temporally and spatially as shown in Figure 2-9. The shear stress is maximum when the bubble wall acceleration is zero i.e. the bubble wall velocity is constant. The maximum fluid shear stress occurred in front of the bubble at the vessel wall ($z > 0$, $r = r_v$) and the maximum circumferential stress happened exactly above the bubble at the vessel wall ($z = 0$, $r = r_v$).
Figure 2-9 Temporal and spatial variation of stresses (a) Fluid shear stress at point $z = 2.4 \, \mu m$, $r = r_v$, (b) The shear stress versus normalized axial direction, (c) Circumferential stress versus time at point $z = 0$, $r = r_v$, (d) Circumferential stress versus normalized axial direction. $E = 5 \, MPa$, $P_a = 2.5 \, P_0$, $f = 1 \, MHz$, $r_v = 5 \, \mu m$, $R_0 = 2 \, \mu m$.

In Figure 2-10a, a $2 \, \mu m$ bubble is located inside an unbound fluid, a $2 \, MPa$, $5 \, MPa$, $10 \, MPa$ and $100 \, MPa$ elastic tubes (where the Young's modulus of $100 \, MPa$ represents a rigid tube). The maximum bubble radius is plotted versus the acoustic frequency at a fixed acoustic pressure. This trend suggests more rigid vessels have a higher damping impact on the bubbles resulting in smaller bubble excursion, and the resonance peak at stiffer vessels shifts towards higher frequencies. In fact, a $16\%$ increase in the resonance frequency is observed when an unbound bubble was placed within a $10 \, MPa$ elastic vessels. In addition, the resonance frequency had an $8\%$ increase when vessel elasticity rose from $2$ to $10 \, MPa$. The rigid vessel had the lowest bubble expansion, with a peak at $5 \, MHz$. To investigate the peak at $5 \, MHz$ for a rigid vessel, Figure 2-10b plots the maximum bubble radius normalized to the bubble initial radius versus the initial radius of different
bubble sizes sonicated at 5 MHz. This curve peaks at 2 µm suggesting that 5 MHz is the resonance frequency of 2 µm bubbles.

Figure 2-10 Maximum bubble expansion as a function of acoustic frequency of a bubble inside different vessels. Pa = 2.5 P₀, rᵥ = 5 µm, R₀ = 2 µm. (b) Maximum bubble oscillation normalized to the initial bubble size versus the initial bubble size inside a rigid vessel (100 MPa) sonicated at 5 MHz.

Figure 2-11 Fluid shear stress versus acoustic frequency for different elastic vessels. Pa = 2.5 P₀, rᵥ = 5 µm, R₀ = 2 µm.

In Figures 2-11 the maximum shear and circumferential stresses of a 2 µm bubble inside a 2 MPa, 5 MPa and 10 MPa elastic vessels are plotted versus the frequency. The shear stress in Fig 2-11 peaks at frequencies higher than the bubble's resonance frequency (e.g. 15% more than the resonance frequency of a 2 µm bubble in a 5 MPa elastic vessel). In more rigid vessels, this peak
was broader and happened at slightly higher frequencies than softer vessels. Shear stress variation with vessel elasticity was trivial below the resonance frequency (less than 1 kPa variation below 2.5 MHz).

In Fig 2-12, the circumferential stress of a 2 μm bubble in a 5 MPa vessel peaks at 23% more than this bubble’s resonance frequency. This stress for each elasticity value has an increasing trend versus the acoustic frequency, while at each frequency there is a dependency on vessel elasticity. As the rigidity increases, the location of the local minimum and maximum peaks shifts towards higher frequencies. Two different trends versus vessel rigidity were observed here. At frequencies lower than 1.5 MHz, a rise in the vessel stiffness resulted in an increase in the circumferential stress. At 1 MHz, a 5 fold elasticity increase (from 2 to 10 MPa) resulted in the circumferential stress increase by a factor of 3.8. However, at frequencies above 2.5 MHz, a rise in the vessel stiffness resulted in an inverse pattern of a decrease in circumferential stress. The largest variation in circumferential stress versus acoustic frequency happens in soft vessels.

![Figure 2-12 Circumferential stress versus acoustic frequency for different elastic vessels. Pa = 2.5 P₀, rᵥ = 5 μm, Rₒ = 2 μm.](image)

Figure 2-13 presents the effects of acoustic pressure at the resonance frequency of a confined bubble inside a 5 MPa tube (3.25 MHz) on maximum bubble expansion, fluid shear and circumferential stress respectively. Linear fits to Fig. 2-13b and 13c give slopes of 7000 and 80000
for shear and circumferential stresses respectively. Therefore the circumferential stress is more sensitive to the bubble expansion than the shear stress.

![Graphs showing maximum bubble expansion and stresses versus acoustic pressure.](image)

**Figure 2-13** Maximum bubble expansion (a) maximum shear (b) and circumferential stress (c) versus acoustic pressure. $E = 5$ MPa, $f = 3.25$ MHz, $r_v = 5 \mu m$, $R_0 = 2 \mu m$.

In Figure 2-14 the microbubble size was varied while keeping the vessel size constant. Figs. 2-14a, b, c and d show the maximum bubble radius, vessel wall movement, shear stress and circumferential stress respectively (at 1 MHz, the off resonance frequency and 3.25 MHz, the resonance frequency of a 2 $\mu m$ bubble in a 5 $\mu m$ vessel). In Fig. 2-14d larger bubbles exerted higher pressures on the vessel wall and therefore there was an increasing trend for the circumferential stress, with a local maxima around 2 or 2.5 $\mu m$ bubbles at 3.25 MHz. At 1 MHz, larger bubbles (e.g. 4.5 $\mu m$) are closer to resonance and for these bubbles the maximum bubble radius and circumferential stress exceed that at 3.25 MHz (Figs. 2-14a and d).
Maximum bubble radius, vessel wall movement, shear stress and circumferential stress are illustrated in Figures 2-15a, b, c and d as a function of vessel radius while the initial bubble radius was kept constant ($r_0 = 2 \mu m$ at 1 MHz and 3.25 MHz). Higher frequencies and smaller vessels resulted in larger shear stresses. In Figure 2-15d when the vessel size is increased the bubble wall is farther away from the vessel and therefore the circumferential stress decreases. However, close to resonance (at 3.25 MHz) the bubble resonated and expanded more in larger vessels, which caused the circumferential stress to increase. In Fig. 2-15d, a 6% increase in the maximum bubble radius at 3.25 MHz resulted in a 42% increase in the circumferential stress.
Figure 2-15 Maximum bubble expansion (a) vessel wall movement (b) maximum shear (c) and circumferential stress (d) versus initial vessel radius. E = 5MPa, Pa = 2.5 P₀, r₀ = 2 µm.

Shear stress was the only stress that changed approximately according to a non-dimensional parameter rᵥ/r₀ (the ratio of the initial vessel radius to the initial bubble radius). Figure 2-16 shows the maximum shear stress versus rᵥ/r₀ at 1 MHz and 3.25 MHz frequencies while either changing the bubble radius or the vessel radius. The shear stress is larger at 3.25 MHz and increases as rᵥ/r₀ decreases.
Figure 2-16 Maximum shear stress versus the vessel radius normalized by the initial bubble radius. $E = 5\text{MPa}$, $P_a = 2.5$ $P_0$.

Another set of simulations were performed using an asymmetrical bubble model. A microbubble was placed off centre (in this case 2.5 µm away from the vessel centre) as shown in Figures 2-1b and 2-1c.

Figure 2-17 Oscillation of a bubble 2.5 µm away from the vessel centre versus time for different points on the bubble wall, (a) at 1 MHz, (b) at 3.25 MHz, $E = 5\text{MPa}$, $P_a = 2.5$ $P_0$, $r_0 = 2$ µm.

Figure 2-17 shows the oscillation of each point (points are defined in Figs. 2-1b and 2-1c) on the bubble wall. Point $R_{r1}$, having the closest distance to the vessel wall, is oscillating with the lowest
amplitude, while $R_z$ (located at $r = r_0$ and $z = 0$) has the largest amplitude of oscillation. In a 3D view, the oscillating bubble forms a mushroom shape. A 2D snapshot of the bubble is given in Fig. 2-18.

![Image](image.png)

**Figure 2-18 A 2 µm bubble 2.5 µm off-centre forming a mushroom shape, at 1MHz, 260 kPa.**

The stresses due to a 2 µm oscillating bubble located at 2.5 µm off-centre is plotted versus the azimuthal angle in Fig 2-19. The highest bubble impact is at zero degrees at the wall closest to the bubble surface. At 90° and 270° degrees the bubble points and vessel wall have the same distance and owing to its symmetry exert the same amount of stress. Also at 3.25 MHz the stresses are higher compared to 1 MHz.
Figure 2-19 Schematic of an off-centre bubble and the azimuthal angle (a), the maximum shear (b) and circumferential stress (c) versus the azimuthal angle for a bubble 2.5 µm away from the vessel centre. \( E = 5 \text{MPa}, \ Pa = 2.5 \ P_0, \ r_0 = 2 \ \mu\text{m} \). The blue line with open circle shows \( f=1 \ \text{MHz} \), and the red line with asterisks represents \( f = 3.25 \ \text{MHz} \).

2.4 Discussion and Conclusion

In this study, we have created a comprehensive three dimensional model of an oscillating microbubble confined within a vessel where fluid/vessel and fluid/bubble wall were closely coupled. Spherical symmetric and asymmetrical bubble models were adapted in this work. Our model was validated with the results of Rayleigh-Plesset equation for an unbound bubble as well as a confined bubble close to a wall (Figs. 2-3, 2-4).

Our model is an extension of other existing confined bubble models and reflects real conditions in which it accounts for the bubble shell properties, vessel wall curvature, fluid viscosity and off-centre bubbles. This work can help us achieve a better understanding of the confined microbubble...
behaviour for imaging purposes and resulting mechanical stresses on the vessel wall with the aim to eliminate any harm on the tissue or to increase local vessel permeability (depending on the application). The vessel elasticity can be changed to model microvessels in different locations e.g. brain, skin or close to a bone which have different capillary types and rigidities [126], [138]–[140].

Fluid streamlines (shown in Fig. 2-8) describe two different situations of a microbubble inside a rigid and a flexible vessel. Similar flow patterns of an expanding microbubble was reported earlier in Ye and Bull 2004 and 2006 (shown in [141] for a rigid vessel and [108] for an elastic flexible vessel). Stagnation points occur exclusively in compliant vessels. On the one hand the bubble expands and pushes the fluid away and at the same time the expanded vessel in front of the bubble “sucks” the liquid from the reservoir into the vessel (in order to conserve the liquid mass). Thus the two opposing flows form a stagnation point. Furthermore temporal and spatial oscillation of stresses in Fig. 2-9 adds to the flow complexity in elastic vessels. The sign reversal of shear stress was calculated numerically from the motion of a gas embolism in a blood vessel [142]. Mukundakrishnan et al. suggested that shear stress sign reversals may contribute to the disruption of endothelial cell membrane integrity and functionality [142]. However, it has yet to be investigated, especially in the context of bubble oscillation in response to MHz frequencies.

Studying the resonance frequency of confined bubbles is important in the medical context as the acoustic energy transfer to the bubbles is maximized at resonance. Bubbles in more rigid vessels had more damping in Fig. 2-10a which is consistent with the work of others [105], [114]. Furthermore, an increase in the resonance frequency of bubbles in elastic vessels was observed (Fig 2-10a) which suggests that bubble oscillation is maximized when sonicated at frequencies higher than unbound bubble resonance frequency. In a recent study, Helfield et al. experimentally observed an increase of about 10% in the resonance frequency of a 1.5 μm (in radius) MicroMarker™ close to an elastic agarose boundary [82]. This shift in resonance frequency versus vessel elasticity could have implications in microvessel imaging and tissue characterization (e.g. a mean to differentiate between bubbles in tumorous rigid tissue from other confined/unbound bubbles).

It is important to note that in an ideal rigid vessel, the infinite vessel wall has absolutely no movement, therefore due to the inertia (bubble has to push/pull two infinite liquid columns)
bubbles would not oscillate. But in case of our simulations, a rigid vessel was defined such that it has 100 MPa elasticity with a finite length therefore excited bubbles undergo oscillations. In Fig. 2-10, bubbles within rigid vessel had a maximum response at 5 MHz. It is also interesting to note that the bubble radius increases at the lower end of the frequency spectrum. This can be due to the fact that at lower frequencies the bubble has more time to expand. Furthermore, bubbles in a rigid vessel might have another peak at lower frequencies (the low frequency peak was not observed here). In fact, in a previous numerical study an unshelled bubble in a rigid finite vessel was reported to have a high and a low resonance peak [112]. However, this situation has not yet been investigated experimentally.

The shear stress versus frequency in Figure 2-11 shows a peak (similar to Fig. 2-10a) however, it happens at higher frequencies than the resonance frequency (e.g. a 2 μm bubble inside 5 MPa vessel has a resonance frequency of 3.25 MHz and the shear stress peaks at 3.75 MHz). The shift in the peak location of shear stress could be explained by the theory derived by Nyborg [51] and Lewin and Bjorno [53], shown in equations (1.1) and (1.2). The velocity gradient, $G$, in equation (1.2) is proportional to $\varepsilon_0^2$ and to $f^2$. On the one hand, the bubble oscillates with maximum amplitude when the frequency is approaching the bubble resonance frequency. On the other hand, the velocity gradient increases with frequency, even passing the resonance frequency. At higher frequencies above 3.25 MHz, the bubble oscillation ($\varepsilon_0$) is slightly lower than at the resonance size -but the frequency is higher, causing the velocity gradient to increase. This causes the oscillation to peak at a higher frequency. When the frequency is further increased, the bubble oscillation dramatically decreases and this makes the velocity gradient drop back down. In the literature, there is a lack of data on experimental shear stress from microbubbles inside micron scale tubes, due to the difficult nature of this type of experiments.

The change in circumferential stress (Fig. 2-12) depends on both the acoustic frequency and vessel stiffness. The rise in circumferential stress versus frequency could be explained by pressure build-up at higher frequencies. Below 1.5 MHz circumferential stress increases with vessel rigidity, and above 2.5 MHz an inverse trend is obtained. From the results presented here, the highest stress on the vessel wall is achieved when the bubble is located inside a softer vessel, and excited at frequencies above 2.5 MHz (or closer to the resonance). At therapeutic frequencies (i.e. below 1.5 MHz) stiffer vessels experienced larger circumferential stresses. This finding could be important
when maximum bubble impact on the tissue is required. For instance, in case of tumour treatment (which vessels are more rigid and ultrasound frequencies are low) circumferential stresses produced on these vasculature are higher than normal vessels. This complicated behaviour of the circumferential stress explains the need for the numerical simulation in order to understand the physical phenomena.

In Figures 2-14 and 2-15, the larger values of shear stress were obtained when the acoustic frequency was high and \( r_v/r_0 \) was low. Shear stress was the only stress that scaled with the vessel to bubble size ratio \( (r_v/r_0) \). The circumferential stress was highest for low \( r_v/r_0 \) values as well. However, the circumferential stress was more sensitive to bubble oscillations itself. As the vessel size increased (in Fig. 2-15d) the less damped bubble oscillations caused the circumferential stress to increase. The predicted vessel wall stresses (Figures 2-11-2-16) could be helpful in enhancing therapeutic outcomes in BBB opening experiments and improving the drug delivery efficacy.

Asymmetrical bubble oscillations were also considered for an off-centre bubble (Fig. 2-17). When the bubble is located closer to one side of the wall it forms a mushroom shape (the closest point to the wall on the bubble oscillates with lowest amplitude). This finding is consistent with the experimental observation of bubbles oscillating closer to one boundary ([81], [133]).

It is worth noting that the physiological shear stress induced on the vessel wall by the flowing blood in the cardiovascular system is about 1 Pa [143] and the corresponding values due to oscillating bubbles reported here are 3 to 5 orders of magnitude larger. van Wamel et al. assessed the membrane permeabilization of endothelial cells \textit{in vitro} due to vibrating microbubbles [55]. A direct correlation between cell deformation and cell membrane permeability was found, which was attributed to the shear stresses induced by oscillating microbubbles. However, for endothelial cells there is no data as to what extent or how long these cells could tolerate shear stress induced by oscillating bubbles.

The vessel wall tensile strength has been reported in the literature in a range of 0.46-3.6 MPa [65], [144]–[146]. However these data were obtained in large vessels. Neal and Michel assessed the vascular strength of frog microvascular endothelium by varying the intravascular pressures [64]. In their study, exceeding a vascular strength of 0.8 MPa caused vascular rupture leading to red blood cell extravasations. The circumferential stress was assumed responsible as the oval rupture
sites had their larger axis along the vessel axis. However, the vascular strengths obtained by Neal and Michel were measured from pressurized vasculature that lasted for 10 seconds. Therefore the link between this vascular strength and the transient circumferential stresses presented here is unclear. Future experimental examinations could aid in calibrating the stress values obtained numerically.

2.4.1 Model Limitations

In this model the acoustic pressure was directly applied on the bubble surface and it was assumed that initial pressure inside the fluid and at the vessel ends were constant: $P_0$. In reality, not only the bubble wall but also the fluid and vessel are subjected to the acoustic pressure (which was ignored in this work). Since for these low pressure amplitudes the ultrasound alone has a small effect on the vessel wall movement (this has been tested in another set of simulations and the difference between the resulting stresses are less than 1%), acoustic pressure was applied directly on the bubble wall to save computational time.

The model proposed here is suitable for relatively low acoustic pressures (~kPa) as de Jong shell model is valid only for small oscillations. Therefore a more complicated shell model for large bubble oscillation is required. Therefore, a confined bubble model applicable at high acoustic pressures (~MPa) is needed where the surface tension influences the bubble wall curvature. In chapter 3 such model is developed, validated and discussed in full details.
Chapter 3
Mechanisms of microbubble–vessel interactions and induced stresses: A numerical study\textsuperscript{2}

3.1 Introduction

Microbubbles could induce mechanical bioeffects on their confining vasculature \cite{50}, \cite{147}, \cite{148}. One major challenge in using bubbles in medical ultrasound is the lack of knowledge about their behaviour in confined geometries, and their impact on the surrounding tissue. It is important to investigate bubble mediated mechanical effects on the vessel wall and to understand the mechanism involved. Previously, the vascular damage was attributed to either the vessel distension or direct impact of a bubble jet on a vessel wall \cite{62}, \cite{114}, \cite{121}. However, recent high speed photographs of \textit{ex vivo} vessels showed that the bubble collapse within a vessel generates a distinct invagination of the vessel wall (\textit{i.e.}, towards the lumen of the vessel) \cite{122}, \cite{149}. This bubble-vessel coupling may be responsible for some vascular damage. Previously, vascular rupture (leading to extravasation of red blood cells) due to bubble activity was observed and reported in different studies \cite{50}, \cite{147}, \cite{150}, \cite{151}.

A numerical simulation of the experimental data could shed light on the bubble-vessel interactions. In particular, such theoretical model could predict non-spherical encapsulated bubble oscillations inside a vessel. It could also contribute to fluid flow information as well as stress levels exerted on the vessel wall during the vessel distension and invagination, thus providing the means to elaborate on one of the mechanisms responsible for cavitation-induced bioeffects.

In chapter 2 we have developed a model that addressed most of these limitations; however, it was only suitable to low acoustic pressures. In this chapter a non-spherical bubble model was developed applicable for relatively high acoustic pressures (~ 1 MPa).

Realistic vessel parameters are critical for bubble/fluid/vessel simulation. In this study, numerical simulations were compared with experimental observations of bubbles within rat mesenteric microvessels \textit{ex vivo}. Chen \textit{et al.} speculated that the tissue viscosity could be significant due to

vessel wall invagination [152]. It was also reported elsewhere that mesenteric tissue behaves in a viscoelastic manner [153]. Also, Swayne et al. observed a non-linear behaviour from mesenteric microvessels when increasing the pressure in increments. They suggested that the basement membrane has the appropriate properties to explain this behaviour [154]. Skalak et al. suggested that the vessel wall could be modeled using a standard viscoelastic solid [124]. As it is crucial to have proper viscoelastic parameters to perform the numerical part, in the first section of this chapter the viscoelastic properties of ex vivo rat mesentery microvessels were assessed. This assessment was done using a standard linear solid (SLS) viscoelastic model. Then these viscoelastic parameters were recruited in the first numerical model, in which the bubble oscillations were dictated to mimic the experimental data sets and vessel wall stresses were calculated. Then, in the second numerical section, we developed a comprehensive encapsulated confined bubble model while accounting for the effects of surface tension within a viscoelastic vessel. Four experimental data sets (cases 1-4) of micron size bubbles inside different rat mesentery microvessels were considered for the comparison with the numerical work. The second numerical part was done with the intention to validate the model, to predict the confined bubble behaviour as well as the associated wall stresses. From both numerical parts, the vessel wall shear stress and circumferential stress were calculated during bubble expansion or compression and conditions in which the stresses were the highest during vascular invagination were predicted.

3.2 Methods

3.2.1 Experimental Data

In a collaborative study (H. Chen and T. J. Matula at Centre for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington) experimental data were obtained. In these experiments high speed photomicrography was used to visualize the direct transient interactions between ultrasound-activated microbubbles and blood vessels within ex vivo tissue. The microscope was aligned confocally with a focused annular ultrasound transducer. Rat mesenteries were used as the vessel model. After the mesentery was flushed clear of blood, the animal was sacrificed and the mesentery with intestine was dissected away from the rat body. Then Definity® microbubbles were mixed with saline and injected into the mesentery segment. The transducer was driven by a single cycle sine wave produced by a function generator (33120A; Hewlett
Packard, Palo Alto, CA, USA) and amplified by a power amplifier (A150; ENI, Rochester, NY, USA). The acoustic pressure at the focus of the transducer was measured with a fiber optic probe hydrophone (FOPH 2000; RP Acoustics, Leutenbach, Germany). Figure 3-1a shows the pressure waveform used here. The ultrasound pulse had a peak negative pressure of 0.8 MPa at 1 MHz and lasted for about 2 µs. During each insonation, images were captured by a camera (Imacon 200; DRS Hadland, Cupertino, CA, USA) using an exposure time of 50 ns. For full details of the experimental setup and image analysis method see reference [133].

From the recorded images the bubble and vessel radii were evaluated using ImageJ software (ImageJ 1.41o; National Institutes of Health, Bethesda, MD, USA). Assuming that the confined bubbles are ellipsoidal (in the image plane), the bubbles' semi-minor and major axes were along r (radial) and z (axial) direction respectively (Figure 3-1b). From the experiments, four data sets were examined in this chapter. Table 3-1 presents the bubble and vessel radii of the four experimental cases. These cases were chosen because of their known initial bubble sizes prior to the ultrasound exposure. The bubble behaviour and accurate numerical simulations highly depended on bubble's initial size.
Figure 3-1 (a) The applied ultrasound pulse with 1 MHz frequency and peak negative pressure of 0.8 MPa. (b) A confined bubble within a microvessel with semi-minor and major axes along r (radial) and z (axial) directions respectively, the scale bar represents 10 µm.

Table 3-1 Experimental cases of single confined bubble within a microvessel.

<table>
<thead>
<tr>
<th>Case #</th>
<th>Bubble Initial Radius (µm)</th>
<th>Vessel Initial Radius (µm)</th>
<th>Frame Interval (ns)</th>
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</tr>
<tr>
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<td>300</td>
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<tr>
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<td>3</td>
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</table>
3.2.2 Viscoelastic Properties of Microvessels

In an earlier study by our collaborators, a number of rat mesentery \textit{ex vivo} experiments were performed in which collapsing microbubbles inside mesenteric venules caused the vessels to invaginate to their maximum extent. The recovery process of the invaginated vessel wall was photographed with the intention of estimating the viscoelastic properties of the vessel \cite{155}.

Microvessels here were venules (diameters larger than 8 µm). They were distinguished from other microvessels by the flow direction as well as vessel branching and collecting. The experimental setup in this part was the same as that used to photograph the confined bubble activity (section §3.2.1), except that longer times were captured to observe the vessel wall recovery \cite{155}. The vessel properties and characteristic were reported recently using a Voigt solid model \cite{155}. In this work the experimental data were fitted with a standard linear solid model (SLS) as it predicts both creep and stress relaxation. Skalak \textit{et al.} suggested that SLS can be used to describe the vessel wall \cite{124}. The SLS model is made up of two branches: one with a single spring, and the other with a spring and a dashpot. The creep function of a SLS model can be written as:

\[
    c(t) = \frac{\varepsilon(t)}{\sigma} = \frac{1}{\mu_0} \left[ 1 - \left(1 - \frac{\tau_\varepsilon}{\tau_\sigma} \right) e^{-\frac{t}{\tau_\sigma}} \right], \text{ where } \tau_\sigma = \frac{\eta_1}{\mu_0} \left(1 + \frac{\mu_0}{\mu_1}\right), \text{ and } \tau_\varepsilon = \frac{\eta_1}{\mu_1} \text{ are time constants.}
\]

\(\sigma\) is the applied stress on the structure, \(\varepsilon(t)\) is the strain, \(\mu_0\) is the spring constant on the first branch, and on the second branch, \(\mu_1\) and \(\eta_1\) are the spring constant and the coefficient of viscosity of the dashpot respectively (Figure 3-2).
Figure 3-2 Viscoelastic model of a generalized Maxwell equation (SLS model), where $\mu_1$ and $\mu_0$ are the elastic moduli and $\eta_1$ is the vessel viscosity.

Since the collected experimental data corresponded to the recovery phase of the vessel wall, the vessel initial position was set to the maximum vessel displacement ($D_{Max}$), during maximum invagination. Therefore $(t) = \frac{D_{Max} - D(t)}{D_{Max}}$, where $D(t)$ is the vessel displacement. Now, the governing equation for the recovery phase would be

$$D(t) = D_{Max} \left(1 - \frac{\tau_\sigma}{\tau_\sigma} e^{-t/\tau_\sigma}\right) \quad (3.1)$$

where $\mu_0$, $\mu_1$ and $\eta_1$ are the three parameters of this model. Smaje reported the elasticity of capillaries and venules in cat mesentery *in vivo* [126]. Based on that, $\mu_0$ (the long term elastic modulus) was set to 50 kPa. The experimental invaginated vessel data were fitted with equation (3.1) while fixing the long term elastic modulus ($\mu_0$ to 50 kPa) in order to evaluate the other unknown parameters $\mu_1$ and $\eta_1$. The fitting was done using Matlab (version 7.11, The MathWorks, Natick, MA) and its lsqcurvefit function (which solves non-linear data-fitting problems in least-squares sense) in the optimization toolbox.

The fitted data sets are shown in Figures 3-3a and 3-3b. The first data set belonged to a small microvessel with a maximum displacement of $D_{Max} = 14 \mu m$ (Fig. 3-3a). The second data set, which represented a larger microvessel (Figure 3-3b), had a maximum displacement of $D_{Max} = 9 \mu m$. The best results for the fit between equation (3.1) and data set 1 gave the values of $\mu_1 = 5$ MPa and $\eta_1 = 1.02$ Pa s. The best values for the fit between equation (3.1) and data set 2 resulted in $\mu_1 = 5$ MPa and $\eta_1 = 0.78$ Pa s. The viscoelastic parameter for the two data sets above were within 26% of each other. In order to implement these findings the parameters obtained from data sets 1...
and 2 were averaged. The average parameters of \( \mu_1 = 5 \text{MPa} \) and \( \eta_1 = 0.9 \text{ Pa s} \) \( (\tau_1 = \frac{\eta_1}{\mu_1} = 0.1817 \mu\text{s}) \) along with \( \mu_0 = 50 \text{kPa} \) were used as inputs into the vessel properties for bubble/fluid/vessel simulation in the next sections. It is worth noting that the viscosity values found here were in the same order of magnitude as that found by Girnyk et al. (i.e. 0.15 Pa s) in liver using ultrasound measurements at 1 MHz [156]. Also the elasticity values are within those reported earlier (i.e. 1-10 MPa) [126], [134].

![Figure 3-3 Fitted standard linear solid model with experimental results. (a) First invaginated vessel data with the best fit of parameters \( \mu_0 = 50 \text{kPa}, \mu_1 = 5 \text{MPa} \) and \( \eta_1 = 1.02 \text{ Pa s} \) (b) Second data with the best fit of parameters \( \mu_0 = 50 \text{kPa}, \mu_1 = 5 \text{MPa} \) and \( \eta_1 = 0.78 \text{ Pa s} \).]
3.2.3 Numerical Simulation

In the experimental data the bubble was nearly in the middle of the vessel, therefore in our numerical model a microbubble was placed at the centre (both axially and radially) of a microvessel (vessels were 2 µm in thickness and 100 µm in length). Also a two dimensional model with axisymmetrical assumption was used, as it was less computational expensive. Figure 3-4 shows a schematic illustration of the numerical model.

![Schematic illustration of the numerical model](image)

**Figure 3-4** Schematic illustration of the numerical model. (a) Forced bubble oscillation in numerical section part I, and (b) Microbubble model in numerical section part II.

3.2.3.1 Forced bubble oscillation and stresses-Numerical section Part I

In this section, an ellipsoidal bubble was forced to oscillate, mimicking the bubble in the experimental cases 1-4 (Fig. 3-4a). The bubble wall was coupled to the surrounding fluid within a viscoelastic vessel. As a boundary condition, the fluid velocity was set equal to the forced bubble velocity while the bubble wall velocity itself was derived from experimental bubble wall displacements. The vessel wall movements, WSS and CS were calculated as a result of this numerical section.
3.2.3.2 Microbubble Model-Numerical section Part II

The focus of this section was on microbubbles’ behaviour in a confined geometry. Therefore, a bubble model was developed considering the effects of surface tension and encapsulating shell. In this section, contrary to the previous section, the bubble oscillation was not dictated anymore. Instead, the bubble oscillated due to the ideal gas law inside the bubble and fluid force surrounding the bubble.

Since the acoustic pressure in this study was relatively high (~ 1 MPa range) and the blood vessels were relatively small, the bubble oscillated in an ellipsoidal fashion. Therefore a non-spherical bubble model was developed. Typically bubble models (e.g. Rayleigh-Plesset equation) consist of a pressure function and an inertia function. In this confined bubble model, the pressure function was applied on the bubble wall while the inertia function due to the surrounding fluid and vessel was numerically solved. A pressure boundary condition at the bubble wall was used (the stress in the tangential direction is assumed to be zero):

\[
\left(-PI + \mu \left(\nabla v_f + (\nabla v_f)^T\right)\right) n = -P_{f-b} n + P_y n
\]

(3.2)

where \(P_{f-b}\) is the pressure on the fluid just outside the bubble wall minus the Laplace pressure, \(n\) is the outward unit normal, \(P_y\) is the Laplace pressure and \(\mu\) is the fluid viscosity. The following equation was applied for \(P_{f-b}\), using the polytropic gas law along with encapsulating shell properties:

\[
P_{f-b} = P_g - \frac{4 \mu \dot{R}_{eq}}{R_{eq}} - \frac{4 \kappa_s \dot{R}_{eq}^2}{R_{eq}^2} - P(t)
\]

(3.3)

where \(P_g = P_{g0} \left(\frac{V_0}{V}\right)^k\) is the gas pressure obeying ideal gas law, \(P_{g0}\) is the gas pressure at the resting state and \(k\), the polytropic index, was set to 1.07 [74]. \(V_0\) and \(V\) are the bubble volume at the initial state and at any other time points, respectively. The bubble volume was calculated at each time step and was then updated in equation (3.3). Similar to chapter 2, \(P_0\) was set to 104.6 kPa. \(P(t)\) was the ultrasound pressure burst shown in Figure 3-1a. Due to the large acoustic wavelength (i.e. a few millimetres) compared to the bubble size, it was assumed that the ultrasonic burst pressure is uniform around the bubble. In equation (3.3), the second and third terms on the right hand side
represented the fluid viscosity effects and the encapsulating shell viscosity respectively. The fluid viscosity of saline, $\mu$, was set to 0.001 Pa s. The shell viscosity, $\kappa_s$, was set to $1.2 \times 10^{-8}$ kg s\(^{-1}\). These two viscous terms were developed under a spherical symmetry assumption on the bubble wall [88]. The fluid viscosity term manifests itself through the boundary condition [72]. The shell viscosity term is proportional to the shell viscosity constant ($\kappa_s$) and the bubble area [157]. Therefore as the viscous terms in equation (3.3) depend on the bubble surface area, we calculated the bubble equivalent radius, $R_{eq}$, by equating the bubble surface area with a sphere area of radius $R_{eq}$.

In order to investigate the effects of surface tension on the bubble, a weak form of the boundary condition in equation (3.2) was applied. In this equation, $P_y = \kappa \gamma$ where $\gamma$ is the surface tension and $\kappa$ is the total curvature ($\kappa$ was twice the mean curvature, where the surface mean curvature was $-\frac{1}{2} \nabla_s \cdot \mathbf{n}$. In that $\nabla_s$ represents the surface gradient operator). After applying the weak form on this boundary condition, the right-hand side of equation (3.2) becomes:

$$
\int_{\Gamma} -P_{f-b} \mathbf{u} \cdot \mathbf{n} \, d\Gamma + \int_{\Gamma} \kappa \gamma \mathbf{u} \cdot \mathbf{n} \, d\Gamma
$$

where $\Gamma$ is the surface boundary and $\mathbf{u}$ is the test function. Using the surface divergence theorem [158], the term on the right hand side above becomes:

$$
\int_{\Gamma} \kappa \gamma \mathbf{u} \cdot \mathbf{n} \, d\Gamma = -\int_{\Gamma} \gamma \nabla_s \mathbf{u} \cdot d\Gamma + \int_{c} \gamma \mathbf{u} \cdot \mathbf{m} \, dc
$$

where $c$ is the contour bounding the surface, and $\mathbf{m}$ is the outward unit normal to $c$ and while on $c$, it is perpendicular to $\mathbf{n}$ ($\mathbf{n} \cdot \mathbf{m} = 0$). The second term on the right hand side in the equation (3.4) (i.e. a contour integral) is equal to zero.

In this model for encapsulating shell surface tension we followed the model proposed by Marmottant et al. [91] with three regimes: buckling, elastic and ruptured state. The surface tension can be described as follows:

$$
\gamma = \begin{cases} 
0 & \text{if } A \leq A_{buckling} \approx A_0 \\
\chi \left( \frac{A}{A_{buckling}} - 1 \right) & \text{if } A_{buckling} \leq A \leq A_{break-up} \\
\gamma_{\text{water}} & \text{if } A > A_{break-up}
\end{cases}
$$

(3.5)
where \( A \) is the bubble area and \( \chi \) is the shell elastic modulus. Based on the work of Tu et al. [159], where they measured the shell elasticity of Definity microbubbles, \( \chi \) was set to 0.7 Nm\(^{-1}\). \( A_{\text{buckling}} \) was the bubble area at which the shell buckles. The surface tension varies in an elastic regime until the shell breaks at \( A = A_{\text{break-up}} \). The surface tension of water, \( \gamma_{\text{water}} \), was set to 0.072 N/m (assuming it is similar to saline).

Here we assumed that the initial surface tension \( (\gamma_{A_0}) \) was zero and bubble radius at rest equals to the radius below which it buckles \( (A_{\text{buckling}} \approx A_0 = 4\pi R_0^2) \). However, \( \gamma_{A_0} \) is an unknown parameter in general and is a subject of research. With the assumption of \( A_{\text{buckling}} \approx A_0 \), the Laplace pressure term \( (2 \gamma_{A=A_0}/R_0) \) vanishes. Thus, \( P_{g0} = P_0 + 2 \gamma_{A=A_0}/R_0 \) reduces to \( P_0 \).

\( R_{\text{break-up}} \), the radius at which the bubble shell breaks, was set to \( R_{\text{buckling}} \sqrt{\gamma_{\text{water}}/\chi + 1} \), and \( A_{\text{break-up}} = 4\pi R_{\text{break-up}}^2 \). At each time step the bubble area was calculated along with its corresponding surface tension. Then, the surface tension from equation (3.5) was implemented in equation (3.4) for \( \gamma \). In this work, and in all of experimental cases, the bubble passed the \( A_{\text{break-up}} \) early in its expansion phase. Exceeding this point, the shell had no influence on the bubble oscillations and the surface tension of water was applied on the bubble wall.

The use of the weak form of the boundary condition in equation (3.2) enables us to simulate non-spherical oscillations of a confined bubble. However, for the case of an unbound bubble the solution using the weak form should converge to the solution of equation (3.3) with the addition of the Laplace pressure. To test the solution of the weak form, a test simulation was performed on an unbound bubble (with spherical oscillations) with and without the weak form. In Figure 3-5a, a 1.1 \( \mu \)m bubble was simulated with the weak form (in dashed blue line), and without the weak form (in solid black line). The solution of the Rayleigh–Plesset equation is shown in Fig. 3-5b for comparison. The bubble oscillation using the weak form was in an excellent agreement with those without the weak form and the Rayleigh–Plesset solution.
3.2.4 Fluid Structure Interaction

The fluid and vessel were coupled in a two-way manner and equation (2.3) was solved in the fluid domain. The continuity equation was satisfied as well: \( \rho \nabla \cdot \mathbf{v}_f = 0 \). The load from the fluid exerted on the vessel boundary was \( -\mathbf{n} \cdot ( -P \mathbf{I} + \mu (\nabla \mathbf{v}_f + (\nabla \mathbf{v}_f)^T) ) \), where \( \mathbf{n} \) was the normal vector to the boundary. This load represented a sum of the pressure and viscous forces.

In the solid domain the following governing differential equation of a viscoelastic vessel, analogous to the standard linear solid model, was solved [160], [161]:

\[
P_i + \frac{\eta_1}{\mu_1} \dot{P}_i = \mu_0 \epsilon(t) + \eta_1 \left( 1 + \frac{\mu_0}{\mu_1} \right) \dot{\epsilon}(t) \tag{3.6}
\]

where \( P_i \) is the intravascular pressure and \( \epsilon(t) \) is the diameter stain. \( \mu_0 \) and \( \mu_1 \) are elastic coefficients (with dimensions of pressure), and \( \eta_1 \) represents a viscous coefficient (with dimension of pressure times time).

The fluid pressure at the vessel wall causes the vessel to undergo displacements. Also, at the vessel wall boundary the normal fluid velocity was set equal to the vessel wall velocity. The latter condition ensures the coupling between the fluid and the structure.
3.2.5 Method of Solution

In the numerical sections (both parts I and II), similarly to chapter 2, the pressure on vessel ends and the initial conditions of the fluid were set to the ambient pressure in the capillaries, $P_0$. The numerical model was solved with a finite element method (FEM) using Comsol Multiphysics 4.2 (COMSOL AB. Burlington, MA). The vessel length was set to 100 µm which was long enough to make the results independent of the vessel length. Triangular meshes for this two-dimensional model were used with 4,000-10,000 elements for cases1-4. Since this numerical model was time dependent, the time resolution was set to 0.005 µs for case 1 and 0.01 µs for the rest. WSS was calculated using $\tau_{rz} = \mu \left[ \frac{\partial u}{\partial z} + \frac{\partial w}{\partial r} \right]$ in cylindrical coordinates, where $u$ and $w$ are the fluid velocity components in $r$ and $z$ directions respectively. The CS was calculated using equation (1.4). It is important to note that this equation was derived for elastic materials. Due to the fact the calculated time constant was small (meaning the elastic restoring force dominated the viscous force) and vessel deformation was in phase with the bubble oscillation, it was assumed that vessels responded elastically. Therefore, recruitment of equation (1.4) can be justified. $P_{out}$ (pressure on the outer diameter of the vessel) in this equation was assumed to be $P_o$ [111].

3.3 Numerical Results

3.3.1 Numerical Section Part I

The numerical results for viscoelastic vessel displacement, as well as the experimental measurement of the vessel wall for cases 1-4 are shown in Figure 3-6a-d respectively. A bubble oscillating in an ellipsoidal fashion within a viscoelastic vessel caused these vessel displacements. Generally there is a good agreement between the numerical wall movements and experimental measurements, except for case 3 where during the bubble compression the numerical results deviate from the experimental data. Histology data of case 3 shows that endothelium has been torn away and this may explain the discrepancy.
Figure 3-6 Radial displacement of the vessel wall. The numerical viscoelastic vessel compared to the experimental of (a) case 1, (b) case 2, (c) case 3, and (d) case 4.

Figure 3-7a presents the WSS at the vessel wall for cases 1-4 obtained from the first numerical simulations. The negative WSS values correspond to the bubble expansion and the positive stress values correlate with the bubble compression. For case 1, the maximum positive and negative WSS values are 73.8 kPa and 18.3 kPa respectively. In cases 2-4 the bubble wall does not approach the vessel wall, and the stress values are lower. Figure 3-7b shows the CS on the vessel wall in all cases. This stress has its maximum value right above the bubble ($r=r_v$, $z=0$). Here, the positive and negative circumferential stresses correspond to bubble expansion and compression phases respectively. In case 1, the maximum positive circumferential stress is 2.2 MPa during the bubble expansion phase and the maximum negative CS is 6 MPa during the bubble compression. Wall stress results are summarized in Table 3-2. Depending on the bubble's initial size and the distance between the bubble wall and the vessel wall, the wall stress values change and can become larger.
during the compression phase of the bubble oscillation. This phenomenon will be shown in the last section of this chapter.

Figure 3-7 Wall stresses (a) Wall shear stress calculated at \( r = r_v \) and an axial distance of \( z = 3 \, \mu m \) (case 1), \( z = 11 \, \mu m \) (case 2), \( z = 13 \, \mu m \) (case 3) and \( z = 9 \, \mu m \) (case 4), (b) The circumferential stress for case 1, case 2, case 3 and case 4 at \( r = r_v \) and \( z = 0 \).

Table 3-2 Ratio of maximum WSS and CS during vascular invagination to expansion.

<table>
<thead>
<tr>
<th>Case #</th>
<th>( \text{WSS}<em>{\text{inv}} ) (kPa) / ( \text{WSS}</em>{\text{exp}} ) (kPa)</th>
<th>( \text{CS}<em>{\text{inv}} ) (MPa) / ( \text{CS}</em>{\text{exp}} ) (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73.8/18.3 = ( 4 )</td>
<td>6/2.2 = 2.7</td>
</tr>
<tr>
<td>2</td>
<td>1.6/1.7 = ( 0.94 )</td>
<td>0.44/0.43 = 1</td>
</tr>
<tr>
<td>3</td>
<td>1.5/1.1 = ( 1.36 )</td>
<td>0.8/0.6 = 1.3</td>
</tr>
<tr>
<td>4</td>
<td>2/2.2 = ( 0.87 )</td>
<td>0.74/0.7 = 1.1</td>
</tr>
</tbody>
</table>
3.3.2 Numerical Section Part II

In Figures 3-8 and 3-9 the results of the second numerical simulations for all cases are plotted. Figures 3-8a-d show the bubbles' semi-minor axis and vessel wall for case 1-4 respectively and bubble's axial displacements (bubbles' semi-major) are plotted in Figs. 3-9a-d. The bubble semi-minor radius and vessel displacement in Figure 3-8a (case 1) are within 4% of the experimental data. The bubble's axial wall in case 1 peaks at a value 25% larger than that measured in the experiments (Fig. 3-9a). The numerical bubble amplitude of oscillation for cases 2, 3 and 4 are within 13 and 15 and 11% of those measured in experiments (Figs. 3-8b-d and 3-9b-d). In case 3 (Figs 3-8c and 3-9c) the numerical simulation stopped during the bubble compression and therefore the last cycle of oscillation is not simulated.
Figure 3-8 Comparison of the numerical simulations with experiments. Bubble radial displacements for cases 1 (a), 2 (b), 3(c) and 4 (d). From the experimental data in case 1, the bubble was not visible in three of middle time points.
The WSS and CS were calculated from the second numerical part as well. The maximum variations of these stresses are compared to the results of the first numerical part. Stresses have trends similar to those reported in Fig 3-7. WSS and CS calculated from the second numerical part for case 1 are 13% and 7% higher than that calculated in the first numerical part. These stresses are higher by 17% (WSS) and 19% (CS) for case 2, 33% (WSS) and 5% (CS) for case 3 and 38% (WSS) and 32% (CS) for case 4.

The stress values obtained for the four experimental cases used here suggest that there is a threshold of bubble wall to vessel wall distance beyond which the stresses are higher during the bubble compression. Numerical simulations (part two) of a 1 µm bubble within various vessel sizes (3-20 µm) were performed to predict this threshold. Figure 3-10 shows the ratio of stresses (invagination over expansion phase) versus a dimensionless metric, $d = \frac{r_v\text{max}-R_{max}}{R_0}$, where $r_v\text{max}$ is the maximum vessel radius and $R_{max}$ is the maximum bubble radius. This metric calculates the bubble wall to the vessel wall distance (at bubble's maximum expansion) normalized to the bubble initial radius. In this case, the bubble size was fixed at 1 µm and $d$ increased with the vessel radius. The shear stress was higher during the bubble compression when the vessel size was 8 µm or smaller. For 7 µm vessels or smaller the CS was higher during the bubble compression.
3.4 Discussion

The experimental data obtained from our collaborators had two sources of measurement uncertainties: 1) uncertainty in measuring the bubble and vessel radii, which was less than 1 µm; 2) frame-to-frame alignment jitter of the camera’s optics, the added uncertainty was about 1 µm. The overall estimated uncertainties in displacement measurements ranged from 1–3 µm [133]. Due to some variability in tissue sample positions, some uncertainties in the timing of the image frames, relative to the ultrasound pulse arrival, were introduced. In most of the observations, the first bubble compression occurred about 1 µs after the arrival of ultrasound pulse. To reduce the uncertainty a common temporal axis was adopted to all photographic observations. In addition, case 2, 3 and 4 had a lower temporal resolution of 0.3 µs. The numerical results in part two (bubble/vessel wall displacements in figs. 3-8 and 3-9) were shifted by less than 0.2 µs to align with the experimental measurements.

It is worth mentioning that in the numerical method section I, wall stresses due to a bubble responding to an acoustic wave passing through the surrounding media could be different from those produced by forced oscillating bubble. However, in the experimental data, the vessel wall only underwent displacements at the bubble site. In fact in locations where the acoustic pressure was acting on the vessel with no bubble present (e.g. a few microns away from the bubble site), the vessel wall did not have any displacements due to the acoustic force itself compared to time...
zero when the ultrasound was off. Therefore, it was assumed that stresses leading to vessel wall expansion and invagination were due only to the oscillating bubble.

In numerical method section II, it was assumed that the acoustic pressure was acting on the bubble wall directly to save computational time. The resultant wall stresses produced due to this assumption could be different from those when an acoustic wave was passing through the surrounding media. In this work the experimental mesentery network was relatively thin and the vessels were soft. Therefore, the impedance mismatch between the vessels and fluid, as well as the attenuation are low. Furthermore, according to the numerical work by Qin et al. in which the transmitted ultrasound pressure was assessed within different microvessels at various frequencies, ultrasound pressure was disturbed the most in the lumen of rigid vessels and at higher frequencies [162]. In this study the vessel wall was soft and the frequency was relatively low. Therefore, it was safe to assume that the acoustic pressure within the lumen acting on the bubble wall was not distorted compared to the transmitted ultrasound burst.

The numerical simulation was performed using an axisymmetrical assumption. The bubble was in the vessel centre for cases 1 and 3. In cases 2 and 4, the bubble was slightly off from the vessel centre. However, there was no bubble deformation due to this asymmetry. Also in this case, the bubble distance to the vessel wall was large enough that the impact of the wall on the bubble oscillation was small. Therefore, the axisymmetrical assumption in our numerical work should be valid.

In order to reveal the importance of a bubble’s non-spherical oscillation, we simulated a bubble with spherical symmetry as well. Case 1 was chosen for this comparison (numerical section part I) and the equivalent bubble radius was used for the spherical bubble. The WSS and CS during bubble compression from the spherical bubble symmetry was 69 kPa and 3.45 MPa respectively. These values were 6% and 43% lower than those obtained from the ellipsoidal bubble. Based on these simulations, the use of a spherical symmetric model for confined bubbles could result in an underestimation of the stresses.

The WSS and CS calculated from the second numerical part (section II of Methods) was higher than those calculated from the first numerical part (fig. 3-7). This could be due to the fact that the bubble oscillation amplitude was higher in the second numerical part compared with the
experimental observations (13% in case 2, 15% in case 3 and 11% in case 4). A reason for this might be that the acoustic pressures experienced by the bubbles were underestimated. In addition the encapsulating shell, or the vessel wall properties might have been stiffer than the ones used in the simulations.

From similar experiments at higher acoustic pressures (1.5-5.6 MPa), our collaborators reported the endothelial cell damage (characterized by the separation of the endothelium from the vessel wall) after performing histology and transmission electron microscopy [163]. The cell damage was attributed to the vessel's invagination-dominated response. From our results at the driving pressure of 0.8 MPa, the stresses during vessel invagination were higher than those during the expansion phase especially when the bubble wall was close to the vessel wall (clearly shown in the case 1). This could indicate that the vascular damage is likely to occur in the compression phase.

To put the stresses into perspective it is worth noting that the wall strength of frog mesentery microvascular was estimated to be 0.8 MPa [64]. In other words, CS exceeding this value in frog mesentery could lead to vascular rupture. The maximum CS calculated for cases 1, 3 and 4 exceeded or was close to this vascular strength. This suggests that the bubble might have ruptured the vessel wall to some extent or if more experiments were to be conducted some vessels would rupture. Also, shear stresses in the range of a few kilopascals is speculated to induce cell lysis or cell detachment [48]. However, the stress values in our numerical model present a transient effect and its direct link to the vascular damage is unclear. Parametric studies of the vascular damage along with using a high speed photomicrography system are required in the future. As a result of such experiments, the stress values found numerically could be correlated to, and calibrated with the vascular damage thresholds.

3.5 Conclusion

Numerical simulations of confined ultrasound contrast agent bubbles within viscoelastic vessels were performed in this chapter and results were compared with experimental observations. Simulations agreed reasonably well with the experimental observations indicating that this comprehensive model can be used to gain new understanding about the physical forces associated with bubble oscillations in small blood vessels. These simulations were used to calculate the WSS and CS. In the first numerical part the bubble was forced to mimic the experimental bubble
oscillations. From this, vessel wall movements and stresses were calculated. In the second numerical part, the confined bubble oscillated due to the ideal gas law and surrounding pressure on bubble's wall considering the effect of surface tension. Using the numerical model, a threshold was calculated beyond which the stresses were higher during the bubble compression. This agreed with experimental results. This model could be used to provide insight into the mechanism behind the bubble and vessel interactions as well as potentially quantify the microbubble induced mechanical stresses on the blood vessel walls.
Chapter 4
A Numerical Study on Acoustic Emissions and Stress Predictions for Microbubble-Mediated Blood Brain Barrier Opening

4.1 Introduction

Previous in vivo experiments have shown that acoustic emissions from bubbles correlate with BBB opening. A significant increase in second and third harmonics of the ultrasound transmit frequency (2f₀ and 3f₀) were observed during sonications with circulating microbubbles that resulted in the BBB opening [164]. In that study, BBB opening occurred without observation of erythrocyte extravasations or wideband emissions, which are indicators of inertial cavitation. Arvanitis et al. [165] recently demonstrated the use of harmonic emissions from Definity microbubbles to monitor BBB opening without broadband emissions. Also, Tung et al. [166] proposed that the fourth and fifth harmonics could be used as indicators of BBB opening.

We have hypothesized that the mechanical stresses generated by microbubbles could play an important role in the BBB opening. Therefore, the objective in this chapter is to use the simulation models of confined microbubbles to assess the harmonic emissions from various bubbles in microvessels, and determine their correlation with the physical stresses induced on the vessel walls. This approach could provide new insight as how to monitor and control the sonications used to induce BBB opening.

4.2 Method

Bubbles confined within microvasculature, as well as unbound bubbles (bubbles unconfined within fluid), were simulated. The coupled bubble-fluid-vessel system was solved numerically. The numerical part was solved using the finite element method (FEM) in Comsol Multiphysics 4.2 (COMSOL AB. Burlington, MA). The same bubble model developed in chapter 3 was recruited here for BBB disruption applications.

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The acoustic frequency was set to 0.551 MHz based on its use in prior experimental studies [34] and its suitability for transmission through human skull [167]. This frequency was used throughout this study unless otherwise mentioned. The acoustic pressure amplitude was varied from 50 to 275 kPa in increments of 25 kPa.

4.2.1 FEM Simulations

A fully coupled model of a confined bubble within a microvessel was developed in chapter 3. This model accounts for non-spherical bubble oscillations as well as the effects of the surrounding confinement on bubbles. For detailed information about the FEM modeling refer to sections §3.2.3.2 and §3.2.4 in chapter 3.

The shell properties used for Definity microbubbles (Lantheus Medical Imaging, North Billerica, Massachusetts) were within the range that has been previously reported [159], [168]. Definity bubbles are poly-disperse [169] and were simulated for various radii. In this work bubbles were either in an infinite fluid, or confined within vessels of various size (radius ranging from 3-10 µm).

Table 4-1 Parameters and Values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polytropic index, k</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Shell viscosity, $\kappa_s$</td>
<td>1.2x10^-8 kg s^-1</td>
<td>Tu et al. [159], Helfield and Goertz [168]</td>
</tr>
<tr>
<td>Fluid viscosity, $\mu$</td>
<td>0.002 Pa s</td>
<td></td>
</tr>
<tr>
<td>Shell elasticity, $\chi$</td>
<td>0.7 Nm^-1</td>
<td>Tu et al. [159], Helfield and Goertz [168]</td>
</tr>
<tr>
<td>Surface tension of water, $\gamma_{\text{water}}$</td>
<td>0.072 N/m</td>
<td>Gilányi et al. [131]</td>
</tr>
<tr>
<td>Vessel elastic modulus, $E_v$</td>
<td>1 MPa</td>
<td>Duck [134], Smaje et al. [126]</td>
</tr>
<tr>
<td>Tissue elastic modulus, $E_t$</td>
<td>0.5 MPa</td>
<td>Duck [134]</td>
</tr>
<tr>
<td>Ambient capillary pressure, $P_0$</td>
<td>104.6 kPa</td>
<td>Burton [170]</td>
</tr>
<tr>
<td>Poisson's ratio, $\nu$</td>
<td>0.49</td>
<td>Melbin and Noordergraaf [171]</td>
</tr>
</tbody>
</table>
The elastic vasculature and the fluid were coupled in a two way manner with a boundary condition of equal fluid-structure velocities at the wall. The Navier–Stokes equations for a viscid incompressible Newtonian liquid and equations for an elastic vessel were solved simultaneously (equation (2.3) and (2.4)). The fluid was assumed to be incompressible. An effective fluid viscosity of 0.002 Pa s (twice that of water) was used to account for thermal damping. The vessel wall was chosen to be 1 µm in thickness. The microvessel was surrounded with a 114 µm layer of tissue structure. Figure 4-1 presents the schematic illustration of the simulated domain. Figs. 4-1a, 4-1b and 4-1c represent the simulations performed on confined bubbles in the middle of microvessels, unbound bubbles and bubbles at a vessel wall, respectively. The parameters and values used in this work are presented in Table 4-1.

Figure 4-1 Schematic diagram of the FEM domain. Bubble, fluid, vessel and flat wall are labeled as B, F, V and W (a) A confined bubble in the middle of the vessel, (b) An unbound bubble inside free fluid, and (c) A microbubble placed close to a flat vessel wall.

The ultrasound waveform was tapered at the start of the burst to avoid any subtle numerical instabilities and to represent a realistic burst from an ultrasound transducer. An example of corresponding ultrasound waveforms, non-spherical bubble oscillations and wall shear stress are shown in Fig. 4-2 for a bubble with 2 µm radius within a 5 µm radius vessel sonicated at 0.551 MHz and 75, 100 and 125 kPa. The changes in the bubble oscillation between the compression dominated oscillations (at 75 kPa, Fig. 4-2b, where the bubble preferentially compresses instead of expanding) to near expansion dominated oscillations (at 125 kPa, Fig. 4-2h) related to the transition from buckling to elastic and rupture states in the shell model, respectively. In Fig. 4-2i it is apparent that the transition to greater expansions is directly linked to greater shear stresses.
Most of the results in the following section were generated from confined bubbles within 5 µm vessels. This vessel size was chosen because the BBB opening experiments with two-photon microscopy revealed that smallest vessels (2.5-12.5 µm in radius) are most susceptible to permeabilization [42], [43].

![Image](link-to-image)

Figure 4-2 A 2 µm radius bubble within a 5 µm radius vessel with the corresponding applied ultrasound pulse (top), bubble wall radial (black solid line) and axial (red dashed line) oscillation (middle), and shear stress (bottom). (a, b and c) at 75 kPa, (d, e and f) at 100 kPa, and (g, h and i) at 125 kPa. At 75 kPa bubble is within the elastic regime of Marmottant shell model and exhibits compression dominated oscillation (b), At 100 kPa bubble enters the rupture state (e), and at 125 kPa bubble shows expansion dominated as it is mainly in rupture state (h).
At certain threshold pressures the bubbles in these vessels underwent collapse along the z axis. Here, the collapse is defined when the opposing bubble wall along the vessel axis rapidly came into contact. The simulations stopped following this point. Figure 4-3a presents an example of bubble collapse from a 4 µm bubble at 125 kPa within a 5 µm vessel during its contraction phase. At the final stage the two axial bubble walls came into contact. At lower pressures, Figure 4-3b shows the stable oscillations of the same bubble at 100 kPa during one cycle. The periodicity of non-spherical bubble oscillations is at the transmit frequency, 0.551 MHz. Figure 4-4 depicts the pressure threshold at which bubbles within 5 µm vessels underwent collapse. The results in the following sections were generated for pressures below the collapse threshold.

(a)

(b)

Figure 4-3 Examples of bubble collapse and non-collapse oscillations during the bubble compression phase, (a) A 4 µm bubble within a 5 µm vessel at 125 kPa with collapse, (b) A 4 µm bubble within a 5 µm vessel at 100 kPa without collapse which continues with stable oscillations shown for one cycle. The periodicity of non-spherical bubble oscillations is at the transmit frequency (e.g. 0.551 MHz).
Figure 4-4 Bubble collapse threshold of different size bubbles driven at 0.551 MHz within 5 µm vessels.

4.2.2 Acoustic Emissions

The acoustic emission radiated from a spherical oscillating bubble at a distance $r$ is normally calculated using the following equation [172]:

$$P_s = \rho_L \frac{R}{r} (2\ddot{R}^2 + R\dddot{R})$$

(4.1)

where $r$ is the radial distance to the bubble in spherical coordinates. However, since a confined bubble within a vessel is oscillating non-spherically, the assumptions leading to equation (4.1) do not hold. Therefore, in this study the Helmholtz equation was solved to calculate the scattered acoustic emissions from confined bubbles.

$$\frac{1}{\rho_L c^2} \frac{\partial^2 P_{ac}}{\partial t^2} + \nabla \cdot \left( -\frac{1}{\rho_L} \nabla P_{ac} \right) = 0$$

(4.2)

where $P_{ac}$ is the acoustic pressure wave. The acoustic waves propagated in a 30×30 µm domain. Figs. 4-1a and 4-1b show a schematic of this model. The non-spherical bubble wall acceleration was coupled to the acoustic model and acted as a source of wave generation. The acoustic emission
from the bubble was calculated at a fixed point (i.e. at point P on Figs. 4-1a and 4-1b). A normal acceleration boundary condition was applied on the bubble wall with the following equation:

\[-n \cdot \left( -\frac{1}{\rho_L} \nabla P_{ac} \right) = -n \cdot a_0 \]  

(4.3)

where \(a_0\) is the bubble wall acceleration which was taken from the bubble oscillation solution in the previous section. It is important to note that point P on Figs. 4-1a and 4-1b might have been angle dependent, however it was not the focus here and this fixed point was used for all of the simulations. At the edge of the numerical domain, a spherical wave radiation boundary condition was used, where an outgoing wave leaves the modeling domain with minimal reflections:

\[-n \cdot \left( -\frac{1}{\rho_L} \nabla P_{ac} \right) + \frac{1}{\rho_L} \left( \frac{1}{c} \frac{\partial P_{ac}}{\partial t} + \frac{P_{ac}}{r} \right) = 0 \]  

(4.4)

\(r\) is the radial axis in the spherical coordinate. Acoustic emissions were assessed for 1.1-3 \(\mu\)m bubbles within 3-10 \(\mu\)m vessels.

4.2.3 Bubble Population

As Definity bubbles are polydisperse, acoustic emissions from a bubble population were also calculated \(P_{sum}\). A Definity bubble distribution in number density was taken from the measurement performed on fresh bubble population by Faez et al. [169]. \(P_{sum}\) was calculated using a summation of scattered pressures from different bubbles, weighted to their distribution: \(P_{sum} = \Sigma \beta P_{ac} \omega_t\). Bubbles with radii ranging from 0.5 to 3 \(\mu\)m were used here, in increments of 0.5 \(\mu\)m.

4.2.4 Signal Processing

The acoustic pressure emitted from a non-spherical bubble was calculated at point P during the sonication (Figs. 4-1a and 4-1b). The time domain signal was 40 \(\mu\)s long (about 22 cycles of oscillation at 0.551 MHz). Frequency spectra of the calculated pressure waveforms were produced using a Fourier transform of the unmodified time domain signal. This Fourier transform was done on \(P_{ac}\) from a single bubble and \(P_{sum}\) from bubble populations. The area under the peaks at the fundamental, second, third, fourth and fifth harmonics around +/-27 kHz (approximately \(\pm 3 \text{ dB}\)) were calculated. Acoustic emission plots represent the area under the curve for each acoustic
signal. This method was in line with the signal processing performed by McDannold et al. [164]. The acoustic emission assessment in this study was performed at pressures below the bubble collapse. In Figure 4-5, the acoustic emissions versus acoustic pressure for a 2 µm bubble within a 5 µm vessel is shown (corresponding to the example given in Figure 4-2).

![Figure 4-5 Effects of acoustic pressure on acoustic emission from a 2 µm within 5 µm vessels at 0.551 MHz.](image)

**4.2.5 Stress Calculation**

On the vessel wall, circumferential stress (along the vessel circumference), shear stress (along the vessel axis) and transmural pressure (pressure in the radial direction) induced by bubbles within 5 µm vessels were calculated. The circumferential stress for a thick wall cylinder was calculated using equation (1.4). The vessel wall shear stress was calculated from equation (2.5). Transmural pressure was calculated as the difference between the inside and outside pressures on the vessel wall.

In a realistic situation, bubbles of different radii are located within different diameter vessels. Wall stresses depend on distance between the bubble and vessel walls, which requires a range of simulations comprised of different bubble and vessel diameter combinations. In order to reduce computational time to feasible levels, and decouple the bubble-vessel distance dependency,
bubbles ranging from 1.1 to 9 µm in radius were placed in proximity to a flat wall (simulating situations in larger vessels) (Fig. 4-1c). Transmural pressure and shear stresses were calculated for bubbles at a flat, elastic wall with the same elastic properties used in the previous sections. All bubbles were initially placed at a distance of 2 µm from the wall. Note that the effect of acoustic radiation force was not taken into account here. Bubbles during their oscillations translated towards the vessel wall and simulations were terminated when bubbles reached a distance of 1 µm away from the wall. At a constant mechanical index, three frequencies of 0.551, 1 and 1.5 MHz were used. The corresponding acoustic pressures were 100, 134 and 164 kPa (constant MI=0.134).

4.2.6 Model Validation

The FEM was validated against ordinary differential equation (ODE) bubble models of two extreme scenarios; 1) when the bubble was within an unbound fluid and 2) when the bubble was within a completely rigid vessel. The ordinary differential equation (ODE) of a modified Rayleigh–Plesset equation for an unbound 1.1 µm bubble was solved using MATLAB (version 7.11, The MathWorks, Natick, MA). Leighton [173] derived an ODE equation of motion for a bubble wall within a finite rigid tube while accounting for the fluid inertia (Equation 12 in Leighton 2011). The rigid tube ODE of a 1.1 µm bubble inside a 4 µm rigid tube was solved with the addition of Marmottant shell terms using MATLAB. Both ODE solutions of bubble wall as a function of time are plotted in Figure 4-6. FEM simulations were performed for the same parameters while adopting unbound bubble and rigid vessel cases (similar to Figs. 4-1b and 4-1a respectively). For the unbound bubble, the plots from the ODE and FEM were identical. In the rigid vessel case, the FEM bubble expansion was 5% lower than the ODE solution.
4.3 Results

4.3.1 Acoustic Emissions

4.3.1.1 Effect of Vessel Radius

Figure 4-7 illustrates the acoustic emissions from confined bubbles within 3, 5 and 10 µm vessels, as well as unbound bubbles. Figs. 4-7a and 4-7b represent a 1.1 µm bubble at 250 kPa and a 2 µm bubble at 150 kPa, respectively. These acoustic pressures were below the bubble collapse threshold (shown in Fig 4-4). Fundamental, second, third, fourth and fifth harmonics are plotted for comparison. Bubble oscillations and consequently acoustic emissions were mitigated when bubbles were confined compared to the unbound case.
4.3.1.2 Effect of Acoustic Pressure

In this section, the acoustic emissions were assessed for bubble radii of 1.1, 1.5, 2, 2.5 and 3 µm within 5 µm capillaries. In order to enhance the effects of acoustic emissions, the results of harmonic emissions are normalized to the fundamental at each corresponding pressure. Normalized harmonic emissions are plotted in Fig. 4-8 for unbound and confined bubbles. The normalized second harmonic had a consistent trend in all bubbles where it reached a minimum ("transition point") followed by an increase. This transition point was associated with the transition from “compression dominated” oscillations in the shell buckling regime occurring at lower pressures, to the “rupture state” for higher amplitude oscillations. An example of this behaviour is shown in Fig. 4-2. For larger bubbles, their normalized second harmonic had its minimum at lower pressures. This transition point occurred at 175, 125, 100, 100 and 75 kPa for 1.1, 1.5, 2, 2.5 and 3 µm bubbles respectively. For larger bubbles, where the bubble to vessel radius ratio is closer to 1, the differences in the normalized emissions of confined and unbound bubbles is more evident.
For a more clinically relevant case, the acoustic emissions from populations of confined and unbound bubbles were calculated. The normalized harmonics are shown in Fig. 4-9. Here, the transition point for the normalized second harmonic occurred at 100 kPa for the unbound case and at 113 kPa, a slightly higher pressure, for the confined case. The absolute value of the second harmonic of the confined bubble population within 5 µm vessels at 100 kPa was 60% larger than that of unbound bubbles. While at this pressure, the normalized second harmonic of the confined bubble population was 73% larger than the unbound population. Also, at 113 kPa and 125 kPa the absolute values of the fourth harmonic were 86% and 80% larger than the unbound case respectively. The normalized fourth harmonic of the confined bubble population was 65% at 113 kPa and 105% at 125 kPa larger than unbound population.
4.3.2 Vessel Wall Stresses

Fig. 4-10 presents stresses induced by confined bubbles within a 5 µm vessel, according to the geometry in Fig. 4-1a. The shear stress, circumferential stress, and transmural pressure versus acoustic pressure induced by 1.1, 1.5, 2, 2.5 and 3 µm bubbles are plotted. Above the transition point (depicted by filled squares in Fig. 4-10) for larger bubbles (i.e. 2, 2.5 and 3 µm), which are closer to the vessel wall, the stresses increase with a steeper slope.
Figure 4-10 (a) Shear stress, (b) Circumferential stress and (c) Transmural pressure exerted by 1.1, 1.5, 2, 2.5 and 3 µm bubbles on 5 µm vessels at different acoustic pressures. Filled squares indicate the pressure at which the transition point occurs.

Fig. 4-11 shows the stress values induced by bubbles on a flat wall, according to the geometry in Fig. 4-1c. While varying bubble size and acoustic frequency, the maximum shear stress and transmural pressure are shown for a mechanical index of 0.134. The stresses in Fig. 4-11 reach a peak at bubble sizes of 3, 4 and 7 µm which are larger than the resonance size at each frequency. Among the three frequencies chosen here, the shear stress and the transmural pressure peaks reached about 12 kPa and 400 kPa respectively.

Figure 4-11 Stresses induced by different bubbles at a flat wall, sonicated at 0.551 MHz (Dash-dotted line with inverted triangles), 1 MHz (Dashed line with diamonds) and 1.5 MHz (Solid line with circles) at a constant MI = 0.134, (a) Shear stress, and (b) Transmural pressure.
4.4 Discussion

4.4.1 Acoustic Emissions

A bubble oscillating within a compliant small elastic vessel will experience increased damping and a resonant frequency shift upwards. For the majority of conditions examined here the bubbles are well below the resonant size (6 µm in the unbound case; 6.5 within a 10 um vessel) at 0.551 MHz. As such the effects of damping and resonant frequency shift will act to reduce the oscillation amplitudes and therefore acoustic emission levels (Fig. 4-7).

A shift in the harmonic energy was observed, and normalized second harmonics had a "transition point" which was bubble size dependent (Fig. 4-8). Simulations of un-shelled bubbles did not exhibit the transition point (data not shown), meaning that the appearance of this feature is attributed to the bubble shell. As shown in Fig. 4-2, this appears to be associated with a transition from compression dominated oscillations, which occur within a regime where shell buckling dominates, to expansion dominated oscillations. The second harmonics in our simulations had a dramatic rise with pressure above the transition point (the absolute values of acoustic emissions presented a dramatic rise as well as shown in Fig. 4-5). This was generally in line with the observations by McDannold et al. [164], where the second harmonic had a dramatic rise at pressure amplitudes that correlated with BBB opening.

The normalized second harmonic to the fundamental were plotted in the results rather than the absolute values of second harmonics. This was done because in the normalized plots the effects of the transition point was more pronounced. For example, the second harmonic of a 2 µm bubble inside a 5 µm vessel decreased by 44% when pressure was increased from 50 to 100 kPa, while this reduction was 70% when the normalized second harmonic was considered.

The emissions from a bubble population were investigated, taking into account the expected bubble size distribution. The transition point for confined bubbles within 5 µm vessels occurred at approximately the same pressure as for unbound bubbles (i.e. 13 kPa difference in Fig. 4-9). The occurrence of the transition point is important since it may provide an indicator for the pressure amplitude delivered to the brain, and could potentially be used to calibrate the in vivo brain ultrasound exposure.
Furthermore, the second and fourth harmonics of confined bubble population (at 100, 113 and 125 kPa respectively) were larger than that of unbound bubbles. In the future, the transition point pressures and acoustic emissions (particularly second and fourth harmonics) from a confined versus an unbound bubble population should be tested experimentally. This would have implications in the development of microvascular imaging techniques as a mean for the confined versus unbound bubble separation.

4.4.2 Vessel Wall Stresses

In Fig. 4-10, a steeper slope in the stresses above the transition point is observed for larger bubbles, which have higher impact on the vessel wall. However a clear trend is not observed for smaller bubbles. From a physical perspective, the transition point is associated with entry into a regime where the expansion phase of the bubble oscillations is more prominent, which is in turn associated with inducing a proportionally higher level of fluid flow (and therefore shear stress) at the vessel wall.

At 0.551 MHz, the experimental threshold for BBB opening occurred from 0.18 MPa (with a mean at 0.28 MPa) [34]. The wall stresses at pressures above the transition point could be correlated to pressures that cause BBB opening. It is important to note that the shear stress, circumferential stress and transmural pressure have similar trends with the acoustic pressure. Therefore, at the current stage it is not clear that which one is responsible for the BBB opening.

The results in Fig. 4-11 show that wall stresses are bubble size and frequency dependent. If large bubbles are considered to be present (especially at 0.551 MHz), then the stress peaks induced from bubbles above resonance (i.e. 3, 4 and 7 μm bubbles) are approximately constant at each frequency. McDannold et al. [174] found that BBB opening was constant at a constant MI. The simulations presented here are in agreement with this result and indicate that the BBB opening is induced by different diameter bubbles (at resonant) at each frequency. This could indicate that bubble induced physical stresses are related to the BBB opening.

It was previously shown that the extent of BBB opening varied with bubble size [175]. They reported thresholds for BBB opening of 0.30-0.46 MPa when bubbles 1–2 μm in diameter were used at 1.525 MHz transmit frequency. This threshold was 0.15-0.30 for the case of bubbles with
a diameter of 4–5 µm. In Fig. 4-10, the calculated stress from larger bubbles, inside 5 µm vessels, was higher. In addition, our results in Fig. 4-11 show that at 1.5 MHz, bubbles of 4-5 µm exert larger wall stresses compared to 1-2 µm bubbles. This agreement between the experiments and the simulations indicate that the bubble size dependent BBB opening is a consequence of the physical stresses inserted on the vessel wall. This knowledge may allow the optimization of the ultrasound frequency, and bubble size for maximum BBB opening.

In the future, BBB opening experiments with high resolution imaging techniques (e.g. two-photon fluorescence microscopy), preferentially using mono-disperse bubbles, together with simulations are needed to bring further insights into the stress values related to the BBB opening and vessel wall damage.

4.4.3 General Discussion and Model Limitations

In experiments performed by O’Reilly and Hynynen [34], ultraharmonics (harmonics of the subharmonic, i.e. 3/2 or 5/2 of the transmit frequency of 0.551 MHz) were detected and used as a control parameter for BBB disruption. In their experiments, ultraharmonics generally appeared later in a 10 ms pulse. However, our numerical simulations were limited to 40 µs (longer pulses are more computationally expensive), and subharmonics or ultraharmonics were not detected. In addition, lipid encapsulated microbubbles with a size between the resonance and twice the resonance have the lowest thresholds for subharmonic emissions (e.g. Katiyar and Sarkar [176]). At 0.551 MHz, an unbound 6 µm bubble in radius is at resonance. Therefore, subharmonic emissions are expected to arise from 6 µm bubbles or larger. The presence of such large bubbles in the microvasculature is debatable as majority of Definity bubbles are below 3 µm in radius [169]. However, the long acoustic bursts which are used for therapeutic ultrasound could cause microbubbles to coalesce [163], [177], [178] or undergo rectified diffusion [179], leading to the formation of larger bubbles. In our numerical model, coalescence or rectified diffusion were not taken into account.

The model used here was 2-dimensional and axis-symmetric, and bubbles were assumed to be in the middle of the vessel to reduce computational complexity. In reality, bubbles may be pushed towards one side of the microvessel wall due to the primary radiation force. However our simulations still account for the general effects of confinement on bubble oscillation.
As shown in Figs. 4-2 and 4-3, bubbles underwent non-spherical oscillations. Note the periodicity of these oscillations, under conditions that produce stable oscillations, is at the transmit frequency. This differs from surface mode oscillations which have oscillation periods that are twice that of the insonation frequencies. Surface modes were not detected with bubble/vessel sizes and acoustical properties used here (e.g. at 0.551 MHz). However, they were observed in the simulations at higher transmit frequencies (an example is shown in section §5.4.1).

It was also observed that bubbles collapsed within 5 µm vessels (Fig. 4-4), where the collapse pressure threshold was lower for larger bubbles. The same unbound bubbles at the corresponding threshold pressures did not collapse, but rather underwent stable oscillations. This implies that confined bubbles within elastic vessels are more likely to undergo inertial cavitation. In previous studies, the appearance of inertial cavitation was associated with bubble fragmentation [180], [181]. Postema et al. [182] reported that in most of the fragmentation cases, other bubbles were present near the fragmenting bubble which may induce surface instabilities. The same analogy can be used where the presence of an elastic nearby vessel wall could have the same effect on oscillating bubbles, thus lowering the inertial cavitation threshold. However, the focus of the current study was on acoustic emissions, and vessel wall stresses evaluated at pressures below the collapse threshold.

For the confined bubbles in this chapter only 5 µm vessels were used and in reality bubbles are located within various vessel sizes. However, the presence of confinement (added damping and shift in the frequency) could have the same impact on bubbles in different vessels, particularly for bubbles at the vessel wall (pushed due to primary radiation force) or targeted bubbles.

The results reported here were necessarily constrained to specific parameters for the vessel properties and microbubble shell properties. The selected values were within a reasonable range of what is expected to be realistic, but the results are influenced by these values. For example, bubble oscillations would be more damped within more rigid vasculature, which may represent a tumorous tissue with high interstitial fluid pressure levels [183]. In this study, we used the Marmottant shell model which is a widely employed model that accounts for strain dependant shell elasticity based on heuristic assumptions (i.e. the presence of buckling, elastic and rupture regimes). It was assumed that the bubble is initially at the buckling state ($\gamma(A_0) = 0$), while in
reality initial effective surface tension, $\gamma(A_0)$, is an unknown parameter and in practice there can be variation in this value. In order to investigate the influence of the initial surface tension on the transition point, a test simulation on a 1.1 µm bubble close to the buckling state in an unbound fluid was performed. The initial surface tension was set to $\gamma(A_0) = 0.005$ and $P_{g0} = P_0 + 2 \gamma(A_0)/R_0$ was replaced with $P_{g0} = P_0$. This was performed at 0.551 MHz and at 100-200 kPa. As the result, for $\gamma(A_0) = 0.005$ the pressure corresponding to the transition point was unchanged compared to the case of zero initial surface tension. Furthermore, in order to investigate if the results and the transition point of a bubble population were sensitive to the initial surface tension, another set of simulations were performed for unbound bubbles using Matlab. The initial surface tension was varied linearly with the bubble area. Table 4-2 and Fig. 4-12a show the initial surface tension values chosen for corresponding bubble radii. The result of the normalized second harmonic to the fundamental is presented in Fig. 4-12b. The transition point from a bubble population with different initial surface tensions occurred at the same pressure, similar to when all bubbles were at the buckling state, even though the normalized second harmonic values at the transition point were different.

Table 4-2 Initial surface tension values versus bubble radius for the test simulation

<table>
<thead>
<tr>
<th>Bubble Radius (µm)</th>
<th>Initial surface tension (N/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.0014</td>
</tr>
<tr>
<td>1</td>
<td>0.0047</td>
</tr>
<tr>
<td>1.5</td>
<td>0.00875</td>
</tr>
<tr>
<td>2</td>
<td>0.015</td>
</tr>
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<td>2.5</td>
<td>0.024</td>
</tr>
<tr>
<td>3</td>
<td>0.035</td>
</tr>
</tbody>
</table>
In the Marmottant model the surface tension at the transition between buckling to elastic and rupture states has sharp edges (shown in Fig. 4-13a). In order to investigate whether this sharp edge influences the appearance of the transition point, a test simulation was performed with a smoothed edge using Matlab. A model proposed by Sijl et al. [184] was used in which two quadratic functions were introduced to smoothen the surface tension. For a 2 µm bubble, the non-linear elastic correction coefficient $\zeta$ was set to 200 N/m (Fig. 4-13b). As a result, the transition point for the smoothed surface tension occurred at 100 kPa as well (similar to surface tension with sharp edges).
Figure 4-13 (a) Surface tension using Marmottant model with sharp edges, (b) Surface tension using Marmottant model with smooth edges as proposed by Sijl et al. [184] the non-linear elastic correction coefficient $\zeta=200$ N/m.

In experimental results from Shankar et al. the normalized second harmonic to the fundamental versus pressure reached a minimum from microbubbles similar to Optison and Levovist in water and saline (Figures 2, 5 and 8 of the reference) [185]. However it was not tested to conclude if the minimum occurred due to the shell properties or if the bubble population was changing. Future experimental interrogations on individual phospholipid bubbles, as well as bubble populations could validate the occurrence of the transition point and calibrate the pressures at which it happens.

4.5 Conclusion

In the context of blood-brain barrier opening, this study aims to assess the relationship between the acoustic emissions and vessel wall stresses. Bubbles confined within small vessels presented with non-spherical oscillations. The acoustic emissions from bubbles excited at pressures below the collapse threshold were assessed. At 0.551 MHz, a bubble oscillating within a small vessel experienced increased damping and a resonant frequency shift upwards. This led to lower acoustic emissions in confined bubbles. The normalized second harmonic to the fundamental increased with acoustic pressure followed by a minimum, called a transition point, which was bubble size dependent. This harmonic could potentially be used for in vivo pressure calibrations to enhance the treatment monitoring techniques. The stresses induced by different bubbles on 5 µm vessels were calculated. Following the transition point the stresses induced by larger bubbles (2, 2.5 and 3 µm) rose with a steeper slope which could be correlated to BBB opening. However, this was not as clear for smaller bubbles. Next, bubbles of different sizes were placed close to an elastic wall.
and driven at various acoustic frequencies (constant mechanical index) to assess the mechanical stresses. The wall stresses were bubble size and frequency dependent. The stress peaks induced by bubbles above the resonance (i.e. 3, 4 and 7 µm bubbles at 1.5, 1 and 0.551 MHz respectively) were constant at each frequency, in agreement with earlier experiments.
Chapter 5
Summary of Contributions, Discussion and Future Directions

5.1 Summary of Contributions

Ultrasound exposures in combination with microbubbles have the ability to transiently enhance the BBB permeability, which has implications for drug and gene delivery in the treatment of brain disorders. Understanding the behaviour of a confined bubble and its interaction with nearby endothelial cells is important in understanding the BBB opening mechanisms and optimizing the exposure conditions. A comprehensive model of confined bubbles within vasculature could shed light on the bubble behaviour, bubble–vessel interactions, vessel wall mechanical stresses and acoustic emissions from the exposed bubble. Due to the complicated nature of bubbles confined within vasculature, the finite element method was used.

5.1.1 A Three Dimensional Confined Bubble Model at Low Pressure (Chapter 2)

A numerical model of a three dimensional confined bubble within a vessel for relatively low acoustic pressures was developed. The model accounts for the bubble shell properties, vessel wall curvature, fluid viscosity and asymmetrical off-centre bubbles. The fluid and vessel wall, as well as the fluid and bubble wall, were coupled in a two way manner. Using the model, a parametric study was performed to investigate the influence of acoustical and vascular properties, vessel/bubble size and off-centre bubbles on bubble oscillations and vessel wall stresses. An oscillating, off-centre bubble forms a mushroom shape with the most damping on the points closest to the vessel wall. The shear and circumferential stresses acting on the vessel had spatial and temporal oscillations. The confined bubbles’ resonance frequency (defined by the maximum amplitude of the response) was different from that of unbound bubbles. The bubble resonance frequency increased as the rigidity of a flexible vessel increased. In general, the more rigid the vessels were, the more damped the bubble oscillations. The synergistic effect of acoustic frequency and vessel elasticity was also investigated. The shear stress reached its maximum at an acoustic frequency above the bubble’s resonance frequency and increased as the bubble wall got closer to the vessel. The circumferential stress increased with the reducing distance between the bubble wall and the vessel wall, as well as with the increased softness of the vessel wall.
5.1.2 Mechanisms of Bubble–Vessel Interactions, Model Validation and Induced Stresses (Chapter 3)

Previously, the vascular damage was attributed to either vessel distension or the direct impact of a bubble jet on a vessel wall. Recent high speed photographs of ex vivo vessels have shown that the bubble compression within a vessel generates a distinct invagination of the vessel wall. The underlying mechanism of bubble interactions within viscoelastic vessels at relatively high pressures (i.e. 0.8 MPa) was investigated. A bubble model was developed considering the effects of surface tension and the encapsulating shell. For a more realistic study, viscoelastic properties of ex vivo rat mesentery were extracted from the experimental data. The numerical model agreed well with experiments. Resultant amplitudes of oscillations calculated numerically were within 15% of those measured in experiments (four cases). Using the model, vessel wall stresses were calculated. Wall stresses were larger during the bubble compression phase, as compared to the expansion phase. Based on this finding, vascular damage could occur during vascular invaginations. Predicted thresholds in which these stresses are higher during vessel invagination were calculated from simulations. Additional experiments on dye leakage or RBC extravasation could help in calibrating and correlating the vascular rupture (i.e. RBC extravasation) thresholds to the stresses obtained numerically.

5.1.3 Microbubbles and BBB Opening: A Numerical Study on Acoustic Emission and Stress Predictions (Chapter 4)

Acoustic emissions from microbubbles in the brain vasculature have been shown to correlate with BBB opening. Using a numerical confined bubble model, we calculated these emissions. In addition, vessel wall stresses from bubbles within 5 µm vessels and bubbles at a flat wall were calculated. A transition in the encapsulated bubble behaviour (versus the acoustic pressure) can explain the experimental observation of a sudden rise in the harmonics. The normalized second harmonic reached a minimum, ("transition point") followed by a rise as a function of applied pressure. Above the transition point, the stresses induced by larger bubbles rose with a steeper slope which could be correlated to BBB opening. Bubbles of different sizes were placed close to an elastic wall and driven at various acoustic frequencies at a constant mechanical index. The resulting stress peaks from bubbles above resonance remained constant, with the mechanical index
in agreement with prior experimental work, possibly indicating that bubble-induced wall stresses are related to the opening of the BBB.

5.2 General Discussions

For the confined bubble model developed in this thesis, a number of assumptions were made which are addressed here. Except for the three-dimensional bubble model in chapter 2, the rest of the simulations were performed with a two-dimensional axis-symmetric model to save computational cost. This assumption is valid for cases where the bubble is in the middle of a small microvessel with a tubular shape; however, a three-dimensional approach is required for bubbles close to the small vessel wall or in complicated geometries. Unfortunately, switching to a three-dimensional model is computationally expensive as the numbers of degrees of freedom increases by two orders of magnitude.

The primary radiation force on bubbles, which leads to bubble translation toward the distal end of the vessel wall, has been ignored in this work. At high pressures and under the application of consecutive pulses, bubble translation could become important. Furthermore, microbubbles' movement with the flow was not taken into account and bubbles were assumed stationary in this thesis. The rate of fluid flow in capillaries is 1 mm/s [186]. A bubble oscillating at 1 MHz moves one thousandth of a micron during each cycle. Since the number of oscillation cycles simulated in this thesis did not exceed twenty two, the assumption of a stationary bubble is a good approximation. When the effects of therapeutic ultrasound with long (millisecond) pulses are considered, though, the bubble's motion with fluid should also be considered.

Throughout the thesis, it was assumed that the acoustic pressure was acting directly on the bubble wall to save computation time. In reality, not only the bubble wall, but also the fluid and vessel, are subjected to the acoustic pressure. In chapter 2, simulations were performed where the whole computational domain was subjected to the incoming pulse. As a result, the difference between stresses from acoustic pressure acting on the whole domain or only the bubble wall was less than 1%. At higher pressures, as was studied in chapters 3 and 4, this assumption still holds if the attenuation and impedance mismatch between the vessels and fluid are low. According to the simulations performed by Qin et al., the transmitted ultrasound pressure was not distorted when
the vessel wall was soft and at relatively low frequencies (i.e. 1 MHz) [162]. This was the case for most of the simulations performed in this thesis.

In order to investigate the sensitivity of the results to the shell elasticity and viscosity parameters, test simulations were performed on an unbound, 1.1 μm bubble at 150 kPa and 200 kPa (corresponding to compression dominated and expansion dominated oscillations, respectively), using the Marmottant model (at 0.551 MHz). When the shell viscosity was reduced six-fold (from 1.2×10⁻⁸ kg s⁻¹ to 2×10⁻⁹ kg s⁻¹, as smaller bubbles were shown to have lower shell viscosity in the range evaluated for Definity bubbles [159]), the maximum bubble expansion increased by 3% at 150 kPa and by 34% at 200 kPa. A decrease in the shell elasticity by 50% (from 0.7 N/m to 0.35 N/m) resulted in a 3% (for both 150kPa and 200 kPa) increase in the maximum bubble expansion. A 50% increase in shell elasticity (0.7 N/m to 1.05 N/m) resulted in a 1% decrease in the maximum bubble expansion (for both 150kPa and 200 kPa); therefore, the results were not sensitive to changes in the shell elasticity, and shell viscosity at lower pressures. At higher pressures, however, the bubble oscillation was sensitive to the reduction in shell viscosity as the maximum bubble expansion increased by 34%.

In order to investigate the sensitivity of bubble oscillations on fluid viscosity, an unbound 1.1 μm radius microbubble was tested at 150 and 200 kPa (at 0.551 MHz) when the fluid viscosity was varied from 0.002 Pa s (which is commonly used in the literature, used in chapter 4) to 0.005 Pa s (due to the inverse Fahraeus–Lindqvist effect, used in chapter 2). With fluid viscosity of 0.005 Pa s the change in the maximum bubble expansion was below 1% (at 150 kPa) and 5% (at 200 kPa), compared to μ =0.002 Pa s. In case of a 1.1 μm bubble confined within a 5 μm vessel, the change in the maximum bubble expansion with a fluid viscosity of 0.005 Pa s was below 1% (at 150 kPa) and 6% (at 200 kPa), compared to μ =0.002 Pa s.

In our model, the bubble pressure inside the gas was assumed spatially uniform. For cases where \( R \ll \lambda \) (\( \lambda \) is the ultrasound wavelength in the gas) and Mach number (the ratio of the bubble wall speed to the speed of sound in the fluid) \( \dot{R}/c \ll 1 \), and for non-collapsing bubbles (where the bubble radius can decrease by an order of magnitude), the uniform gas pressure assumption holds to a good approximation [187].
In this thesis, fluids were assumed incompressible. Previously, different bubble models were derived to consider the effects of fluid compressibility (such as Gilmore [85] and Trilling [87]). The applicability of these models depends on the Mach number ($\frac{\hat{R}}{c}$). The most extreme case, with the highest bubble wall velocity, is our simulation in Case 1 in chapter 3. In this case, the maximum bubble wall velocity was 80 m/s, with a Mach number of 0.05. This Mach number is relatively small and we can safely assume that the Gilmore equation reduces down to the RP equation. The incompressible fluid assumption may hold, even until the very last instant of the bubble collapse [188].

The rectified diffusion (the bubble growth due to the mass transfer into the bubble during the oscillation) was not incorporated into the current model. The amount of gas in the bubble is assumed to remain constant during each oscillation cycle, and there is no rectified diffusion during the course of short ultrasound exposures [189]; however, for longer ultrasound pulses (ms time scale), such as in therapeutic ultrasound, rectified diffusion could become significant [190].

The thermal effects due to bubbles was not a focus of this thesis and the energy deposition into the tissue which leads to temperature rises on the surrounding vasculature was not taken into account. When short ultrasound pulses are used (e.g. in imaging), temperature changes around the bubble are insignificant. However, in therapeutic ultrasound with long pulses, local heat deposition in the presence of microbubbles is enhanced [46]. Therefore, thermal effects as well as mechanical effects need to be considered.

In reality, bubbles confined within the vasculature are surrounded by red blood cells (RBCs) and other bubbles (depending on the concentration). In our model, the bubble-RBC and bubble-bubble interactions were ignored. It was assumed that the bubble concentration was sufficiently dilute to have a single bubble in the domain, and that the RBCs were a few microns away from the bubbles, with minimal impact on the bubble behaviour. The presence of an RBC can disrupt the downstream flow field and might become important for drug delivery. Through numerical simulations, Wiedemair et al studied the impact of an RBC close to a confined bubble on the mechanical stresses [116]. They found that localized shear stress peaks appear in the presence of RBCs and spatial gradient patterns of stresses were altered noticeably. A neighbouring bubble could also affect the main bubble’s oscillation and acoustic emissions.
The shell models used in this thesis were developed for soft lipid-coated bubbles. Although the Marmottant model takes into account the nonlinear relationship between the effective shell surface tension and the bubble area, a nonlinear theory for shell viscosity needs to be taken into account as well [191]. This is due to recent experimental studies that have shown the shell viscosity to be dependent on the bubble’s initial size, and that lipid-shelled bubbles exhibited shear thinning (a phenomena that the shell viscosity decreases as the shear rate, $\dot{R}/R$, increases). In a recent study, even though for MicroMarker agent a clear shear thinning behaviour was observed, the relationship between shell viscosity and maximum shear rate for Definity was shown to be less evident [192]. However, this study was performed at different transmit pressure amplitudes and the shear thinning property of the Definity bubbles was shown to be pressure-dependent [159]. Therefore, in the future appropriate shell modelling should be adapted in order to account for the agent type and acoustic properties.

5.3 Validation and Calibration

In order to validate and calibrate the numerical results presented in this thesis, several experiments are proposed for the future.

5.3.1 Bubbles in Different Tubes

A bubble’s resonance frequency (defined by the maximum oscillation in chapter 2) was shown to shift upwards as the bubbles were placed inside elastic microvessels, depending on the elastic moduli. This shift could have implications in tissue characterization. Future experiments on individual bubbles inside different elastic/rigid tube are proposed. Optical and acoustical recordings from such bubbles (in comparison with the same unbound bubbles) while sweeping the frequency could confirm the frequency shift and correlate the shift to vessel elasticity. It is important to note that the resonance frequency of the maximum bubble response (calculated in chapter 2, the frequency at which the bubble exhibits maximum radial excursion) could differ from the resonance frequency of the maximum scattering [193]. Therefore, such experiments should be accompanied by additional simulations to calculate the frequency shifts from maximum scatterings.
5.3.2 Mechanical Stress Calibration

The bubble-vessel interaction was described in chapter 3, and corresponding vascular stresses were calculated; however, vascular rupture thresholds were not evaluated experimentally. In the future, high speed photomicrography experiments (similar to those described in chapter 3) of individual bubbles within mesentery vasculature could be combined with dye leakage or red blood cell extravasation assessments (i.e. an indicator of the vascular damage). Parametric studies of such experiments could correlate the stresses found numerically to the vascular damage thresholds and calibrate the stress values.

5.3.3 Experiments on Single Bubbles

In order to validate the acoustical emissions calculated in this thesis, we propose comparing our model with experimental results. A unique and large experimental dataset of individual bubbles close to the Opticell and agarose (a more compliant) boundaries is available in our laboratory. Helfield et al. recorded the linear and nonlinear responses from bubbles [82], [194]. They found that fundamental scattering increased while subharmonic scattering decreased as the bubble approached the agarose boundary. Currently, this experimental dataset is un-matched with the bubble models close to a boundary. In the future, recruiting our model with a bubble at the wall and calculating the acoustic emissions for different offset distances would open up an opportunity to match the numerical results with the experimental data.

Moreover, it was shown theoretically that the normalized second harmonic to the fundamental (scattered from bubbles) versus the acoustic pressure exhibited a minimum called a transition point (chapter 4). The presence of such a point could further guide the monitoring of BBB opening, and calibrating the in vivo pressures. From a physical perspective, this transition is associated with an entry into a regime where the bubble expansion phase is more prominent. Future experiments to interrogate individual phospholipid microbubbles optically and acoustically (as the transition point was bubble size dependent), as well as bubble populations, could validate the occurrence of the transition point and calibrate the pressures at which it happens. Furthermore, in the simulations, the second and fourth harmonics scattered from confined bubbles at the transition point were larger than those of unbound bubbles. This could have implications in separating confined from unbound...
bubble populations. These observations, however, require experimental verification to estimate its clinical potential.

5.3.4 The BBB Opening Experiments with Two-Photon Microscopy

Future experiments on blood brain barrier opening thresholds with high resolution imaging techniques (e.g. two-photon fluorescence microscopy) combined with mono-disperse bubbles are proposed. Acoustic emissions assessed from such experiments could validate the presence of the transition point (as predicted in chapter 4) and calibrate the pressures at which the transition point occurs and its relationship with the BBB opening threshold. From such experiments, in which the bubble and vessel sizes are known, the physical stress values calculated numerically could be correlated to the BBB opening thresholds.

5.4 An Examination of Non-spherical Oscillation Phenomena

Two important classes of ultrasound contrast agent oscillation have previously been implicated in microbubble mediated drug delivery: shape oscillations and jets. In the future, using the current numerical model, these phenomena could be investigated more in depth. Some examples are given in the following subsections.

5.4.1 Shape Oscillations

From the simulations, it was found that in some situations the confined bubble underwent shape oscillations at half the insonation frequency. In this type of oscillation, the bubble wall location is dependent on the polar angle. The occurrence of shape oscillations in ultrasound contrast agents is important as it may have implications in imaging, provide insight into the shell properties, and induce intense microstreaming fields close to the bubble (i.e. enhancing the drug delivery efficacy) [195]. The phenomena has been well documented in the free bubble literature and have also been observed optically for encapsulated microbubbles [72], [195]–[198]. The shape oscillations of ultrasound contrast agents were observed and reported to be significantly present in medically relevant ranges of bubble sizes and acoustic pressures [198]. Furthermore, these shelled bubbles optically exhibited subharmonic behaviour [198].
An example of the shape oscillations is shown in Fig. 5-1 from a 4 μm bubble within a 6 μm vessel, sonicated at 1.653 MHz and 0.1 MPa. To date, the experimental studies of subharmonic emissions have not been linked to shape oscillations. However, in this example the shape oscillation was coupled to bubble's volume that underwent oscillations at half the insonation frequency (Fig. 5-2). The acoustic emissions in Fig. 5-3a shows subharmonics, whereas the same bubble in an unbound fluid did not exhibit subharmonics (Fig. 5-3b). The engagement of the bubble's volume, which leads to monopole emissions, may result in acoustically observable subharmonic energy. Therefore, further numerical and experimental investigations are required to determine whether this kind of bubble oscillation may be one potential mechanism in subharmonic emissions.

Figure 5-1 The shape oscillation of a 4 μm bubble within a 6 μm vessel at 1.653 MHz and 0.1 MPa over a 2 cycle oscillation at times 20, 20.2, 20.4, 20.6, 20.8, 21, 21.2 μs. At each time step, the image on the left shows a 2-dimensional cross section view with the vessel border and the image on the right shows the corresponding 3-dimensional bubble.
Figure 5-2 Bubble's volume versus time showing oscillations at half the insonation frequency, a 4 µm bubble within a 6 µm vessel at 1.653 MHz and 0.1 MPa.

Figure 5-3 FFT of the acoustic pressure at 1.653 MHz and 0.1 MPa (a) from a 4 µm bubble within a 6 µm vessel, (b) from a 4 µm bubble in an unbound fluid.

5.4.2 Bubble Jets

Bubbles close to an elastic wall could form a liquid jet either towards or away from the boundary. This has been the subject of intensive research for free (unshelled) bubbles [81], [199]–[201]. The fluid jet towards an elastic boundary, observed experimentally from free and encapsulated bubbles,
was proposed as a mechanism for drug delivery or in severe cases vessel damage [202], [203]. However, the study on micro-jets observed from encapsulated bubbles, in *ex vivo* rat mesentery microvessels, showed that bubbles jet away from the nearest vessel wall [204]. The laser-induced bubble jet towards or away from an elastic wall depends on a non-dimensional standoff parameter (defined as the distance between the laser focus and the boundary by the maximum bubble radius), and it has been speculated that the jetting behavior arises from the interaction between the opposing forces induced by the rebound of the elastic boundary, and the Bjerknes attraction force towards the boundary [81].

In some situations in our model, when different encapsulated bubbles at resonance were placed at a constant standoff distance from a flat elastic wall at a constant MI, bubble jets towards or away from the wall were observed (Figure 5-4). In the final cycle during collapse, 2.5 µm and 3.5 µm bubbles at 1.5 MHz and 1 MHz jet away from the elastic wall (or the wall bounces them back), while a 6.5 µm at 0.551 MHz bubble jets towards the wall forming a mushroom shape (bottom right image in Fig. 5-4). The jet formation in Fig. 5-4, which depended on the bubble size and acoustic parameters, could be the subject of future investigations, as it could have implications in the enhancement of vascular permeability for drug delivery and/or vascular damage.

![Figure 5-4 Bubble shape oscillations close to an elastic vessel wall during the bubble's last two cycles of oscillation. Columns from left to right present bubbles at expanded, contracted, expanded and contracted phases respectively. (a) 2.5 µm bubble at 1.5 MHz, (b) 3.5 µm bubble at 1 MHz, and (c) 6.5 µm bubble at 0.551 MHz.](image-url)
5.5 Model Expansion

The confined bubble model developed in the thesis has certain limitations (discussed in section §5.2). In the future, several approaches can be taken to expand the current model. In this thesis, a linear (with bubble area) viscoelastic shell model was used. As for future work, more complicated nonlinear shell models (e.g. the model proposed by Paul et. al. [205]) should be recruited with a nonlinear relationship between surface tension and bubble area. To account for rheological effects of the shell, numerical modeling could improve by adding a viscoelastic layer around the gas. Additionally, for the promising techniques of magnetic bubbles [206] or drug-loaded magnetic bubbles [207] in dual-modality applications, different shell models and materials could be incorporated into the model to account for magnetic particles.

Throughout this thesis, the stresses shown were the maximum stress values. Other methods of stress assessment, such as stress integration over time, could provide additional metrics for mechanical bioeffects. In addition, calculating the wall surface divergence was proposed as a measure relevant for sonoporation [208], a process of transient membrane permeabilization [61].

In the current model, the presence of a red blood cell (RBCs) or another bubble are ignored. In future studies, for a more realistic outcome, bubble-RBC and bubble-bubble interactions should be accounted for. A preliminary study on a RBC close to a bubble was performed. The RBC had a biconcave shape with dimensions shown in Figure 5-5a. The RBC diameter was set to 8 µm. Its cross section had a peanut shape with a maximum width of 2.8 µm and a minimum width of 1.4 µm [209]. The RBC was treated as an elastic solid with an elastic modulus of 1350 Pa [209]. It was coupled to the fluid flow and allowed to translate freely. The bubbles had a radius of 2 µm within a 5 µm vessel and acoustical parameters were set to 1 MHz and 260 kPa. Preliminary results showed that the presence of a RBC disrupted the downstream flow (Fig 5-5b). Also, WSS peaked at the bubble location (WSS = 2.662 kPa) and at the RBC location, as well (WSS = 2.082 kPa). This finding is in line with the results reported in [116] where the wall shear stress peaks at the location of the red blood cell, with the amplitudes of the same order of magnitude. Controlled parametric simulations are needed to show the dependence of bubble-RBC distance and RBC elasticity on vessel wall stresses.
Figure 5- 5 A red blood cell used in simulations and its dimensions, (b) Numerical domain showing half of the geometry of a bubble and a RBC in its vicinity.

5.6 Applications

5.6.1 Model Applications for Blood Brain Barrier Opening

The transfer of acoustic energy to the bubble is maximized at resonance, therefore, understanding the resonance frequency for confined bubbles is important (chapter 2). Furthermore, a shift in the resonance frequency (between unbound and confined bubbles or between bubbles in various elastic vessels) could be beneficial for the development of microvascular imaging strategies, and tissue characterization (e.g. a mean to differentiate between bubbles in tumorous rigid tissue from other confined/unbound bubbles). The results from vessel wall stress calculation and finding ultrasonic parameters in which stresses are maximized may have significance in improving the efficacy of drug delivery into the brain (chapters 2 and 3). The vessel wall stresses calculated
numerically (chapter 3) in combination with experimental calibrations (section §5.3.2) could provide a mean to predict vascular damage at various exposure conditions. The acoustic emissions calculated numerically (specifically, the transition point in the second harmonic) could provide feedback to online monitoring techniques and calibrating the \textit{in vivo} pressures (chapter 4). Furthermore, the difference in the acoustic information from unbound versus confined bubbles (i.e. stronger second and fourth harmonics from confined bubbles) could have implications for the development of microvascular imaging techniques (chapter 4).

5.6.2 Model Applications in General

Although the model developed in this thesis was motivated by BBB opening, the application of the model is not limited here and could extend to other organs with different types of vasculature, and to the diagnostic use of microbubbles.

Ultrasound imaging in combination with microbubbles provides functional, anatomical and molecular information. Some of the imaging applications include the evaluation of myocardial perfusion [210]–[212], delineation of the myocardial wall [213], measurement of cardiac output [214], [215], the identification of neo-vascularized regions in carotid atherosclerotic plaques [216], improvement in the detection of small vessel [217], and solid tumour detection in the abdomen and prostate [218]–[221]. Development of targeted microbubbles in combination with ultrasound has opened many new opportunities in molecular imaging. By incorporating ligands and antibodies on the bubble surface that bind to cell receptors, bubbles can be targeted to specific disease sites. This allows imaging of intravascular molecular changes associated with inflammation, ischemia-reperfusion injury, angiogenesis, and thrombi [222]–[227]. The feasibility of microbubble injection into the lymphatic system has been assessed as well [228]. This technique allows lymph node imaging for local control and staging breast cancer.

Nearly all of these imaging techniques take advantage of the nonlinear behavior of stimulated microbubbles. A shift in the confined bubble’s resonance frequency (chapter 2) and different acoustic characteristics of confined bubbles (in harmonics shown in chapter 4) could improve contrast imaging techniques by capturing maximum nonlinear signal. Furthermore, a shift in the resonance frequency and distinct acoustic characteristics of confined bubbles can bring a way for unbound/confined bubble separation in targeted molecular or microvascular imaging techniques.
The shift in resonance frequency varies with the vessel elasticity (chapter 2). This could further have implications in in vivo tissue characterization techniques, and thus provide a means by which to separate bubbles in tumorous vasculature from those elsewhere.

Sonoporation, the process of transient membrane permeabilization [61], [229], is one of the mechanisms by which bubbles enhance drug delivery in therapeutic ultrasound. Aside from BBB opening, sonoporation has applications in cancer and cardiovascular treatment [230], [231]. Since it increases the local drug concentration, this technique may allow for the use of lower systemic drug dosages, thereby minimizing side effects, and improving efficacy. Therapeutic bubbles for cancer treatment can be combined with other modalities as well. For example, the use of ultrasound and microbubbles in combination with radiation has been proposed as a radioenhancing modality [232]–[234].

For the therapeutic applications mentioned above, the outcome of a confined bubble model developed in this thesis could elucidate the microstreaming fields around bubbles (chapter 2) and bubble-vessel interactions, as well as the vessel wall stresses (chapter 3). Vessel wall stresses depend on the vessel elasticity and ultrasound exposures (chapter 2). Therefore, using the numerical results, one may be able to obtain the optimal ultrasound parameters to achieve the maximum bubble impact on the vessel wall and improve its therapeutic efficacy.

5.7 Final Words

Microbubbles excited by ultrasound can transiently open the blood brain barrier to facilitate drug delivery in the treatment of brain pathologies. Understanding the mechanisms of bubble-vessel interactions brings insight into the blood-brain barrier opening experiments and further guides the optimization of the exposure conditions. A theoretical model can be used to explore bubble behaviour under various conditions, with significantly reduced costs when compared with experimental approaches. In this thesis, a theoretical model of a complex bubble-fluid-vessel system was developed to investigate the confined bubble behaviour, bubble–vessel interactions, vessel wall mechanical stresses and the bubbles' acoustic emissions. Additional experiments, however, are required to calibrate the numerical stresses and validate the acoustic information for the development of future real-time monitoring techniques.
References


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