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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**A STUDY OF SOFT-MATERIAL MECHANICAL
PROPERTIES USING MICROFLUIDIC BUBBLE**

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SCHOOL OF ELECTRICAL AND ELECTRONIC
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2014**

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SUMMARY

The rapid development of microfluidics plays a key role in the study of soft materials. Microfluidic systems provide several key advantages such as the precise control of the sample volume and the physico-chemical conditions. This doctorate thesis focuses on the theoretical and experimental study on soft materials, including water-in-oil droplets, cell membrane and high viscous liquids. Specially, droplet fusion, single-cell membrane poration and tensile strength measurement of liquids have been studied using microfluidic systems driven by laser-induced cavitation bubbles.

Droplet fusion is demonstrated using a microfluidic chip actuated by a pulsed laser-induced cavitation bubble. The theoretical studies of the mechanism of the droplet fusion and the “neck” growth are carried out and a simplified model is established for the droplet generation using the microfluidic chip with a T-junction and a collection chamber. For droplet fusion, a cavitation bubble is created with the pulsed laser beam focused into one droplet. High-speed photography of the dynamics reveals that the droplet fusion is induced within a few tens of microseconds and caused by the rapid thinning of the continuous phase film separating the droplets. Finally, the cavitation bubble collapses and re-condenses into the droplet. Droplet fusion is experimentally demonstrated for static and moving droplets, droplets of equal and unequal sizes, and droplets in the hexagonal structure. Furthermore, the diffusion is discussed for the fusing droplets and the transport is demonstrated for a single encapsulated cell into a fused droplet.

Single-cell membrane poration is demonstrated using a microfluidic chip driven by a pulsed laser-induced cavitation bubble. The theoretical analyses of the

microjet generation and the single-cell membrane poration due to the fast liquid microjet are carried out and a simplified model for single cell trapping is established. Based on the theoretical analyses, the microfluidic chip is designed with an array of single cell trapping structures and the single cell trapping is demonstrated. A laser-induced cavitation bubble is created at a quantified stand-off distance from the target cell. The asymmetrical growth and collapse of the cavitation bubble lead to the formation of the microjet, which deforms and porates the cell membrane. In the experiments, the membrane porations of myeloma cells are probed with the uptake of trypan blue. Time-resolved studies of the diffusion of trypan blue show a marked dependency on the bubble dynamics, i.e. the standoff distance. The penetration length of the dye increases with shorter distance. Numerical simulations of the diffusion process agree with larger pores formed on the cell membrane.

The shock pressure and tensile strength measurement of water and glycerol is demonstrated using the laser-induced cavitation bubble in a microfluidic chip. The principle of the tensile strength measurement is theoretically analyzed. In the experiment, an air-liquid interface is created in a partially filled microchannel. A shock wave is generated by focusing an infrared pulsed laser into the liquid medium. Then, the expanding shock is reflected by the interface as a tensile wave and the liquid under the tension is ruptured. The shock pressures are determined by measuring the velocity of the interface and the tensile strength of water and glycerol are determined as -33.3 ± 2.8 MPa and -59.8 ± 10.7 MPa at 20 °C, respectively. The significant effects of the repetitive tensile strength, the microparticles and the surfactant on the cavitation nucleation are presented and discussed.

The successful implementation of three microfluidic systems has evidenced the effectiveness of employing microfluidic systems driven by the pulsed laser-induced cavitation bubbles in the study of soft materials.

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NOMENCLATURE

DNA	Deoxyribonucleic acid
PDMS	Polydimethylsiloxane
HepG2	Hepatocellular carcinoma, human
NA	Numerical aperture
SDS	Sodium Dodecyl Sulfate

CHAPTER 1

INTRODUCTION

1.1 Motivation

This PhD research topic is motivated by the potential applications of the microfluidic systems in the study of soft materials. Soft materials are a class of materials, including liquids, colloids, emulsions, foams, gels, polymers, grains materials and biological materials [1]. Two major features of the soft material are complexity and flexibility [2]. The rapid development of microfluidics plays a key role in the study of soft materials [3-4]. Microfluidics is the science and technology to precisely control and manipulate a small volume (from 10^{-9} to 10^{-18} Liters) of fluids in microchannels with sub-millimeter dimensions [5-7]. It is a real multidisciplinary research, which involves fundamental knowledge, ranging from fluidics, optics, electromagnetics and chemistry to biology. Microfluidics provides many unique properties, such as ultra-small minimal amount of samples and reagents, ultra-high resolution and sensitivity, low cost and rapid analytical time.

A microfluidic system usually consists of several integrated microfluidic components, such as mixers, valves, micro-reactors and fluidic sensors. Various fabrication methods for different materials have been developed, which originate from the well-developed photolithography and associated technologies in the silicon microelectronics or Micro-electro-mechanical systems (MEMS) [8]. Microfluidic channels can be fabricated on silicon substrates using wet (chemical) etching [9] or

dry (plasma) etching [10-11]. Glass is also widely used as the substrate or structure for microfluidics due to its good optical properties and the exploitation of the electro-osmotic flow [12]. More and more microfluidic channels are fabricated using polymers or plastics, such as poly(dimethyl siloxane) (PDMS) etc, due to low cost, good optical characteristics, biochemical reliability and biocompatibility. Fabrication methods include soft lithography at room temperature [13-14], hot embossing at the temperature up to the glass-transition temperature [15] and injection molding with low cost and high volumes [16].

One subcategory of microfluidics is droplet microfluidics [17], including the generation and manipulation of droplets in the microfluidic system. It has several significant advantages, such as compartmentalization of reagents and reactions into individual droplets to enhance the sensitivity by increasing the concentration of reagents and decreasing the reaction time, low cost due to the ultra-low consumption of reagents and high throughput using the high parallelized reactions in multiple individual droplets. Many applications have been demonstrated using the droplet microfluidics, such as chemical reactions [18-19], single cell analysis [23-24], protein and DNA analysis [25-27] and simple Boolean logic function [28-29]. Various methods have been developed for droplet generation, sorting, mixing, fission and fusion in microfluidic systems [30-34]. One focus of our study is the droplet fusion. Even through various passive and active droplet fusion methods have been demonstrated, the rapid expanding of pulsed laser-induced cavitation bubbles provide the possibility to fuse droplets in microfluidic systems.

Microfluidic system provides a powerful tool for cell biological researches [35], such as mechanical and chemical microenvironment control [36-37], precise chemical spatiotemporal concentration gradients [38], precise temperature control

[39] and mechanical force control [40]. Many cell biophysical studies have been carried out in microfluidic systems, including cell mass measurement [41-42], living cell refractive index measurement [43-47], cell rheological study [48], cell adhesion force [49], cell stress investigation [50] and cell Young's modulus measurement [51], etc. One focus of our study is single-cell membrane poration. Several physical techniques for single-cell membrane poration using microfluidic system have been developed, including electroporation [52], sonoporation [53] and opto(photo)poration [54]. The formation of the microjet due to the interaction between the pulsed laser-induced cavitation bubbles and the solid boundary provides another possible technique to porate a single trapped cell.

Microfluidic systems can also be used to investigate physical and chemical properties of liquids, such as refractive index, concentration, density, viscosity, pH value and dissolved oxygen, etc. Refractive indices of liquids are measured in the microfluidic channels with a grating structure based on the transmission of the incident light [55-56]. The concentration is measured based on the ratio of the total internal reflected light over the transmitted light [57-58]. The density and concentration of liquid samples are also measured using an integrated vacuum-sealed resonating silicon microtube in a microfluidic channel [59]. The viscosity and density of 5-pL liquid sample are measured using a suspended microchannel resonator with high resolution [60]. Using integrated sensors, the pH value and dissolved oxygen of liquids in microfluidic channels are detected [61-62]. Microfluidic systems combined with the pulsed laser-induced cavitation bubble have great potential in the shock pressure and tensile strength measurement of liquid samples. In addition, the precise control of the volume and concentration of

reaction agents using microfluidic systems provides the possibility to investigate the impurity effects on the tensile strength and cavitation nucleation.

For microfluidic systems, many kinds of forces and external fields have been employed to control and manipulate the flows. The manipulations can be realized by applied forces macroscopically or locally generated by the integrated components inside microfluidic channels [5]. Pressure-driven flow [63-64] and electro-osmotic flow [65-67] are the two most common methods for the microfluidic flow manipulation. External fields such as dielectrophoresis using electric field [68-69], magnetic field [70], acoustic field [71] and gravity field [72-73] are also used to control fluid flows in microfluidic channels. Other manipulation approaches include capillary or surface tension [74-77] and the gradient of the interfacial tension along the free surface [78-80] by shrinking the dimensions, and the movement of biological organisms such as the kinesin protein motors [81] and the mixer using bacterial carpets [82].

Another main approach for flow control in microfluidic systems is bubble driven flows. Bubbles generated using high frequency acoustic waves have been demonstrated to drive the flows with high velocity [83-84]. Laser-induced surface bubbles have also been used to reconfigure plasmofluidic lenses [85]. And optothermally generated bubbles have also been demonstrated to trap and manipulate particles [86] and concentrate and pattern particles and cells [87]. Pulsed laser-induced cavitation bubbles have also been employed, such as high speed microfluidic switch [88], fluorescent activated cell sorting microfluidic system [89], high frequency and high throughput droplets generator [90] and micro-pumps [91-92]. The pulsed laser-induced cavitation bubble provides several unique advantages, including rapid energy absorption from nanoseconds to femtoseconds based on the

duration of a single laser pulse, fast energy conversion into mechanical power and large force and displacement. These advantages provide the possibility of the potential applications in the studies of droplet microfluidics, cell biophysics and physical and chemical properties of liquids.

In summary, the studies and development of microfluidic systems for soft materials studies have been a goal actively pursued by both academia and industry. The study of microfluidic systems for droplet fusion, single-cell membrane poration and tensile strength measurement of liquids is chosen as the topic of this thesis.

1.2 Objectives

The main objective of this research is to develop microfluidic systems based on pulsed laser-induced cavitation bubbles for ① droplet fusion, ② single-cell membrane poration and ③ tensile strength measurement of liquids. These microfluidic systems can be widely employed in the research of microfluidic mechanics, dynamics and single cell physics. The primary target is to develop microfluidic systems, which can provide functionalities of droplet, cell and liquid manipulation by microfluidic designs. Research investigations include theoretical analyses, system designs, fabrication process development and experimental studies. Theoretical models and analyses guide the designs of the corresponding microfluidic systems. Designed microfluidic components are integrated into the microfluidic systems to facilitate droplet generation and collection, single-cell trapping and formation of micro-scale uncontaminated environment for homogeneous nucleation. Soft lithography fabrication process is applied for the fabrication of the microfluidic systems in this PhD research. Experimental studies are carried out using the developed microfluidic systems to observe and study droplet fusion, single-cell membrane poration and tensile strength measurement of liquids.

The microfluidic system for droplet fusion is designed, fabricated and experimented. Droplet fusion is realized via the rapid expansion of the pulsed laser-induced cavitation bubble. The theories of droplet fusion and droplet generation are studied. A T-junction forms part of the microfluidic system to facilitate droplet generation. The soft lithography fabrication process is developed. A cavitation bubble is created by focusing a pulsed laser beam into the target droplet. High-

speed photography is employed to reveal the dynamics. The experiment results are discussed and compared with the theoretical analyses.

The microfluidic system for single-cell membrane poration is designed, fabricated and experimented. The cell membrane poration is realized via the microjet generated by the interaction between the laser-induced cavitation bubble and the rigid flat boundary. The theories of cell membrane poration and single cell trapping are studied. An array of trapping structures forms part of the microfluidic system to facilitate single cell trapping. A cavitation bubble is created by focusing a pulsed laser beam in vicinity to a trapping structure with a trapped cell. High-speed photography is employed to record the membrane poration process. Numerical simulation is used to validate the formation of the porated pore. The experiment results of single-cell membrane deformation, poration, restoration and swelling are presented and discussed.

The microfluidic system for tensile strength measurement of liquids is designed, fabricated and experimented. The measurement of shock pressure and tensile strength of liquids is realized via the reflection of the shock wave induced by the optical breakdown. The theory of the measurement principle of shock pressure and tensile strength is studied. An air-liquid interface is formed in a partially filled microchannel. An optical breakdown induced shock is generated in the liquid medium. The expanding shock is reflected by the interface as a tensile wave and the liquid under the tension is ruptured. The shock pressures are determined by measuring the velocity of the interface and the tensile strength of water and glycerol are determined using the critical standoff distance method. The effects of repetitive tensile waves, microparticles and surfactant on the cavitation nucleation are presented and discussed.

1.3 Major contributions

The major contributions of this PhD thesis lie in various aspects of the theoretical analyses and experiments of the study of three soft materials using microfluidic systems driven by pulsed laser-induced cavitation bubbles. The details are listed below:

- 1) The fusions of water-in-oil droplets are realized under different conditions, including two equal-sized droplets and unequal-sized droplets, in a continuous droplets stream and in a hexagonal structure. The pulsed laser-induced cavitation bubbles are employed to actuate the fusion and drain the continuous phase between the fused droplets. (See Chapter 3)
- 2) The diffusion dominant mixing flow between two fusing droplets is experimentally studied and compared to the theoretical model. The fusion of droplets with encapsulated cells is also demonstrated and discussed. (See Chapter 3)
- 3) Fabrication process for the microfluidic systems is developed using PDMS as the chip material. SU8 photoresist is used as the mold material and patterned on a silicon wafer by lithography. (See Chapter 3)
- 4) A microfluidic system for single-cell membrane poration is developed. The deformation, poration, restoration and swelling processes of the single trapped suspension cell are experimentally studied. Particularly, the deformation process is also studied using a boundary element method simulation. (See Chapter 4)

- 5) The interaction of the cavitation bubble and the rigid boundary is experimentally studied. Particularly, the velocities of the generated microjets as the function of the width of the trapping channel and the standoff distance are studied. (See Chapter 4)
- 6) Reflected shock wave based measurement principle is analyzed theoretically and acted as the guidance for the measurements of the shock peak pressure at various standoff distances and the tensile strength of liquid samples. (See Chapter 5)
- 7) The tensile strength of water is determined as -33.3 ± 2.8 MPa at 20 °C using the critical distance method. The tensile strength of glycerol with ultra-high viscosity is also measured as -59.8 ± 10.7 MPa at 20 °C. (See Chapter 5)
- 8) The effects of repetitive tensile waves, microparticles and surfactant on the cavitation nucleation are experimentally studied and the current experimental results show that the cavitation nucleation is significantly affected by these factors. (See Chapter 5)

1.4 Organization of the thesis

The thesis is organized into six chapters. The introduction of the thesis covers the motivation, objective, and major contributions as presented in this chapter. The motivation section explains why and how the PhD research is carried out. The objective states the main focus of this thesis, and the contribution section lists the innovations and important findings in both the theoretical and technological aspects.

In Chapter 2, literatures of the passive and active methods for droplet fusion employed in microfluidic systems are reviewed. Then, the developed traditional methods and cavitation bubble driven methods for cell membrane poration are reviewed. In the third section, the measurement of tensile strength of water and glycerol and theories of the homogeneous and heterogeneous nucleation are reviewed. In the final section, microfluidic systems driven by the cavitation bubbles are reviewed. All these important background works provide the guidance and impetus for the research, and also lay the technological foundations on which the work of this thesis is built.

In Chapter 3, the design, fabrication and experiments of the microfluidic system for droplet fusion are demonstrated and discussed. The mechanism of droplet fusion and “neck” growth are theoretical analyzed. Then, the microfluidic chip design and experimental setup are presented and discussed. Subsequently, the experimental results of droplet fusion under different conditions are presented and discussed. Finally, the diffusion process and the droplet fusion process with encapsulated cells are presented and discussed.

In Chapter 4, the design, fabrication and experiments of the microfluidic system for single-cell membrane poration are elaborated. The theoretical analysis of the microjet generation and the single-cell membrane poration are presented. Then, the design of the microfluidic chip for the single cell trapping is illustrated. Subsequently, the experimental results of the bubble-boundary interaction induced microjets and the visualization of the flow field are presented and discussed. Finally, the experimental results of single-cell membrane poration, including the deformation, poration, restoration and swelling processes, are presented and discussed.

In Chapter 5, the design, fabrication and experiments of the microfluidic system for the tensile strength measurement of liquids are elaborated. The principle of shock pressure and tensile strength measurement is theoretical analyzed. Then, the design of the microfluidic system and the experimental setup are illustrated. Subsequently, the experimental results of the measurements of the shock pressure and tensile strength of water and glycerol are presented and discussed. Finally, the experimental results of the effects of repetitive tensile waves, microparticles and surfactant on the cavitation nucleation are presented and discussed.

Chapter 6 concludes the major contributions of this thesis, and follows by the recommendations for future work.

CHAPTER 2

LITERATURE SURVEY

This chapter is divided into four parts. The first part focuses on the techniques being employed to fuse droplets in microfluidic systems. Two categories of methods, the passive and active methods, are discussed and the pros and cons of each fusion method are presented. The second part presents the current techniques employed in cell membrane poration, from bulk system for group of cells to microfluidic systems for single cell. The third part focuses on the techniques being used to measure the tensile strength of water and glycerol, together with the homogeneous and heterogeneous theories. The fourth part presents the development of the microfluidic systems driven by laser-induced cavitation bubbles. All these works lay the foundation for the contributions of this thesis in the development of microfluidic systems for soft material studies, i.e. droplet fusion, cell membrane poration and tensile strength measurement of liquids.

2.1 Development of droplet fusion in microfluidic systems

The fusion process of droplets dispersed in another immiscible liquid medium has been studied for centuries [93]. It plays an important role not only in the basic physical problems such as the size distribution of rain droplets [94], but also in the industrial applications such as printing and sintering processes [95], besides many chemical or biological processes, e.g. in emulsification and extraction. Recent developments in the research of droplet microfluidics provide a further need to study this phenomenon [5, 17, 29, 96-104]. There have been a series of reports demonstrating droplet generation [105-107], fission [108] and mixing [109-110], as well as interrogating [111] and sorting [112]. A basic operation in droplet microfluidic systems is the merging or fusion of droplets. The numerous techniques developed so far [34, 113-126] can be divided into two categories: passive and active droplet fusion techniques. In this section, all these techniques are reviewed and discussed, together with their pros and cons.

2.1.1 Passive droplet fusion

Several passive techniques for droplet fusion using microfluidic systems have been developed based on channel geometries, partial surface modification and direct injection using side channels. Several different channel geometry designs have been implemented to fuse droplets, such as sudden expansion with rectangular shape [113-114], disk shape [115], tapered expansion [116] and fluid resistance elements [117]. Rectangular sudden expansion structure in a microfluidic channel removes fluids from the expansion channel part at equal volumes through the top and bottom channels [113-114]. The drained volume between two droplets can be controlled by adjusting the flow rates, which supports to control the number of droplets to be fused. Controlled chemical reaction in a droplet microreactor is realized using the disk-shape expansion microchannel design [115]. The volumetric ratio between the continuous phase and dispersed phase is controlled by adjusting the flow rate ratio before their contact. The synthesis of the cadmium sulfide (CdS) nanoparticles has also been demonstrated in microchannels with a tapered shape expansion design by fusing two droplets with different reagent solutions [116]. Fluid resistance elements have also been employed to fuse droplets [117]. As one functional element, the fluid resistor is combined with injectors, segment splitters and elements for transformation to form a complex fluidic micro reactor. In general, those geometric methods make use of the same basic mechanism: the expanded portion in the microchannel thins the continuous phase, i.e. drains the film between droplets, through decreasing the droplets flow rate, while the film is ruptured downstream in the contracting channel.

The second passive technique applies surface modification to change the surface energy [118], e.g. using a partly hydrophilic microchannel to trap and fuse droplets. A pair of droplets with distilled water and a dye solution is demonstrated to be trapped by the hydrophilic part of the microchannel, and then they are fused and mixed. The fusion process time is in the order of tenths of milliseconds to milliseconds. In high flow rate, the droplets will directly flow through the hydrophilic stripes without the occurrence of droplet fusion. The detachment of the fused droplet or the fusion of more droplets can be controlled by adjusting the flow rate of the continuous phase fluid.

The third passive technique uses side channels to directly inject additional reagents into the target droplet [119]. Different reagents are pushed into the passed-by droplets through a set of three side channels. Using the side channel method, it reduces the probability of cross-contamination and prevents the generation of small droplets of additional reagents. The side channel method has also been used to inject a third immiscible liquid as spacers between droplets to prevent droplets fusion [120].

Passive droplet fusion has several unique advantages, such as simple design, non-active control and low contamination probability. However, the mechanism of the passive fusion limits the fusion rate at a low level. And all passive fusion methods are not suitable for on-demand droplet fusion. Any droplet flowing through the functional microchannel will be fused. Active fusion method can overcome the limitation and realize the on-demand droplet fusion.

2.1.2 Active droplet fusion

Several active techniques for droplet fusion using microfluidic systems have been developed and demonstrated using electrical [111, 121-125], heat-based [117] and optical methods [126]. For electrical methods, electrodes are usually integrated with microchannels to induce electrical forces on the target droplets, which lead to an imbalance of the interfacial stress forcing the fusion of adjacent droplets. With parallel electrodes oriented perpendicular to the droplet flow direction, the electric field (500 kV/m, AC voltage) parallel to the flow direction is generated to induce the force to deform two adjacent droplets and the electrocoalescence [111]. The measurement of an enzyme kinetic constant has been demonstrated using this method with high throughput at rates of thousands droplet per second coalescence.

The fusion of tightly packed droplets has been demonstrated using a low voltage potential pulse [121]. A pair of gold electrodes is fabricated perpendicular to the microchannel. When a potential pulse with 50~100 ms and 1~3 V DC voltage is applied, the pair of droplets between the two electrodes coalesce in 100 μ s or less. After the optimization of the electrode geometry and the applied potential, the rupture of the single lamellae within highly ordered droplet arrangements has been demonstrated. Droplets and cell electrofusion are also demonstrated using continuous DC voltage [122]. The electrodes are fabricated parallel to the microchannel with the narrowest section of 200 μ m and the generated electrical field is 120 kV/m. When a pair of linked cells based on biotin-streptavidin interaction flows through the microchannel with geometry variation, the electrofusion is conducted. The geometry of the microchannel effectively avoids the fusion of more than three cells.

Dielectrophoresis (DEP) has also been applied to fuse droplets [123]. The liquid medium of droplets is dielectrically distinct from the continuous phase liquid [123]. Under non-uniform electric stresses induced by the electrodes integrated at the bottom of a microchannel, droplets are deformed and stretched. Any two droplets within sufficiently close distance will coalesce. In another approach, a droplet is driven by turning on and off of a series of electrodes sequentially to move toward another droplet and start to coalesce [34]. The aqueous droplet fusion in immiscible and low-permittivity continuous phase is demonstrated by both moving droplets with an array of two-dimensional electrodes and the dielectrically-activated injection of droplets.

Droplet fusion has also been demonstrated by the combination of the sudden expanded channel design and the aligned parallel electrodes. Using the sudden expanded channel design, the stabilized surfactant droplets are moved to together and the fusion process is activated by the pulsed electrical field [124]. Droplet fusions for different sizes from nano-liter to pico-liter are realized and the start of the fusion can be precisely controlled and determined. Electrical methods have also been applied in the fusion of more stabilized vesicles such as cells and liposomes [125]. Using an array of asymmetric electrodes with the electric field intensity approximately 6 kV/cm, the fusions of a large fraction of liposomes are observed. When DC pulses are applied to the vesicles, small pores are formed on the membrane of the liposomes vesicles. When two vesicles are close to each other, the two porous membranes will be rearranged and fuse together.

The second active method uses heating elements combined with a microfluidic resistance element [117]. The basic principle is based on that the drainage rate as the function of the fluid temperature. The increasing temperature

induces the decrease of the viscosity due to the thermodynamic property of liquids. Based on this mechanism, the drainage rate of the continuous phase liquid can be dynamically controlled by adjusting the temperature. Incorporating with a microfluidic resistance element, the lower viscosity liquid is allowed to flow with the activation of the heating elements. The drainage of the continuous liquid slows the movement of the droplets and triggers their fusion.

The third active method uses optical tweezers in which on-demand droplets are fused by direct optical manipulation [126]. Lower refractive index droplets are generated using the microfluidic platform in the immiscible continuous phase. Optical vertex traps are used to manipulate and transport the generated droplets. Through shrinking the droplet volume and increasing its refractive index, droplets can be trapped by the optical tweezers and the facile fusion is demonstrated. However, due to the low forces caused by the optical tweezers, this method is only suitable in a stationary liquid or at very low flow rates.

2.2 Development of cell membrane poration

Permeabilization of cell membrane is a widely used biological method to transport large molecules into cell cytoplasm, such as gene transfection. Several methods have been developed and can be classified into three categories, including biological, chemical and physical methods. The typical biological methods are the retroviral and adenoviral systems. Retroviral systems are suitable for many different cell types and the long-term gene expression is possible [127-128]. However, the limitation of delivering genes to dividing cells makes it only efficient *ex vivo* but inefficient *in vivo* [129]. Adenoviral systems can be used for both dividing and non-dividing cells [130]. However, this method may trigger an immune response [131-132].

Chemical method applies chemical stimuli to porate cell membranes with high transfection efficiency [133-135]. For example, using small unilamellar liposomes containing a cationic lipid, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) interacting with DNA, lipid-DNA complexes are formed and DNA is uptake and expressed with the facilitation of the fusion between the complex and cells plasma membranes [133]. However, the toxicity of the chemicals and the specific uptake with different cell types limit its applications. Moreover, the spatial or temporal control of the method is difficult to realize.

Physical methods include microinjection, electroporation, sonoporation and opto(photo)poration. Microinjection uses glass capillary micropipettes to directly penetrate cell membranes [136-139]. This method allows highly efficient injection of different drugs into different cells with accurate dosage. It is also easy to carry

out single cell membrane poration. However, the main limitation of microinjection is the size of the micropipette tip, which typically in a few hundred nanometers. Materials larger than the tip cannot be injected into or withdrawn from cell due to clogging or damages induced by shear forces. The requirement for skilled technical personals and low throughput also limit its application.

Electroporation applies an external and pulsed electrical field to porate cell membranes [140-141]. Widely used electroporation devices have been miniaturized into microfluidic systems to enhance the process control for single-cell membrane poration [52, 142-145]. One example is a developed polymeric chip for immobilizing and locally electroporating single cells [52]. The designed chip can focus the electrical field and allows parallel single cells electroporation. The electroporation of HeLa cells is demonstrated using low voltages (< 1 V) and the membrane poration is probed using the uptake of the calcein AM or trypan blue. Electroporation is effective for almost all cell types with high efficiency. Some of the drawbacks of this method, including unpredictable and uncontrollable size, number and locations of membrane pores, have been overcome by the recent developed nano-electroporation (NEP) [146], which can precisely control the dosage of biomolecules into a cell. However, a relatively complex fabrication process is needed due to the integration of electrodes for a micro/nano-electroporation device [52, 142-147].

Sonoporation employs ultrasonic cavitation microbubbles to increase the transient permeability of plasma membrane and enhance the introduction of foreign macromolecules or DNA into cells [53, 148-155]. The employed ultrasound can be focused on the target cells or tissues to perform a localized therapy and enhance the uptake of the molecules with minimal invasion [154-155]. The membrane poration

of 3T3 mouse cell is demonstrated by applying ultrasound [53]. Under various acoustic energy dosages and frequencies, the creation and oscillation of gas bubbles may induce cell membrane permeabilization. However, the locations of the induced pores are randomly distributed on the cell membrane, which is similar to electroporation. In addition, a combination of electroporation and sonoporation has been demonstrated to yield higher efficiency [153-154].

Opto(Photo)poration utilizes direct laser irradiation to create pores on cell membranes at the laser focus point [54, 155-158]. Both continuous wave [54, 155] and pulsed including nanosecond [156-157] and femtosecond [158-159] lasers have been applied in this method. The laser beam is usually focused on cell outer membrane through a high numerical aperture objective lens. The laser beam modifies the membrane permeability and creates transient pores to allow foreign DNA or molecules to enter the target cell. For instance, the plasma membrane poration of a Chinese hamster ovary cell is demonstrated [54]. After the irradiation of an argon ion laser (488 nm), small circular dark spots on the cell membrane appear immediately and then disappear within 5 minutes. The phenol red dye is used to enhance the laser beam absorption. However, the ultra-high temperature at the focal point may directly damage cells and other biochemicals.

In addition to the above discussed techniques, cell membrane poration can be achieved by using fluidic mechanical forces induced by pulsed laser-induced cavitation bubbles [160-164]. For instance, a single cavitation bubble is generated to porate a group of adherent cells [160]. Both the detachment and poration of adherent cells are observed with different standoff distances. However, for this method, the target cells are randomly distributed and the poration parameters such as pores size and location cannot be precisely controlled.

One more precisely controllable method for single adherent cell membrane poration has been developed using the tandem bubbles induced rapid microjets [161]. Two laser-induced cavitation bubbles are created at a distance of 40 μm with a 4 μs time delay. Two microjets at the time of the first bubble collapsing and the second bubble collapsing are generated due to the interaction between the two cavitation bubbles. Cells membrane poration are tested for different angles and standoff distance and probed by the uptake of trypan blue dye. This method provides a new approach for highly controlled and localized method for single adherent cell poration. In addition, another approach is using the transient shear stress induced by the cavitation bubble, controlled and confined by a micro-pipette with coated metallic nanostructures [162]. A photothermal nanoblade is fabricated using a pulling micropipette coated titanium (Ti) and a magnetron sputter deposition system. When a nanosecond laser pulse is excited, an explosive vapor bubble is generated by the heated Ti coating and a high speed, localized flow is generated since the transient expansion and collapse of the bubble are faster than 1 μs . The delivery of high concentrated cargo is demonstrated and this method provides a new approach for delivering different and difficult cargos into cells.

Nanoparticles served as nucleation centers for laser-induced cavitation bubbles have been demonstrated for adherent cells poration [163]. Cells are seeded on a glass substrate with immobilized gold nanoparticles and routinely cultured. The gold nanoparticles exposed to pulse laser irradiation are heated in nanoseconds and explosive vapor bubbles are generated. The high shear stress induced by the transient expansion and collapse of the bubbles changes the cell membrane permeability temporary for foreign molecules uptake. However, all these three

techniques are applicable only to adherent cells. Suspension cells in any non-confinement environment will flow away due to the bubble-induced flow.

One solution for membrane poration of suspension cells is by using the laser-induced cavitation bubbles in a confined microfluidic device [164]. Membrane porations of suspension HL60 (human promyelocytic leukemia) cells are tested by the releasing out of the calcein or the uptake of trypan blue. A critical interaction distance is defined and this method provides the alternative to other physical poration method. However, the coarsely control of the cell location limits its application in quantitative analysis. A small change in the distance can lead to largely different effects on the bubble dynamics and the cell poration process. Therefore, it is crucial to control the position and size of the cavitation bubble together with the location of the cell in the microfluidic environment for efficient and precise single-cell membrane poration.

2.3 Tensile strength of liquids

Liquids subjected to negative pressure, or under tension, are of interest because of the formation of cavitation in the stressed liquids. The tensile strength of a liquid is the maximum negative pressure that it can withstand. To analyze the cavitation phenomena, tensile strengths of liquids, especially water, need to be estimated under the homogeneous nucleation. The theoretical study of the homogeneous nucleation predicts the tensile strength of water at -140 MPa at 25 °C [165-166] and the spinodal breakdown theory suggests it at -200 MPa at 35 °C [167-168]. Several methods have developed to detect the tensile strength of water, including Berthelot tube method [169-172], mineral inclusion method [173-175], centrifugation method [176-177], acoustic method [178-183] and shock wave reflection method [184-190]. The tensile strength of glycerol [191-194] and other viscous liquids [195-197] are also measured using several different methods. The homogeneous [198-213] and heterogeneous nucleation [214-227] models are also discussed in this section.

2.3.1 Tensile strength of water

2.3.1.1 Berthelot tube techniques

The technique to measure the tensile strength of water is firstly developed under static conditions [169]. A sealed cylindrical glass tube is almost completely filled with water. Water expands to fully fill the tube under heating and then is allowed to cool. Water breaks under the increased tension due to the competing between the adhesion between water and the wall of the tube and the contracting of the cooling water. Once water breaks, the release of the tension induces water volume to increase. The tension is estimated approximately -5MPa based on the volume increased ratio. A series of techniques have been refined based on the Berthelot's original work [170-172]. One refined approach is attaching a pressure transducer to the Berthelot tube to directly measure the pressure and tension changes [170]. Another approach is demonstrated using the coil shape tube and a distance meter to overcome two difficulties in the conventional technique: the determination of the true temperature and the influence of the glass tube on the tension [171]. The third improved method uses a helix glass capillary to monitor the pressure change in the stressed liquid [172]. These techniques allow for the maximum tension of -10 MPa.

2.3.1.2 Mineral inclusions

Another method with the similar principle of the Berthelot tube method is the mineral inclusions method [173-175]. Water with a desired density is trapped in synthetic inclusions with fractured crystals, such as quartz, calcite and fluorite, under different temperatures and pressures [173]. To detect the tensile strength, the inclusions are studied following the Berthelot's method: water in the inclusions is

cooled down in an isochoric process until water is stretched. The tensile strength of water based on this technique is estimated at -140 MPa at 40 °C. In another work on inclusions, Brillouin scattering method is used to measure the sound speed in the stretched liquid medium and the results indicate the tension strength of water beyond -100 MPa at 20 °C [174]. The tensile strength of water can also be detected from the kinetic aspects by measuring one inclusion's statistical lifetimes (following a Poisson distribution) at fixed temperatures [175], and the measured value is -16.7 MPa at 258.3 °C.

2.3.1.3 Centrifugation

Another group of static techniques are the centrifugation methods [176-177]. Reynolds first employs this method [176]. Using this method, the testing liquid is contained in a Z-shaped horizontally mounted glass capillary with two open ends, which is spun with the angular velocity ω . A pressure field with the gradient of $\rho\omega^2r$ is formed in the outward direction. At the two ends of the tube, the pressure is one atmosphere and it decreases with approaching the axis through the tube center. With the increase of the ω , the pressure becomes negative and eventually the liquid at the center ruptures first when the ω is sufficiently large. The tensile strength of water in a Pyrex glass capillary is measured under a centrifugal field to be approximately -28 MPa at 10 °C and -22 MPa at 50 °C [177].

2.3.1.4 Acoustic cavitation

Negative pressure can be generated by an acoustic wave at its negative swing [178-183], and water can be quenched under the induced tension. The tensile strength of water at different temperatures has been studied using both standing wave [178-179] and high amplitude bursts [180-183]. Using the standing wave, a spherical resonator is generated in water and the amplitude is measured using a

microphone [178]. The measured tensile strength for air-saturated distilled water is -0.1 MPa and that for degassed distilled water is -20 MPa. Another study using standing wave in a stainless steel cylinder gives out -16 MPa for clean and degassed water with an average several minutes of waiting time [179]. The focused high frequency ultrasound has also been used to study the tensile strength of water [180-183]. The threshold is defined based on the highly reproducible statistical cavitation measurements, and it monotonically increases from -26 MPa to -17 MPa when the temperature increases from 0.1 °C to 80 °C [180]. This is one of the most negative values among acoustic measurements.

2.3.1.5 Reflected shock wave

Another group of methods to measure the tensile strength of water are using a tension wave, which is generated when a compressional wave is reflected by a suitable boundary. One classic example is the bullet-piston (B-P) method [184-188]. For instance, the tensile strength of water is measured using a modified B-P experimental setup for the pulse reflection [188]. The B-P experimental setup consists of a vertical tube and a piston at the end of the tube. When a bullet strikes the piston, a pressure pulse is generated and travels up the liquid column in the tube. When it reaches the free surface, it is reflected as tension, and several dynamic pressure transducers are used to monitor the pressure variation.

The underwater explosion method is also based on the reflection of a shock wave in liquid by the free interface [189-190]. An explosive charge below the liquid water surface is detonated. Using high-speed motion photography, the displacement of the spray dome above the free surface is measured and its initial velocity can be calculated. The shock peak pressure can be estimated from the particle velocity using the *Rankine-Hugoniot* equations. By extrapolating the graph of the initial

velocity vs. the depths to the initial velocity equals to zero, the tensile strength can be estimated as the double of the shock peak pressure. The measured tensile strengthes of ordinary water and glycerol are the same of -0.8 MPa using this method [190]. The possible reason is that liquid samples are bulk and heterogeneous nucleation dominates in the measurements.

However, most of the experimental measured values except that based on the mineral inclusions method are much lower than the theoretical predictions. The possible reason is that the impurities or cavities from the container surface induce the heterogeneous nucleation, which causes a significant decrease of the tensile strength.

2.3.2 Tensile strength of viscous liquids

2.3.2.1 Tensile strength of glycerol

Glycerol is one of the most common high viscous liquids. Its tensile strength has been studied for several decades. The first measurement of glycerol tensile strength is -6.3 MPa at the room temperature, using the modified B-P method with an electrical pressure bar in 1956 [190]. Then, it is measured as -25 MPa at the room temperature, using the method of the partial reflection of shock at a glycerol/octane interface with the extremely high stress rate in 1971 [191]. Later, it is measured as -0.8 MPa at the room temperature, using the under-water explosion method in 1975 [192].

A method using the interferometry is also developed to measure the tensile strength of glycerol [193-194]. The pulsed electron beam deposition is used to generate stress in a solid target and the velocity interferometry is applied to measure the interaction between the stress wave and the glycerol-solid interface. The tension wave propagates from the solid to the liquid glycerol with a slight effect due to the

Table 2.1. Comparison of different tensile strength measurement for Glycerol.

Method	TS (PMa)	Interface	Ref
Modified Bullet-Piston	-6.3	Air-Glycerol	190
Shock reflected (Transducer)	-25	Octane-Glycerol	191
Underwater explosion	-0.8	Air-Glycerol	192
Electron beam deposition & velocity interferometry	-60/-81	Mylar Stretcher-Glycerol	194/193

acoustic impedance matching between the solid and liquid glycerol. The tensile failure occurs in the liquid glycerol under the tension wave with sufficient magnitude. A velocity interferometer is used to monitor the dynamic response of the aluminized Mylar film and the tensile strength can be determined by using the velocity data. The tensile strength of glycerol is measured as -60 MPa at the room temperature [194]. The tensile strength of glycerol at the room temperature (20 °C) is measured as -81 MPa by interpolating the values between 220 ~ 350 K [193]. All the measured values of the tensile strength of glycerol are listed in Table 2.1, ranging from -0.8 MPa to -80 MPa. Except the effect of impurities in glycerol samples, this large range can be accounted for glycerol's high temperature-sensitivity around the room temperature and the fact that different tensile strength rate are employed in different measurements.

2.3.2.2 Tensile strength of viscous liquids

The tensile strength of a liquid is affected by many fundamental physical properties, such as viscosity and surface tension. Several studies have been conducted to investigate the relation between the tensile strength TS and the viscosity μ [188, 193-197]. All those results can be expressed using the empirical equation $TS = k\mu^n$, where k is a constant and the value of the exponent n is variable for different liquids investigated using different methods. A group of fluids, consisting of water, olive oil, glycerol and syrup, with different viscosities are studied using the modified B-P method [195] and the exponent n is estimated as 0.2. Glycerol with different viscosities at different temperatures is investigated [194] since glycerol is a highly temperature-sensitive liquid when the temperature is close to the room temperature. Using the velocity interferometry method, the exponent n is estimated as 0.3 for glycerol viscosities from 0.02 to 19 Pa·s. Several groups of

oils with different viscosities are also studied using modified B-P method [188, 196-197]. The exponent n for two groups of silicone oils (Dow Corning 200) with viscosities from 1, 50, 100 to 350 mPa•s [188, 197] and from 1, 10, 100 to 1000 [196] are estimated to be approximately 0.1. The petrol oils, 10w-40 diesel oils and 50w-30 diesel oils with varied viscosities at different temperatures are investigated and the exponent n is estimated as 0.43, 0.34 and 0.22, respectively [197].

One possible reason to explain how viscosity affects the tensile strength is that the viscosity suppresses (retards) the initial growth of cavitation bubble [195]. However, another explanation is that the relationship between the tensile strength and viscosity is due to the effects on the relaxation time for homogeneous nucleation [194]. The non-equilibrium expansion effects might only influence the number density of nuclei. However, until now, no experimental photographs directly show the difference of the homogeneous nucleation for different viscosities liquids. The use of a large volume of liquids in those experimental studies easily introduces heterogeneous nucleation, which causes a remarkable decrease of the tensile strength.

Table 2.2. Comparison of tensile strength measurements for viscous liquids.

Liquids	Method	Exponent n	Ref
Water, olive oil, glycerol and syrup	Modified Bullet-Piston	0.2	188
Glycerol at different temperature with different viscosities	Electron beam deposition & velocity interferometry	0.3	194
Silicone oils (1, 50, 100 to 350 mPs•s) (1, 10, 100 to 1000 mPs•s)	Modified Bullet-Piston	0.1	196
Petro oils	Modified Bullet-Piston	0.43	197
10w-40 diesel oils	Modified Bullet-Piston	0.34	197
50w-30 diesel oils	Modified Bullet-Piston	0.22	197

2.3.3 Cavitation nucleation

2.3.3.1 Homogeneous nucleation

Most of the measured values of the tensile strength of water are much lower than the theoretical predictions as discussed in the section 2.3.1. This phenomenon can be explained by the preexisting of the cavitation nuclei in water. Based on the homogeneous nucleation model [165, 198-201], there are tiny gas or vapor nuclei in the liquid water due to the random distribution of thermal energy. However, such gas or vapor bubbles are unstable, which are continuously forming and disappearing due to the statistical fluctuations and cannot act as cavitation nuclei without any stabilization mechanism.

One stabilization model is proposed by Fox and Herzfeld [202]. In this model, very small spherical gas bubbles are stabilized by a skin consisting of organic molecules, which mechanically prevents the diffusion-induced loss of gas and so the chemical purity is considered as one key factor to determine the cavitation threshold of liquid samples. However, it is not supported by the later experimental results on the measurement of the cavitation threshold of distilled and tap water [203-204]. The experiments show that there are no difference of the cavitation threshold between the distilled and tap water. Later, a more successful variable permeable model is proposed [205-206]. In this model, an elastic impermeable skin consisting of surface-active molecules stabilizes the nuclei in liquids. This model is first used to explain the formation of cavitation bubbles in gelatin, but it can also be applied to water and solutions with surfactants as well.

Surfactants reduce the surface tension and prevent the mass transport at a liquid-gas interface [207-209]. These two mechanisms increase the stability of

nuclei against dissolution [210]. The effects of SDS surfactant on the inertial cavitation activity are experimentally studied [211]. It is shown that the SDS strongly affects the sonoluminescence and inertial cavitation. Another numerical study based on the molecular dynamics simulation has shown that the shape of the nanobubble is reorganized by the position of the head group (-OH) of the surfactant (1-Heptanol) [212-213].

2.3.3.2 Heterogeneous nucleation

Another stabilization model in the heterogeneous nucleation is the crevice model [179, 213, 214-217]. It is proposed that tiny gas bubbles are trapped in the hydrophobic conical cracks and crevices on surface of the solid particles or motes [218-219]. The earliest proof can be dated back to the observation that the enhancement of the cleanliness of the surface increases the possibility of the liquid to wet the solid surface [214]. This model has been considered in the experimental measurement of the cavitation threshold [203]. The measured tensile strength of water is approximately -20 MPa with the removal of the particles with a diameter larger than 200 nanometers [179]. This result supports the model that the nuclei, which induce cavitation, are highly relevant with the solid particles suspended in plain water. Another research of the acoustic cavitation in the distilled water also supports the model [216].

Several theoretical studies have also been conducted based on the Harvey's original model [218-219]. The effects of the pre-pressurization in the liquid samples [203] and the size and shape of the motes are also theoretically considered [215]. For small and wetted motes, the surface tension and the size of motes affect the cavitation nucleation, which is independent of the gas content and its history. However, for big and imperfectly wetted motes, the conclusion is the contrary.

Subsequently, a modified Harvey-type model is proposed that the interfacial voids are formed at locations with the concave curvature [220].

Recently, a series of researches on effects of size, shape and concentration of the micro-particles on the cavitation nuclei have been carried out [221-225]. The shape of the natural particles usually is highly random and the local curvature on the surface of these particles has a large range [216]. The effects of the mono-sized particles with different diameters and wettability on the tensile strength of water are studied [221]. The random shaped natural particles with a diameter larger than 1 μm are removed before seeding the spherical solid particles with diameters of 3 μm , 20 μm , 30 μm and 76 μm , respectively. The 3- μm particles, both hydrophobic and hydrophilic, do not influence the measurement of the tensile strength. The larger particles, both hydrophobic and hydrophilic, obviously decrease the measured value of the tensile strength. Those experimental results support the model that the existence of cavitation nuclei on the surface of the particles.

One further experimental research investigates the effects of the surface structure and the chemical component [224]. The particles with the hydrophobic and corrugated surface enhance the cavitation, whereas the particles with the hydrophilic and smooth surface do not facilitate the cavitation. For the consecutive experiments, the number of cavitation bubbles decrease exponentially, which suggests that the number of nucleation sites on the surface of particles is limited. The dynamics of the accelerated microparticles after the cavitation inception at the particle surfaces is further studied [223-224]. For the corrugated and hydrophilic particles with diameters ranging from 30 to 150 μm , cavitation cavity is induced on the surface of the particles, which are exposed to a strong tensile stress wave. The particle is accelerated by the growing cavitation cavity in the directions opposite to

the cavity growth. Eventually, the particle detaches from the cavitation cavity when the tensile stress decreases and the expansion of the cavity slows down. The dynamics is studied using the model to consider the momentum of the particle and the liquid medium, and it is also studied and simulated using the boundary element method for further understanding. The effects of artificial cavitation nuclei with different concentrations on the dynamics of the cavitation cluster are studied as well [225]. The phospholipid shelled microbubbles with a mean diameter of 2.5 μm are used as the cavitation nuclei. With a higher concentration of the artificial cavitation nuclei, the life time of the cavitation cluster is longer. The extent of the cavitation cluster enlarges with an increase of the concentration.

The tensile strength is also highly affected by the experimental conditions. Under non-controlled lab conditions, the measurement is highly difficult to reproduce and the measured results are highly scattered for a large number of measurements under the same experimental conditions [203]. The system cleanliness is an important factor, which affects the measurement results of cavitation threshold [226-227]. Paying attention to the details of the preparation and handling of the liquid samples, and the setting up of the environment, helps to reproduce the experiments.

2.4 Laser-induced cavitation bubble driven microfluidic system

When a high power pulsed laser beam is highly focused into a liquid medium, a larger energy injection into the liquid at the focal point happens and a strong optical field is created to breakdown the liquid molecules [228-230]. Extremely high temperature (6000~15000 K) ionized hot plasma is generated near the focal point [231], and the hot plasma recombines in few nanoseconds which releases energy for instant heating the liquid medium. This heating produces an explosive vapor bubble with a high expanding speed exceeding 100 m/s and an initial internal pressure up to hundreds of MPa or even higher [232]. After the vapor bubble grows to its maximum, it will collapse. Both the theoretical and experimental estimations show that about 15% of the total injected energy is dissipated by the initial pressure emission, less than 5% energy is transformed to bubble and approximately 80% is transferred to the heating process [233]. Using the pulsed laser to generate cavitation bubbles has several unique properties, including exact positions of bubbles, bubble size control by adjusting the laser pulses energy [234] and rapid energy conversion into mechanical power with large force and large displacement [88]. Recently, wide varieties of micro- and nanofluidic systems driven by pulsed laser-induced cavitation bubbles are proposed and demonstrated [88-92, 235-241].

Bubble expansion has been utilized to drive several different microfluidic systems. One approach is the high speed microfluidic switch [88], which makes use of the rapid expansion of the bubble with a speed faster than 100 m/s. The PDMS

microchannel wall is deformed by the bubble expansion to form a high speed switch with switching time less than 10 μ s. Based on this rapid switching, a fluorescent activated cell sorting microfluidic system is developed [89]. When a fluorescent particle is flowing through the detection area, a laser pulse is triggered to create the cavitation bubble and the expansion of the bubble deflects the fluid and the cells. Another approach is droplet manipulation in microfluidic systems. The high throughput on-demand droplet generation has been realized using the bubble expansion [90]. For a stable oil-water surface in a nozzle structure, the rapid expansion of the bubble can push water into oil phase to generate water droplets. Up to 10 000 droplets per second can be generated and the volume from 1 pL to 150 pL can be continuously tuned by controlling the laser energy.

Two micro-pumps driven by the bubble expansion have been developed [88-89]. First, in the peristaltic membrane pump [91], fluid sample in one microchannel is driven by the deformation of the peristaltic membrane, which is induced by the expansion of the cavitation bubble. This pump eliminates any contamination potential since the driven source is separated from the pumped fluid. The other approach is to generate cavitation bubbles next to a nozzle structure [92]. The bubble expansion pushes out liquid in the chamber into the nozzle structure. The interaction between the boundary and the cavitation bubble induced a jetting flow may also contribute to the liquid pumping.

The mixing of two fluids and initiating chemical reactions within a laminar flow has implemented using laser-induce cavitation bubbles [235]. The expansion and collapse of the cavitation bubble disrupt the laminar flow with two parallel flow streams and introduce a localized mixing region. The experimental results show that the mixing is initialized by the bubble collapse-induced jet and the whole mixing

process is in millisecond time scale. This method provides a fast and localized on-demand mixing solution for laminar flow with multiple streams without any specialized microchannel geometries or on-chip integrated instrumentations. The displacement of particles is also realized using laser-induced tandem bubbles [236]. Two laser-induced cavitation bubbles are created with 4 μs delay using an infrared (wavelength 1064 nm) and a green (wavelength 532 nm) laser pulses. Two directional microjets and a pair of vortices are generated due to the asymmetrical collapse of tandem bubble, which can drive single polystyrene particles with a maximum velocity of 1.4 m/s in 30 μs in a microfluidic channel. This method may have high potential for the high-throughput cell sorting in microfluidic systems.

The pulsed laser induced-cavitation bubbles have also been used in cell physics studies, such as cell lysis [229, 237-238], collection and separation of adherent cells [239] and red blood cell stretching [240]. A laser-induced cavitation bubble is created above confluent monolayer cells. When the transient shear stress induced by the expansion of the laser-induced cavitation bubble is greater than the critical value, cells within the stress field are lysed [232]. This critical shear stress is varied from the cell surface density and independent of the pulse energy. The lysis of non-adherent BAF-3 cells is also demonstrated using laser-induced cavitation bubbles in a microchannel [237]. Time-resolved fluorescent photographs show the lysis occurs on the sub-microsecond time scale. Another approach for cell lysis is using microbubbles generated around gold nanoparticles [238]. When a pulsed laser is focused on the nanoparticles as nucleation centers, vapor bubbles are generated. The target cells (> 99%) are directly killed by bubbles but surrounding normal cells are alive.

The mechanism of the collection and separation of adherent cells are investigated and demonstrated by using the micropallets release with laser-induced cavitation bubbles [239]. SU-8 photoresist micropallets are fabricated on glass coverslips. A pulsed laser beam is focused at the interface through a high numerical aperture. The subsequent optical breakdown and bubble formation produce the micropallets detachments. The viscoelastic properties of red blood cells (RBCs) are also investigated using laser-induced cavitation bubbles [240]. A strong shear flow in an ultrashort time (smaller than 40 μ s) is created in a microfluidic chamber during the expansion-collapse cycle of a laser-induced cavitation bubble. The micrographs of the stretch and recovery of the red blood cells (RBCs) within a radius of 600 μ m from the bubble center are recorded using a high speed camera. This study indicates that wheat germ agglutinin treated RBCs are stiffer compared with normal RBCs and the neuraminidase treatment reduces the deformability of the RBCs since the overall surface charge is affected.

The homogeneous nucleation in water and ethanol is also demonstrated using laser-induced cavitation bubbles [241]. In a partially filled microchannel, a cavitation bubble is created by focusing a pulsed laser beam in the liquid medium away from the free (air-liquid) surface at a standoff distance. The laser-induced shock wave is reflected by the free surface as a tension wave. The nucleation images have been captured and the rupture of water has been observed with very strong tensile waves. Combination with the Euler flow simulations, the tensile strength of DI water has been estimated to be -60 ± 5 MPa, which is significantly higher than earlier researches. In this method, the ultra-small volume and the ultra-short time under a tension significantly reduce the probability of heterogeneous nucleation. However, the shock peak pressure estimation depends on the numerical

simulation. It is only suitable for liquids with low viscosity because the viscous effects can be neglected in the simulation. The simulation for high viscous liquids is extremely difficult and the tensile strength of high viscous liquids cannot be estimated using this method.

2.5 Summary

Based on the literature survey, several methods for droplet fusion, cell membrane poration and tensile strength measurement of water and glycerol as well as the effects of particles and surfactants in microfluidic systems have been presented. However, new methods need to be developed to overcome the limitations of the existing methods. Cavitation bubble driven microfluidic systems have many unique advantages as compared to the microfluidic systems driven by other methods, such as rapid energy absorption from nanoseconds to femtoseconds based on the duration of single laser pulse, fast energy conversion into mechanical power and large force and displacement. With the development of pulsed laser-induced cavitation bubble driven microfluidic systems, it is capable to develop microfluidic systems for droplet fusion, single-cell membrane poration, tensile strength measurement of water and glycerol and effects of repetitive tensile waves, microparticles and surfactants on cavitation nucleation.

CHAPTER 3

DROPLET FUSION

This chapter presents the design, fabrication and experiment of the microfluidic system for cavitation bubble based droplet fusion. The theoretical analyses are presented, including the mechanism of the droplet fusion using laser-induced cavitation bubbles, the “neck” growth theory between two fusing droplets and the simplified model for droplet generation. Subsequently, the microfluidic design using a T-junction for droplet generation is discussed, and the fabrication processes and experimental setup are presented for cavitation bubble generation and high-speed imaging. Then, the experimental results of droplet fusions under different conditions are presented and discussed, including the development of a single bubble inside a single droplet, the fusion of static and moving droplets, the fusion of droplets of equal and unequal sizes and the fusion of multiple droplets in a hexagonal structure. Finally, the diffusion dominated mixing between two fusing droplets and the transport of a single encapsulated cell from one droplet to the other droplet are presented and discussed.

3.1 Theoretical analysis of droplet fusion

This section presents the theoretical analyses of the two equal-sized droplets fusion, the “neck” growth theory between the two fusing droplets and the generation of droplets using a microfluidic chip with a T-junction.

3.1.1 Fusion mechanism

In general, the fusion process of two droplets can be split into three consecutive steps [242-250]. The first step is the film drainage of the continuous phase, and the second step is film rupture. The third step is the growth of the connecting neck between the two fusing droplets. In this chapter, a rapid thinning of the continuous phase film during the bubble growth is achieved by creating a cavitation bubble inside one droplet, which is in proximity to a second droplet. The rupturing of the film is observed during the shrinkage of the bubble, while the growth of the connecting neck is occurring on a much longer timescale after the bubble has vanished.

These three stages of laser-induced droplet fusion are illustrated from left to

Figure 3.1: Schematic mechanism of two equal-sized droplets fusion using a laser-induced cavitation bubble.

right in Fig 3.1. First, a laser-induced cavitation bubble is created inside one of the two droplets (droplet A) (Fig. 1(b)). The cavitation bubble center is focused to the center of the droplet (droplet A). The expanding bubble contains vapor of the dispersed phase, which “blows up” and pushes droplet A into the neighboring droplet B. The expansion of droplet A is not isotropic but more pronounced towards the lower droplet B. This can be explained due to the higher viscosity of the oil leading to a higher normal stress difference sideways across the interface. In contrast, the effective viscosity of the fluid in droplet B is smaller. Therefore, the expanding droplet A primarily deform towards droplet B. Thus, droplet B deforms into a heart shape (Fig. 1(b)). The initial continuous film is thinned rapidly by the impulsive force of the expanding bubble. After the bubble reaches its maximum volume, the pressure imbalance reverses its motion and the bubble starts to contract and finally collapse. Thereby, the thin film between the two droplets is pulled back towards droplet A (Fig. 1(c)). It is speculated that this reversal of motion, i.e. the acceleration, induces the film rupture. Thereafter, a neck connecting the two droplets becomes visible and the two droplets fuse on a much slower timescale into a large droplet (Fig. 1 (d–f)). Due to the interfacial tension, the surface energy between the two liquids is minimized, which is achieved through an increase of the diameter of the neck. A single convex shaped droplet is formed as shown in Fig. 1(f) and eventually becomes spherical with a minimal surface energy.

3.1.2 “Neck” growth theory

In droplet fusion process, when two droplets approach, the thin film of the continuous phase between these two droplets drains away under the action of the applied initially gravitational or inertial force [242, 244]. The drainage rate may be reduced by the electrostatic repulsive forces with the thinning of the film. When the film is very thin (i.e. below a certain droplet-droplet separation), the drainage rate becomes extremely slow. London-van der Waals attractive forces cause the film to rupture, while a spontaneous connection occurs, which is mediated by the capillary waves and faster than further drainage, such that the fusion between these two droplets occurs [243, 248].

Following the rupture of the film, the fusion begins with the formation and growth of the neck between the pair of droplets [245-246]. The growth speed of the neck is slowed down due to the competition between the capillary and viscous forces. The capillary force drives the fusion process but the viscous forces slow it down. Formulating these two forces (i.e. normalizing the capillary number to unity) results in a time dependence of the radius of the neck R_t , which is expressed as [240]

$$R_t(t) \propto \frac{\gamma}{\eta} t \quad (3.1)$$

where γ is the surface tension, η is the viscosity and t is the time. The fusion mechanism leads to large speeds for ordinary liquids. The capillary velocity of water is approximately 70 ms^{-1} .

The full theory predicts a logarithmic correction to the above scaling argument [240], and the modified Eq. 3.1 is expressed as

$$R_t(t) = -\frac{\gamma}{\pi\eta} t \ln\left(\frac{\gamma}{R_0\eta} t\right) \quad (3.2)$$

where R_0 is the radius of the underformed droplet at the beginning time. This prediction is valid for viscous droplets in the inviscid surrounding.

The main force governing the fusion dynamics is either the viscous or inertial force, which depends on the ratio of inertial forces to viscous forces, i.e. the Reynolds number Re , which is expressed as

$$Re \sim \rho \gamma R / \eta^2 \quad (3.3)$$

where ρ is the density of the fluid, γ is the capillary velocity as the characteristic speed and R is the radius of the neck as the characteristic size. When $Re > 1$, the inertial fusion occurs. When $Re < 1$, the viscous fusion occurs.

Based on the theoretical analysis, the microfluidic system will be designed and fabricated using an external force to drain the continuous phase between two fusing droplets. The growth process of the connection neck between the two droplets is captured using an ultra-high speed camera. The radius of the neck as a function of time is measured and discussed based on the high speed micrographs.

3.1.3 Droplet generation

Before the fusion process of two droplets, they should be generated first. Several microfluidic systems have been developed to generate droplets, and the two most common methods for generating droplets by injecting aqueous phase into an immiscible carrier fluid are using the T-junction geometry [17, 101, 105, 107, 251-254] and the flow focusing geometry [17, 254]. In this study, the geometry of T-junction is chosen to produce the immiscible fluid segments, i.e. droplets.

In the microfluidic system with T-junction geometry, the microchannel for dispersed phase is perpendicularly connected to the main channel containing the continuous phase. An interface is formed between two phases at the T-junction, and as the fluid flows continue, the tip of the dispersed phase in the microchannel containing the continuous phase. Shear forces and the subsequent pressure gradient generated by the continuous phase cause the elongation of the head of the dispersed phase. Then, the elongated dispersed phase head flows into the continuous phase channel and the thickness of the dispersed phase neck decreases gradually. Eventually, the neck is broken and the stream of dispersed phase is separated to discrete segment.

For predicting the size of the discrete fluid segment, a simple scaling law is expressed as [107]

$$\frac{L}{w} = 1 + \alpha \frac{Q_{in}}{Q_{out}} \quad (3.4)$$

where L is the length of the dispersed phase slug, w is the width of the microfluidic channel, Q_{in} and Q_{out} are the flow rate of the dispersed and continuous fluids, respectively, and α is the geometry constant, which depends on the T-junction geometry.

Capillary number Ca is defined as the ratio of viscous forces to surface tension acting across an interface between two immiscible liquids. It can be expressed as

$$Ca = \mu \frac{u}{\gamma} \quad (3.5)$$

where μ is the viscosity, u is the mean speed of the continuous phase and γ is the interfacial tension. For microfluidic systems, the typical flow rate is between 10^{-2} to $1 \mu\text{L s}^{-1}$ and the characteristic dimension is on the order of $100 \mu\text{m}$. Therefore, the capillary number is typically small ($\sim 10^{-2}$) and the scaling relation (Eq. 3.4) is independent of the material parameters of the fluids: the viscosity and interfacial tension.

3.2 Droplet generation and imaging

This section presents the design and fabrication process of the microfluidic chip for droplet generation and the experimental setup for cavitation bubble generation using the pulsed laser and high speed imaging.

3.2.1 Droplet generation

A microfluidic chip with a T-junction is designed and fabricated to generate water-in-oil droplets as shown in Fig 3.2 [101, 105-107]. The microfluidic chip has two inlets and one outlet. The microchannel for the dispersed phase is perpendicular to the microchannel for the continuous phase to form a T-junction, where the dispersed phase is injected to form water-in-oil droplets. The widths of the channels of the dispersed and continuous phases are 30 and 100 μm , respectively. The main channel expands downstream to 200 μm first, and then expands to 800 μm to

Figure 3.2: Schematic of the microfluidic chip with a T-junction. The channel widths are 30 and 100 μm for the dispersed and continuous phases, respectively. Downstream of the main channel expands from 200 μm to 800 μm as the droplet collection chamber. All channels have the same height of 100 μm .

Figure 3.3: Micrographs of generation process of water-in-oil droplets in a microfluidic chip with a T-junction. The scale bar is 50 μm .

form a droplet collection chamber. All channels have the same height of 100 μm . For the dispersed phase, an aqueous refill ink for inkjet printers (Magenta color, Mextec) is used, with a density 1.046 g cm^{-3} and a viscosity of approximately 2 cSt at 23 $^{\circ}\text{C}$. Immersion oil (Cargille) with 2 wt% surfactant (Span 80, Sigma) is used as the continuous phase with a density 0.923 g cm^{-3} and a viscosity 1250 cSt at 23 $^{\circ}\text{C}$.

The formation process of water-in-oil droplets in a microfluidic chip with a T-junction are shown in Fig 3.3. Two immiscible fluids, including the immersion oil with surfactant and the inkjet printer ink, are pumped into the microfluidic chip from the two inlets, respectively. The immersion oil serves as the continuous phase and the printer ink is the dispersed phase. An interface is formed at the junction of the printer ink and oil channels. The ink penetrates into the oil channel and a series of ink droplets are generated. As shown in Fig. 3.3 (a), when the injected printer ink

arrives at the T-junction, the ink begins to enter the immersion oil channel and the formation of droplets starts. From Fig. 3.3 (b) to (f), the whole oil channel is gradually filled with the swelling of the printer ink. When the ink/oil interface moves to the edge of the ink channel, the neck connecting the droplet with the ink breaks. The formed ink plug flows downstream in the oil channel, while the tip of the ink solution retracts to the end of the inlet, waiting for the generation of the next plug. When the formed plugs flow downstream and encounter a sudden expansion in the oil channel, they become circular droplets under the interfacial tension as shown in Fig. 3.3 (g). The radii of the droplets and the distance between two generated droplets can be adjusted by coordinating the flow rates of two solutions. In Figure 3.3, the flow rates of the ink and the immersion oil are $0.035 \text{ ml min}^{-1}$ and $0.056 \text{ ml min}^{-1}$, respectively

3.2.2 Fabrication process

The microfluidic chip is fabricated using the soft lithography technique and the polymer material is Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) [255-257]. PDMS has many advantages, such as low cost, low temperature curing, excellent elasticity and so on. The transparent property in the visible and near-UV (below 280 nm) optical regions makes PDMS an excellent choice for applications in wavelength visualization and spectroscopy [258]. PDMS is non-toxic and also penetrable for air from the biological point of view. Thus it can be used in cell culture and *in vivo* devices. These features make PDMS suitable for optical and biomedical applications [259].

Figure 3.4: Fabrication process flow of the microfluidic chip using PDMS material.

The microfluidic chip is designed and drawn using a computer-aided design (CAD) program (L-Edit 12.0). A sodalime glass or plastic mask (dark field clear feature) is commercially fabricated based on the CAD drawing (Infinity Inc., Singapore). The resolution is approximately 3 μm for glass mask and 30 μm for plastic mask. The process flow for fabricating the microfluidic chip is shown as in Fig. 3.4. Before the fabrication of the mold, a 6-inch silicon wafer (one side

polished) is cleaned using a piranha clean ($\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$) and rinsed with DI water. A photoresist SU8 (MicroChem) is used for the mold fabrication. For different thicknesses of SU8 photoresists, the detailed parameters can be obtained in the data sheets of SU8 photoresists. For example, a microfluidic chip with a height of 100 μm can be fabricated as follows. A 100- μm thick layer of SU8 photoresist (SU8-50, MicroChem) is spin-coated using a spin-coater (CEE200, Brewer Science) onto the 6-inch silicon wafer. After 10-minute pre-bake at 65 °C and 30-minute soft-bake at 95 °C using a hot plate, the photoresist is then exposed to a UV light for 19 s using a mask aligner (506, OAI). Then, 1 minute at 65 °C and 10-minute at 95 °C post exposure bake are applied. Afterwards the mold is developed for 6 minutes using the SU8 developer and hard-baked for 10 minutes at 120 °C.

After the mold is fabricated, a degassed mixture of PDMS base and curing agent (10:1) is poured over the mold. After degassed for 30 minutes and baked for 2 hours at 75 °C, the PDMS layer is polymerized and peeled off from the mold. The inlet and outlet holes are punched manually using a Harris unicore sampling punch (0.75 mm). Then, the PDMS device is exposed to air plasma for 15 s using a corona treater (BD-25, Electro-Technic Products) to bond it to a PDMS coated glass substrate and baked overnight at 75 °C.

After the PDMS base and curing agent are mixed, the cross-linking or curing process starts. The base is made of dimethylvinyl-terminated dimethyl siloxane (CAS: 68083-19-2, more than 60 wt% of base), whereas the main chemical constituent of the curing agent is dimethyl methylhydrogen siloxane (CAS: 68037-59-2, 40-70 wt% of curing agent) [260]. In the curing process, the methylhydrogen siloxane units (from the curing agent) cross-links with the terminal vinyl groups of the base polymers, and then an elastomeric network is formed from the short liquid

polymer chains. The curing time depends on the curing temperature and PDMS will shrink slightly ($\sim 1\%$) after the curing.

After cured, the surface of the PDMS chip is hydrophobic with a very low surface energy with repeating groups of $-\text{O}-\text{Si}(\text{CH}_3)_2-$. The PDMS surface can be changed by exposing the sample to oxygen plasma. Under the oxygen plasma, the methyl groups ($\text{Si}-\text{CH}_3$) on the surface are attacked by reactive oxygen radicals and substituted by silanol groups ($\text{Si}-\text{OH}$), and then the PDMS surface becomes hydrophilic. However, the state of the hydrophilic surface is not stable. The hydrophilic surface can be kept approximately 30 minutes and then it returns to the hydrophobic surface.

The bonding is important for the chip, and a strong bonding can be realized between the two PDMS surfaces or PDMS/glass surfaces. After the oxygen plasma surface treatment, two treated surfaces are pressed together and the condensation reaction between silanol groups forms the $\text{Si}-\text{O}-\text{Si}$ bonds with the removal of H_2O molecules. The strong covalent bonding is the basis of the irreversible sealing between two PDMS replica or one PDMS replica and one glass slide.

Mostly, water based solvents can be used in PDMS fabricated microfluidic chips, such as water, ethylene glycol, nitromethane, DMSO and glycerol, which do not swell PDMS. However, organic solvents, like chloroform, swell the PDMS in various degrees from about 5% to greater than 30%. Diisopropylamine, the most swelling solvent for PDMS, can cause the 2.13 times expansion of PDMS [260].

3.2.3 Bubble formation and high-speed imaging

The schematic of experimental setup for laser-induced cavitation bubbles generation and high-speed imaging is depicted in Fig. 3.5. The setup consists of a pulsed laser, mirrors and lenses, an inverted microscope and a high-speed camera [262-266]. The Nd:YAG laser (Orion, New Wave Research, Fremont, CA) creates a

Figure 3.5: Schematic of the experimental setup for the droplet fusion using laser-induced cavitation bubbles and the recording of the rapid dynamics with a high-speed camera.

single laser pulse at the wavelength of 532 nm with a duration of approximately 6 ns and 100 μ J energy. The beam is expanded with the lens group consisting of lenses L_1 ($f = -30$ mm) and L_2 ($f = 400$ mm). The energy of the laser pulse is controlled by changing the Q-switch delay. The variation of the laser energy is about $\pm 5\%$. The laser beam is reflected by the mirror into the back aperture of the microscope objective (20 $\times$, NA = 0.75, Olympus), which is integrated in an inverted microscope platform (IX-71, Olympus). The illumination light from the

Figure 3.6: Time sequence of the illumination, the laser pulse and the exposure time of the high-speed camera. For the recording, the illumination is always on, the duration of the laser pulse is 6 ns, the typical exposure time of the high speed camera is 370 ns and the time interval between two images is 2.5 μs in this case.

microscope condenser propagates through the dichroic mirror of the microscope and the images are recorded by a high-speed CMOS-based camera (SA-1.1, Photron). A notch filter at 532 nm (NF02-532S-25, Semrock) is mounted before the sensor of the CCD camera to protect it from the intense laser light.

The time sequence of the illumination, the laser pulse and the exposure time of the high-speed camera is plotted in Fig 3.6. To record the high speed images, the illumination light from the microscope condenser is always on. The duration of the laser pulse is 6 ns and the typical exposure time of the high speed camera is 370 ns. A function generator (BNC, Berkeley Nucleonics) is used to generate synchronous signals to turn on the high-speed camera and the pulsed laser. As shown in the Fig. 3.6, the illumination light is turned on first and in the constant-on state in the whole recording process. Then, the pulsed laser and the high-speed camera are turned on

Figure 3.7: Relationship between the radius of the laser-induced cavitation bubble and the laser energy. The error bars denote the standard deviation of 10 distinct measurements.

by the synchronous signals from the function generator. The single laser pulse is used to generate the cavitation bubble and the high-speed camera is used to record the whole process of the droplet fusion. Usually, one additional micrograph is recorded before the pulsed laser is turned on to show the micrograph before the cavitation bubble generation. This can be realized by adjusting the setting of the function generator.

The maximum radii of laser-induced cavitation bubbles as a function of the laser energy are shown in Fig 3.7. It is seen that the radius of the bubble increases almost linearly with the increment of the laser energy. The radius of the bubble is defined as the projected radius imaged onto the high-speed camera. The generated cavitation bubble in an ink film is confined in a tiny chamber as shown in the up-left inset in Fig. 3.7. The length, width and height of the chamber are 20 mm, 20 mm and 15 μm , respectively. The tiny chamber is constructed by two microscope

cover slips and the aluminum foil is used as a spacer to separate two cover slips. The magenta inkjet printer ink is used to generate bubbles, because the inkjet printer ink enhances the absorption of the green laser pulse. The transparent property of the ink is also sufficient for the optical setup to obtain an ultra-high speed framing with the illumination light from the microscope condenser. The density of the aqueous ink is 1046 kg/m^3 and its dynamic viscosity is $2.14 \text{ mPa}\cdot\text{s}$ at room temperature. Micrographs of laser-induced cavitation bubbles for different laser energies are shown in the insets of the Fig. 3.7. With the increasing of the laser pulse energy from 12.9, 26.7 and 40.6 to 66.7 mJ, the maximum radius of the cavitation bubble increases from 52, 74 and 85 to 123 μm . Therefore, the maximum radius of the generated cavitation bubble can be easily controlled by adjusting the energy of the laser pulse.

3.3 Experimental results and discussions

This section presents single bubble dynamics under different conditions, and fusions of two equal-sized droplets, two unequal-sized droplets, droplets in a continuous droplets stream and droplets in a hexagonal structure.

3.3.1 Bubble dynamics

3.3.1.1 Bubble dynamics in single phase liquid

The dynamics of a single cavitation bubble is examined first in a liquid film confined in a tiny chamber. It consists of two microscope cover slips, which are separated with an aluminum foil spacer as shown in the upper-left inset in Fig. 3.7. The micrographs of a single cavitation bubble at different time points are shown in Fig. 3.8. The micrograph with the maximum cavitation bubble is defined as time $t = 0$. The pulsed laser with energy $8.58 \mu\text{J}$ at wavelength 532 nm is turned on at the time $t = -8 \mu\text{s}$. The bubble expands to its maximum at $t = 0 \mu\text{s}$ and the maximum radius of the bubble is approximately $36 \mu\text{m}$. Then, the bubble starts to shrink. It becomes smaller at $t = 8 \mu\text{s}$ and it vanishes finally.

Figure 3.8: Selected frames of the dynamics of the single cavitation bubble in the confined chamber. The scale bar depicts $40 \mu\text{m}$. The micrograph with the maximum bubble is defined as time $t = 0$.

Due to the fact that the height of the chamber is $15 \mu\text{m}$, the bubble in Fig.

Figure 3.9: Comparison of the experimental and theoretical radii of the 2D cavitation bubble at the different time points. Markers are from high-speed camera recordings and the line is from the theoretical analysis.

3.8 can be considered as a 2D bubble. The 2D bubble dynamics can be calculated using the following modified Rayleigh-Plesset equation [267-269]

$$(R\ddot{R} + \dot{R}^2) \log \frac{R}{R_\infty} + \frac{1}{2} \dot{R}^2 = \frac{P}{\rho} \quad (3.6)$$

where ρ is the density of the liquid, R_∞ is the distance at which the velocity in the fluid drops to zero, and P is the pressure far from the bubble, which is assumed as a constant $P = 10^5$ Pa. The parameter R_∞ comes from the geometrical constraints. In this experiment, the bubble is generated in the chamber $20 \times 20 \text{ mm}^2$ with a height of 15 μm . Thus R_∞ is equal to 10 mm due to the chamber width of 20 mm . The radii of the cavitation bubble at different time points are shown in Fig. 3.9. The experimental results show good agreement with the theoretical prediction.

Since the experiments are conducted in a small height channel, the bubble dynamics is affected by the viscosity of the liquid. The vorticity growing distance

Figure 3.10: Comparison of the measured collapsing radii for different laser energy. Markers are from high-speed camera recordings.

from the wall into the channel, d , is roughly estimated as

$$d = \sqrt{\nu t} \quad (3.7)$$

where ν is the kinetic viscosity of the liquid and t is the life time of the bubble. In this case, the bubble life time is approximately 18 μs , and the estimate distance $d \approx 4.2 \mu\text{m}$, which is smaller than the half height of the channel 7.5 μm . Therefore, the viscosity effect can be ignored in this case.

The collapsing processes of three bubbles generated with different laser pulse energies (8.58 μJ , 12.9 μJ and 26.7 μJ) are plotted in Fig. 3.10. It is seen that when the energy is higher, the maximum radius of the bubble is larger and the collapsing time is longer. For the larger bubble, it finds that the expansion process is shorter than its collapse. It may be due to the fact that the expansion is driven by the vapor pressure from the initial hot bubble, while the hot vapor cools down as the bubble expands. That induces the asymmetry in the plotted expansion and collapsing radius-time curve [265]. In addition, the increase of the laser energy

enlarges the probability to create more non-condensable gas, which elongated the collapsing process, too.

3.3.1.2 Bubble dynamics in two parallel immiscible flows

When two immiscible liquids, silicon oil and inkjet printer ink, are pumped into the microfluidic channel with a width of $100\ \mu\text{m}$, two unsteady parallel flows are formed at the initial time as shown in Fig. 3.11(a). The width of the ink flow is approximately $36\ \mu\text{m}$ and that of the oil flow is approximately $64\ \mu\text{m}$. When the green pulse laser ($532\ \text{nm}$) with the energy $100\ \mu\text{J}$ is focused in the ink flow, a cavitation bubble is created as shown in Fig. 3.11(b). The bubble reaches its maximum size (with the length $l \approx 100\ \mu\text{m}$ and the width $w \approx 36\ \mu\text{m}$) at $t = 0$ as shown in Fig. 3.11(c). Then it collapses in $12\ \mu\text{s}$ (see Fig. 3.11(d-f)). It is also observed that the slight deformation of the interface between immiscible flows and the PDMS microchannel wall at $t = -4\ \mu\text{s}$ since the expansion of the cavitation bubble (see Fig. 3.11(b)).

Figure 3.11: Selected frames of the dynamics of the single cavitation bubble in two parallel immiscible flows. The scale bar depicts $40\ \mu\text{m}$. The micrograph with the maximum bubble is defined as time $t = 0$.

Compared to the bubble in a single phase liquid, the shape of the bubble is not spherical like. It is due to the fact that the growth of the bubble is confined in the ink flow. The expansion of the bubble towards the oil phase (x -direction) is far

Figure 3.12: Evolution of the dimension of the non-spherical cavitation bubble at x - and y -directions.

smaller than that along the ink flow (y -direction). The dimensions of the droplet along x - and y -direction as a function of time are plotted in Fig. 3.12. The maximum length of the bubble along y -direction is approximately $49\text{ }\mu\text{m}$, which is almost 3 times of that along x -direction ($17\text{ }\mu\text{m}$). This is induced by the strong viscosity ratio, i.e. 1250:1, between silicon oil and ink flows.

3.3.1.3 Bubble dynamics inside a single water droplet

The dynamics of a single cavitation bubble in an aqueous droplet is examined in this section. A laser-induced cavitation bubble within an aqueous droplet is created as shown in Figure 3.13(b). The bubble is created a few micrometers above the bottom of the microfluidic channel. When the laser hits the center of the droplet in the microfluidic channel, the bubble is generated due to water and dye molecular absorption. The micrographs are recorded by the high-speed camera at framing rates of up to 250,000 frames per second and the exposure

Fig 3.13 Selected frames from the dynamics of the single cavitation bubble in an aqueous droplet and the bubble located at the center of the droplet. The scale bar depicts 20 μm . The first micrograph with a bubble present is defined as time $t = 0$.

Fig 3.14 Selected frames from the dynamics of the single cavitation bubble in an aqueous droplet and the bubble located in vicinity to the lower boundary of the droplet. The scale bar depicts 20 μm .

time is 370 ns. The first micrograph with a bubble present is defined as time $t = 0$. A bubble forms quickly after the laser pulse hits the droplet. It reaches the maximum diameter at $t = 0 \mu\text{s}$ as shown in Fig. 3.13(b) and the droplet is expanded to the maximum at the same time. After that, the bubble starts to shrink, and at $t = 16 \mu\text{s}$, the bubble within the droplet collapse completely (Fig. 3.13(c-f)). When the created cavitation bubble is located in vicinity to the lower boundary of the droplet, the dynamics of bubble is totally different as shown in Fig 3.14. Since the boundary-bubble interaction effect, one jet towards the boundary is formed at $t = 4 \mu\text{s}$ as (See Fig. 3.14(c)). Then, the bubble starts to shrink and the bubble splits to two sub

Fig 3.15 Evolution of the diameters of the droplet and the bubble inside the droplet.

bubbles at $t = 12 \mu\text{s}$ (See Fig. 3.14(d-e)). At $t = 16 \mu\text{s}$, the residues of the two sub-bubbles can still be observed and then they collapse completely (See Fig. 3.14(f)). The deformation of the part of the droplet, which is in vicinity to the cavitation bubble, is also observed from $t = 0 \mu\text{s}$ to $t = 16 \mu\text{s}$.

The radius of the droplet in Fig. 3.13 is measured and the variation of the diameters as a function of time is plotted in Figure 3.15. The black square represents the radius of the droplet and the red dot represents the radius of the laser-induced cavitation bubble inside the droplet. Using the spherical volume equation, the bubble radius R_b at different time can be given by

$$R_b = (R_d - R_{d0})^{1/3} \quad (3.8)$$

where R_d is the radius of the droplet, R_b is the radius of the bubble inside the droplet and R_{d0} is the radius of the droplet at the beginning. Two assumptions for this equation are (1) the shapes of the droplet and the cavitation bubble inside the droplet are ideal spherical shapes in the whole experimental process, and (2) the volume of the droplet is a constant in the whole experimental process, even there is

Fig 3.16 Selected frames from the evolution of a single bubble inside a single flowing plug confined in a microfluidic channel with a width $25\ \mu\text{m}$. The scale bar depicts $25\ \mu\text{m}$. The first micrograph with a bubble present is defined as time $t = 0$.

a few mass losses in the process of the cavitation bubble generation (a few volume of the droplet is vaporized to generate bubble).

The dynamics of a single cavitation bubble inside a flowing aqueous plug droplet confined in the microfluidic channel is shown in Fig 3.16. A flowing aqueous plug is formed in the microfluidic channel at $t = -4\ \mu\text{s}$. The negative sign here means the micrograph is recorded before the bubble is created. When a green (532 nm) laser pulse is focused into the plug, a cavitation bubble is created and its radius reaches the maximum at $t = 0\ \mu\text{s}$. Both the plug and the PDMS microfluidic channel are deformed by the expanding bubble. Then, the bubble starts to shrink gradually from $t = 4\ \mu\text{s}$ to $t = 20\ \mu\text{s}$. The bubble collapses at $t = 16\ \mu\text{s}$ and the residue of the bubble still can be observed at $t = 20\ \mu\text{s}$.

3.3.2 Fusion of two equal-sized droplets

Fig 3.17 Selected frames from the fusion process of two nearly equal-sized droplets (the upper droplet with a diameter of $92\ \mu\text{m}$ and the lower droplet with a diameter of $96\ \mu\text{m}$). The scale bar depicts $40\ \mu\text{m}$. Please note the largely different intervals between frames. The first micrograph with a bubble present is defined as time $t = 0$. The fusion process starts only 65 ms after the bubble is created.

To form equal-sized droplets in the microchannel, the flow rates of the ink and the immersion oil are $0.035\ \text{ml min}^{-1}$ and $0.056\ \text{ml min}^{-1}$, respectively. The droplets are generated in the main channel and then stored in the droplet collection chamber after the flow has been switched off. Experimental micrographs selected from a high speed recording taken at 250,000 frames per second of the fusion of two equal-sized droplets in a stagnant liquid are shown in Fig. 3.17. The sequence covers the time from $4\ \mu\text{s}$ before bubble creation to the fully developed neck at $t = 1\ \text{s}$. Two aqueous droplets separated by a liquid oil film of $6\ \mu\text{m}$ are shown in the first micrograph in Fig. 3.17. The laser pulse spot is focused at the center of the upper

droplet and a vapor bubble is created due to the absorption of the light in the dispersed phase. The initially hot vapor of the dispersed phase causes an explosive growth of the bubble. The vapor inside cools down quickly because of the rapid volume growth, and thereby, the internal pressure is reduced. This leads to a deceleration of the radial expansion, and the bubble reaches its maximum size at $t \approx 0 \mu\text{s}$, thus within $4 \mu\text{s}$.

The expansion of the upper droplet is not isotropic but more pronounced towards the lower droplet. This behavior is explained due to the higher viscosity of the oil leading to a higher normal stress difference sideways across the interface. In contrast, the effective viscosity of the fluid below the expanding droplet is smaller. Therefore, the expanding droplet primarily deforms towards the neighboring droplet. The bubble begins to shrink and collapses at $t = 32 \mu\text{s}$. A remaining and slowly dissolving gas bubble is visible inside the upper droplet. Between $t = 32 \mu\text{s}$ and $t = 20 \text{ ms}$, the sucked in and now bulged interface from the lower droplet slowly retracts back (downwards). Interestingly, the neck of the unconnected droplets shrinks between $t = 30 \text{ ms}$ and $t = 50 \text{ ms}$. The limited spatial resolution ($2 \mu\text{m}$ per pixel) is not sufficient to detail the flow leading to fusion, yet it is interesting to note that the fusion occurs during the retraction of the interface [273]. The fusion process with the formation of a clear connecting neck is visible, starting from approximately $t = 65 \text{ ms}$. The thickness of the neck grows gradually until $t = 1 \text{ s}$ that is when the neck has vanished.

The growth rate of the neck is one key observation in the fusion process. The initial neck growth depends on the balance between the capillary and viscous forces. For low viscosity liquids and short times, a logarithmic power law can be used to describe the neck growth process provided that $R_n \ll R_0$ [244], and it can be

Figure 3.18: Radius of the neck after fusion occurs. The arrow indicates the start of the fusion at the time 65 ms as shown in Fig. 3.17. One error bar represents exemplarily the measurement error of ± 1 pixel accuracy, which is representative for all data points and shows the error in determining the radius of the neck.

expressed as

$$R_n(t) \propto t \ln t \quad (3.9)$$

where R_n is the radius of the neck and R_0 is the radius of the fused droplet. At later times, inertia becomes dominant and the growth rate follows a power law. When comparing this model to the experimental data, the satisfactory agreement cannot be achieved because of the highly viscous continuous phase in the present study. A modified model for the neck growth based on a purely viscous flow, i.e. the Stokes' equation, is proposed [247]. This model suggested that the entire evolution except just after the fusion can be reasonably fitted to the modified equation, which is expressed as

$$R_n(t) = A + B \times \exp\left(-\frac{t}{t_1}\right) \quad (3.10)$$

Figure 3.19: Selected frames of the un-fusion process of two nearly equal-sized droplets. The scale bar depicts 10 μm . The first micrograph with a bubble present is defined as time $t = 0$. The upper droplet with a diameter of 15 μm and the lower droplet with a diameter of 14 μm .

where $A = -44.9 \mu\text{m}$, $B = -51.3 \mu\text{m}$, and the time $t_l = 0.329 \text{ s}$ in this particular case.

Hence, the neck growth rate decreases due to the viscous dissipation. Fig 3.18 shows the evolution of the neck radius together with the fitted result (Eq. 3.10) denoted by the solid line. The reasonable agreement indicates that viscous dissipation, rather than inertial effect, is dominating and it slows down the neck growth. Therefore, the fusion process is not only affected by the droplet size and the distance between two droplets, but also by the liquid properties, such as viscosity, density, and surface tension. With the demonstrated technique, the effects of these parameters to the fusion process can be investigated in future.

Further, when the continuous phase between two droplets has not been drained completely, these two droplets cannot be fused as shown in Fig. 3.19. A cavitation bubble is created in the lower droplet using a green (532 nm) laser pulse and reaches its maximum at $t = 0 \mu\text{s}$. Then, the bubble starts to shrink and it collapses at $t = 36 \mu\text{s}$. Two droplets detaches clearly at $t = 20 \text{ ms}$ and these two droplets become spherical shapes due to the interfacial tension at $t = 40 \text{ ms}$. The main reason for the un-fusion is that the thin oil film between two droplets does not drain completely.

3.3.3 Fusion of two unequal-sized droplets

The fusion process of two unequal-sized droplets is investigated by creating a cavitation bubble inside the smaller droplet as shown in Fig 3.20. To generate droplets of different sizes, the flow rates of water and oil are set to $0.04 \mu\text{l min}^{-1}$ and $0.056 \mu\text{l min}^{-1}$, respectively. Subsequently, the inlet of water is switched off slowly to generate a series of droplets with decreasing diameters. Droplets in Fig 3.20 are located in the droplet collection chamber and approximately $400 \mu\text{m}$ away from the closest wall. The oil film drainage and the fusion initiation are very similar to the case of two equal-sized droplets. However, when the neck between the droplets is established, the smaller droplet is shrinking quickly while the bigger droplet is growing. This behavior can be explained by the difference in interfacial pressure jump between the smaller and bigger droplets. The pressure jump across the interface obeys the Young–Laplace equation, which can be expressed as

Figure 3.20: Fusion process of two unequal-sized droplets. The first micrograph with a bubble present is defined as time $t = 0$. The scale bar is $20 \mu\text{m}$. The radii of the bigger and smaller droplets are $18 \mu\text{m}$ and $8 \mu\text{m}$, respectively.

$$P_{droplet} = P_{oil} + \frac{2\gamma}{R} \quad (3.11)$$

where $P_{droplet}$ is the pressure inside the water droplet, P_{oil} is the pressure in the oil phase, γ is the surface tension between the water droplets and oil continuous phase, and R is the radius of the droplet. Based on the Eq. 3.11, the pressure within the smaller droplet is higher than that of the bigger droplet. Therefore, a flow will be driven from the smaller to the bigger droplet when they are fused because of the pressure difference.

In contrast to the fusion of two equal-sized droplets, the neck does not grow noticeably once it is formed. This stark difference as compared to two equal-sized droplets is due to the generation of the directed flow, which is driven by the pressure difference in the formerly unconnected droplets and from the smaller droplet to the bigger one. This observation allows the formulation of a simple model for the radial dynamics of the droplets by assuming the neck length and diameter to be constant. It is assumed that the flow, from the smaller droplet 2 with radius R_2 to the bigger droplet 1 of radius R_1 , through the (cylindrical) neck is laminar and fully developed. Hence, the flow rate is given by

$$Q = \frac{\pi d^4 \gamma}{64 l \mu} \left(\frac{1}{R_2(t)} - \frac{1}{R_1(t)} \right) \quad (3.12)$$

where d and l are the diameter and length of the neck, respectively, and μ is the viscosity of ink. The flow rate balances with the rate of change of the droplet volumes,

$$Q = 4\pi R_1^2 \frac{dR_1}{dt} = -4\pi R_2^2 \frac{dR_2}{dt} \quad (3.13)$$

From Eqs 3.12 and 3.13, ordinary differential equations for the radii of droplet 1 and 2 are obtained.

Figure 3.21: Evolution of the radii of the (a) bigger and (b) smaller droplets as a function of time.

In Fig 3.21, the measured radii of the droplets are plotted as a function of time. The evolution is also predicted from Eqs 3.12 and 3.13 with $l = 10 \text{ } \mu\text{m}$, and $\mu = 2 \text{ mPa}\cdot\text{s}$. The coefficient of interfacial tension $\gamma = 1.6 \times 10^{-4} \text{ N}\cdot\text{m}^{-1}$ and the neck diameter $d = 2.85 \text{ } \mu\text{m}$ are varied to fit both curves. The overall model captures the growth and shrinkage of the two droplets including the timescales, yet a more

careful model for the flow at the later stage of bubble shrinkage deems necessary, especially as the drop radius approaches the radius of the neck. In addition, the model should be tested in future with different liquids of known interfacial tension.

3.3.4 Fusion of droplets in a continuous stream

The fusion of droplets transported in a flowing stream is studied in this section. The fusion dynamics of two equal-sized droplets translating in a continuous flow of droplets is presented in Fig 3.22. The flow rates of water and oil are 0.03 and 0.056 $\mu\text{l min}^{-1}$, respectively. The velocity of the droplets along the flow direction is 90 $\mu\text{m s}^{-1}$. A very similar dynamics of the fusion process is observed after the cavitation bubble is induced in the central droplet B as shown in Fig 3.22.

Figure 3.22: Fusion process of two aqueous droplets in a droplets flow stream inside the 100 μm wide oil channel. The first micrograph with a bubble present is defined as time $t = 0$. The diameter of droplets is 44 μm and the inter-droplet spacing is 6 μm . The scale bar is 40 μm .

Figure 3.23: Strain of the droplet B in x - and y -directions. The x - and y -directions are indicated in Fig 3.22 at $t = 0$.

However, the droplet B deforms now the two neighboring droplets, the trailing droplet A and the preceding droplet C. Despite the remarkable symmetry of the deformation, only the fusion of the central droplet B with the lower droplet C occurs. The upper droplet A continues to trail at a new shorter distance. This is due to the fact that the bubble is created slightly closer to droplet C inside the droplet B. It is not affected by the flow direction or the flow rate because the bubble expansion velocity is several orders of magnitude larger than that of the droplet.

The strain of the central droplet B, $\Delta L/L$, in the flow (x -direction) and perpendicular (y -direction) directions is plotted in Fig 3.22. The maximum strain in the y -direction occurs at $t = 4 \mu\text{s}$, which is measured as 3.2 while only 1.5 in the x -direction. When the bubble collapses at $t = 36 \mu\text{s}$, both strains are the same since the droplet B obtains a circular shape again. At later time ($t > 64 \mu\text{s}$), the strain in the y -direction becomes negative, since the droplet B shrinks in the flow direction. This

unequal stretching of the droplet B is due to the strong viscosity ratio, i.e. 1250:1, between the dispersed phase and the continuous phase.

3.3.5 Fusion of droplets in a hexagonal structure

When the generated water-in-oil droplets flow into the collection chamber, droplets self-assemble into a hexagonal structure as shown in Fig. 3.24. The width of the collection chamber is 800 μm . The surfactant in the oil continuous phase prevents droplets from the fusion. Usually, the hexagonal structure is not perfect and there are some defects due to the mismatch of the assembly of droplets as shown in Fig. 3.24.

Figure 3.24: Monodisperse droplets in the self-assembled hexagonal structure formed inside the collection chamber with the width of 800 μm . The diameter of droplets is 70 μm and the inter-droplet spacing is 4 μm . The scale bar is 60 μm .

The controlled multiple droplets fusion in the droplets array with the hexagonal structure can be realized as shown in Fig. 3.25. A cavitation bubble is generated in the central droplet at $t = 0$ s (The white spot is the hot plasma). The cavitation bubble has not been captured in the Fig. 3.25 due to the mismatch between the high speed camera capturing sequence and the evolution of the cavitation bubble. The central droplet expands and the other six surrounding droplets are deformed by the rapid expansion of the created cavitation bubble at $t =$

Fig 3.25 Fusion process of multiple aqueous droplets in a hex structure inside the 800 μm wide oil channel. The first image with a bubble present is defined as time $t = 0$. The scale bar is 60 μm .

0 s. The continuous phase drains between the central droplet and the other six surrounding droplets and the connection between the central droplet and the surrounding droplets is initialized at $t = 8 \mu\text{s}$, and they begin to fuse and finally merge to a single larger droplet at $t = 384 \text{ ms}$. It is speculated that when the power of the laser pulse is higher, droplets in the larger scope will be deformed and fused.

3.4 Diffusion and fusion of droplets with cell

This section presents the bubble transfer between two droplets, the diffusion dominant mixing between the two fusing droplets and the fusion of droplets with an encapsulated cell.

3.4.1 Bubble transfer between two droplets

The break-up and transfer processes of the bubble between two equal-sized droplets are shown in Fig. 3.26. Two equal-sized droplets A and B are generated using a microfluidic channel with T-junction. A cavitation bubble is created inside droplet B ($t = 0 \mu\text{s}$) using a focused green (532 nm) laser pulse. The droplet B is expanded by the expanding bubble and the droplet A is suppressed and deformed by the expanding droplet B. The bubble reaches its maximum and the tip of the bubble

Figure 3.26: Selected frames of the bubble transfer process between two equal-sized droplets. The first micrograph with a bubble present is defined as time $t = 0$. The diameter of droplets is $70 \mu\text{m}$ and the inter-droplet spacing is $4 \mu\text{m}$. The scale bar is $40 \mu\text{m}$.

penetrates the thin oil film between two droplets at $t = 4 \mu\text{s}$. Then, the bubble splits up into two parts at $t = 8 \mu\text{s}$. One part still stays inside droplet B and the other part is transported into the droplet A from $t = 8 \mu\text{s}$ to $t = 40 \mu\text{s}$. Different with the fusion in the previous section, the tip of the bubble does not contract to the droplet B, but the tip breaks down. The half part of the bubble stay in the droplet B and the other half part of the bubble is trapped in the droplet A. In this process, materials in droplet B can be successfully transferred from the droplet B to the droplet A using the broken bubble and the two droplets are still separated after the transportation. It shows a great potential in the applications such as the mass transfer between droplets and the pumps driven by the pulsed laser-induced cavitation bubble.

3.4.2 Diffusion mixing of two droplets

The diffusion dominated mixing process between two fusing droplets is shown in Fig 3.27. The sequence of mixing process is presented by two different-colored droplets: one is made of aqueous red dye and the other contains DI-water. A modified version of the microfluidic chip for the droplet generation is employed. It consists of two T-junctions, which are combined into the same microchannel to generate two different-colored droplets at the same time. The flow rate of the continuous phase is $0.05 \mu\text{l min}^{-1}$ and those of the aqueous red dye and DI water both are $0.1 \mu\text{l min}^{-1}$. For this configuration, a continuous stream of equal-sized droplets of alternating color for much larger droplet volumes is obtained. These droplets span the whole channel, and therefore become severely deformed into

Figure 3.27: Mixing process of two aqueous Taylor droplets. The upper droplet is red ink-jet printer ink and the lower droplet is DI water. The frame rate is 10 000 frames per second with an exposure time of $100 \mu\text{s}$. The scale bar is $40 \mu\text{m}$.

Taylor droplets [270]. Subsequently, when a cavitation bubble is created just above the thin layer of oil, the expanding bubble (not shown in this figure) leads to the injection of a small portion of the red dyed fluid from the upper droplet into the lower droplet at $t = 100 \mu\text{s}$. The oil layer ruptures, and the dye diffuses gradually from the upper droplet into the lower droplet, and *vice versa*. It is not observed that the residual liquid flow shortly after the bubble has vanished. Thus, the diffusion can be described using Fick's law, which is expressed as

$$\frac{\partial c}{\partial t} = D \nabla^2 c \quad (3.14)$$

where c is the mass concentration, t is the time and D is the constant of mass diffusivity. If a stepwise change of the one dimensional concentration field at $x = 0$ is imposed, the diffusion process (in infinite space) follows

$$x_{th} \propto \sqrt{t} \quad (3.15)$$

Figure 3.28: Position of x_{th} as a function of the square root time. The data points are fitted to Eq. 2.7 (solid line).

where x_{th} denotes the position of the threshold concentration. In Fig 3.28, the evolution of the measured concentration field is fitted to a square root of t . Reasonable agreement is seen during the measurement period except at the initial period. This is due to the presence of the remaining fluid motion ($t = 100 \mu s$ in Fig 3.26) from the film rupture between the two droplets and this may lead to the measured enhanced mixing. However, the agreement suggests that the mixing within the fused droplets is governed mainly by diffusion and can thus be described by Fick's law. It is also instructive to note that for predicting the diffusion process properly at later times, the boundary effects have to be considered.

3.4.3 Fusion of droplets with single cells

In cell analysis, it is critical to control the volume of the reagents. The proposed fusion method allows for introducing quantified reagents into droplet reactors which encapsulate a single cell. The on-demand fusion technique is demonstrated in Fig 3.28. The micrographs selected from a high-speed recording present the successful fusion of one droplet with a single HepG2 (Hepatocellular carcinoma, human) cell with the second droplet containing the magenta ink dye. The frame rate is 10,000 frames per second and with an exposure time of 100 μ s. The minimum distance between the two droplets is measured as 4 μ m. The white dash circles follow the movement of the HepG2 cell and the black dash circle shows the location of the laser-induced cavitation bubble. Both droplets are stagnant in the droplet collection chamber. The bright spot at the laser focus is due to the continuum emission from the short lived plasma created by the focused laser.

Figure 3.29: Fusion process of two droplets containing HepG2 cell and aqueous dye.

The flow rate of the continuous phase is $0.05 \mu\text{l min}^{-1}$ and those of the aqueous red dye and cell culture solution with cells are both $0.1 \mu\text{l min}^{-1}$. The laser pulse is focused into the droplet without the cell at $t = 0 \mu\text{s}$. The white point is the hot plasma created by the focused laser pulse and then one cavitation bubble is formed. The continuous phase between the two droplets is again drained by the cavitation bubble at $t = 100 \mu\text{s}$. Then, the connection neck between the two droplets is formed at $t = 2 \text{ ms}$. Under the mild driving force from interfacial tension, both droplets fuse and mix gradually from $t = 25 \text{ ms}$ to $t = 1.5 \text{ s}$.

During the fusion process, the permanent poration of the cell membrane may lead to cell death when the cell is in vicinity to the expanding bubble [174, 271-272]. However, in this case, it is not expected that the destructive effect of bubble dynamics on the cell. This is because the oil film between the droplets dampens the bubble dynamics and the cell is located in the distant droplet. This experiment is far from exhaustive, *e.g.* a viability test of the cells has not been conducted. The cell apoptosis and proliferation will be investigated in future.

The laser-induced cavitation bubble also has a great potential in cell fusion. The fluidic jet generated by the collapse of the bubble in vicinity to a solid boundary can be used to penetrate cell's membrane to trigger fusion process. However, more experiments are necessary to find the optimized parameters for the poration of the cell membrane and the fusion processes.

3.5 Summary

This chapter focuses on the development of the microfluidic system for droplet fusion using laser-induced cavitation bubbles, including theoretical analysis, microfluidic design, fabrication process development, experimental setup, and experimental results. The theoretical analysis explores the droplet fusion mechanism using laser-induced cavitation bubbles, the neck growth theory between two fusing droplets and the droplet generation mechanism using the microfluidic chip with a T-junction. A microfluidic chip with a T-junction is designed and fabricated to generate droplets. An experimental setup is developed for cavitation bubble generation and high speed imaging. Experimental results of the bubble dynamics in bulk liquid, two parallel immiscible flows and a single droplet are demonstrated and discussed. Using the developed droplet fusion technique, the droplets under different conditions are fused and the experimental results are presented and discussed, including the fusion of the static and moving droplets, the fusion of the droplets of equal and unequal sizes and the fusion of multiple droplets in a hexagonal structure. The transport process between two droplets, the diffusion dominant mixing between two fusing droplets and the fusion of droplets with a encapsulated cell are also presented and discussed.

The innovation of the developed droplet fusion technique using laser-induced cavitation bubbles is summarized as follow,

- a) This is the first effort demonstrated to fuse droplets using laser-induced cavitation bubbles in microfluidic channels. Fast on-demand droplets fusion is realized via the transient and rapid flow from a short-lived laser-induced cavitation bubble inside one of droplets. Droplet fusion

under different conditions is demonstrated and the neck growth between fusing droplets is discussed.

- b) Unlike other fusion techniques in microfluidics, the developed technique not only can be employed in two equal-sized droplets fusion, but also can be employed in the fusion of the two unequal-sized droplets, the droplet fusion in a continuous stream and on-demand multiple droplets fusion in a hexagonal structure. The potential applications are presented with the mixing of two reagents and the merging of droplets containing a encapsulated cell.
- c) The developed droplet fusion technique has several advantages, including the good controllability in time and space, simple fabrication as only optical access is necessary, and very fast actuation leading to the rapid droplet fusion.

CHAPTER 4

SINGLE-CELL MEMBRANE PORATION

This chapter presents single-cell membrane poration using a high-speed liquid microjet. The microjet is created during the collapse of a pulsed laser-induced cavitation bubble in vicinity of the cell. The cell is trapped in a converging structure within a microfluidic chip. The asymmetrical growth and collapse of the cavitation bubble in vicinity of the single cell trapping structure lead to the formation of the microjet, which deforms and porates the cell membrane. The single-cell membrane poration is probed with the diffusion of the trypan blue. Time resolved studies show a marked dependency on the bubble dynamics. The penetration length of the dye increases with shorter distances of the bubble from the cell. Numerical simulations of the diffusion process can explain this behavior with a larger pore created in the plasma membrane. First, theoretical analyses of microjet generation and the mechanism of single-cell membrane poration are presented. Subsequently, the design of the microfluidic chip with single-cell trapping structures is illustrated. Finally, the experimental results of microjet generation and single-cell membrane poration are discussed in details.

4.1 Theoretical analysis of single-cell membrane poration

Previous investigations of the cavitation bubble dynamics have shown that a fast jet is created when a laser-induced cavitation bubble collapses near a rigid flat boundary [234, 273-275]. In the collapsing process, the bubble is elongated in the direction normal to the boundary and the asymmetric flow around the bubble pushes the bubble towards the boundary due to the secondary Bjerknes force. Then, a microjet towards the boundary is formed, which penetrates the bubble to a toroidal shape. In this chapter, an array of cell trapping structures are used to trap and locate single cells, and also act as rigid boundaries to induce the microjet for single-cell membrane poration. The strength of the microjet is strongly affected by the maximum bubble size and the standoff distance, which is the distance from the bubble center to the upper boundary of the trapping structure (see Fig. 4.1(a)). Thus the standoff distance among other parameters determine the size of the pores and the cell viability.

Figure 4.1: Schematic illustration of the working mechanism of single-cell membrane poration using the cavitation bubble-induced microjet.

The working mechanism of single-cell membrane poration is illustrated in Fig. 4.1. The cell is trapped within a converging microchannel in the shape of an equilateral triangle as shown in Fig. 4.1(a) (details of the geometry are available in the inset of Fig. 4.2). Each side of the equilateral triangle has a width w_1 of 20 μm

and the width of the rectangular microchannel w_2 below is 5 μm . 0.4% saline trypan blue solution (w/v) (T5184, Sigma-Aldrich, prepared in 0.81% sodium chloride and 0.06% potassium phosphate, dibasic) is flushed into the microfluidic chip as the extracellular medium. Trypan blue is not only used as a biomarker to indicate the cell membrane poration, but also used to increase the laser absorption to facilitate bubble generation. A single cavitation bubble is created by focusing a green (532 nm) pulsed laser beam at a location away from the cell with a standoff distance as shown in Fig. 4.1(a). Both the expansion and shrinkage of the cavitation bubble are non-symmetrical due to the presence of the trapping structure as shown in Fig. 4.1(b) and (c), respectively. During bubble expansion, the cell is pushed towards the trapping structure and flattens. Subsequently, during bubble shrinkage, the cell elongates towards the bubble. In addition, a fast microjet is formed pointing towards the trapped cell (see Fig. 4.1(d)). After the bubble collapses, the flow of the microjet develops into two counter-rotating vortices (see Fig. 4.1(e)). The first four events happen within 10 – 20 μs , while the deformed cell recovers its original round shape subsequently on a millisecond timescale. The diffusive uptake of trypan blue molecules into the cell's cytosol occurs on a second timescale as shown in Fig. 4.1(f).

4.2 Single cell trapping and imaging

This section presents the design and fabrication of the microfluidic chip with an array of structures for single cell trapping, the routine culture of the myeloma cells and the preparation for the cell membrane poration. The experimental setup for the formation of cavitation bubbles and the experimental results of the single cell trapping are also presented and discussed.

4.2.1 Microfluidic chip design and fabrication

Figure 4.2: Schematic illustration of the microfluidic chip with an array structures for single cell trapping (not to scale). The inset denotes the design parameters w_1 , w_2 and w_3 of the trapping structure.

The schematic illustration of the microfluidic chip with an array of structures for single cell trapping is shown in Fig. 4.2. The trapping structure consists of a converging microchannel in the shape of an equilateral triangle towards a rectangular microchannel (see inset of Fig. 4.2). The length of the equilateral triangle w_1 is $20\text{ }\mu\text{m}$ and the widths of the rectangular microchannel w_2

Figure 4.3: Schematic of the single cell trapping mechanism using a converging and a rectangular microchannel. (a) Structure allows the solution with cells to flow into the converging microchannel. (b) After a cell is trapped in the converging microchannel, solution with cells flows around the trapping structure. This induces the single cell trapping at a high proportion.

are varied from 5 μm , 7.5 μm to 10 μm . The height of the microfluidic chip is 27 μm . The microfluidic chip is fabricated using PDMS materials.

For the design of the trapping structure [52, 278-279], the converging and the rectangular microchannel are employed to allow the solution with suspension cells to flow through (see Fig 4.3(a)). Once a cell enters the converging microchannel and is blocked by the rectangular microchannel, the solutions with other cells will flow around the trapping structure (see Fig. 4.3(b)). This induced single cell trapping at a high proportion. The trapping structure has the other important application: When the cell is trapped by the trapping structure, a microjet towards the trapped cell is induced by the cavitation bubble. Therefore, the trapping structure is not only used to facilitate single cell trapping, but also enable microjet for membrane poration.

The fabrication is based on the soft lithography technique and the fabrication process flow is similar to the one described in Chapter 3. In short, the mold is made of SU8 photoresist (SU8-100, MicroChem), which is spun on a silicon wafer substrate (1000 rpm, CEE 200, Brewer Science). After the 10-minute prebake at 65 °C and 30-minute soft-bake at 95 °C, the photoresist is exposed to UV light (506, OAI). Then, 1-minute at 65 °C and 10-minute at 95 °C post exposure bake are applied to minimize the stress. Finally, a mold with a thickness of 85 μm is fabricated with the SU-8 being developed using the SU-8 developer for 10 minutes. A mixture of PDMS (Sylgard 184, Dow Corning) pre-polymer and curing agent (10:1) is poured over the master. After degassed using a vacuum machine and 2-hour bake, the PDMS layer with micro-structures is peeled off and punched for inlets and outlets (0.75 μm, Harris Unicore). Then, the PDMS layer treated using a corona surface treater (BD-25, Electro-Technic Products, USA) is bonded with a glass or PDMS substrate to form the microfluidic chip.

4.2.2 Cell culture and preparation

Myeloma cells are cultured in a medium consisting of 86% (v/v) DMEM (Sigma-Aldrich, USA), 10% (v/v) fetal bovine serum (FBS, Gibco, USA), 1% (v/v) penicillin/streptomycin (Gibco, USA), 1% (v/v) sodium pyruvate, 1% (v/v) L-Glutamine and 1% (v/v) non-essential amino acid. Cells are grown in a T-75 cell culture flask (NUNC, Denmark) at 37 °C in a humidified environment with 5% CO₂. Before the cell membrane poration experiments, cultured cells in culture medium are concentrated at 2000 rpm for 2 min (Velocity 18R, Dynamica). Then, the culture medium is replaced by a 0.4% trypan blue saline solution (T5184, Sigma-Altrich). The trypan blue solution with myeloma cells at 1×10^6 cells/ml

concentration is pumped into the designed microfluidic chip with a syringe pump (New Era 1000, USA) with a flow rate $0.5 \mu\text{l min}^{-1}$.

4.2.3 Single cell trapping

A micrograph of cell trapping using the converging structures is shown in Fig. 4.4. Myeloma cells with the concentration of 10^6 cells/ml and suspended in the 0.4% trypan blue solution are pumped into the microfluidic chip at a flow rate of $0.5 \mu\text{l/min}$. After the cell solution is fully filled up the entire microfluidic chip, the pump is switched off and each trapping structure may have trapped none, one, two, or even more cells. The statistical result of cell trapping for total 94 structures is plotted in Fig. 4.5. The fraction of the structures contained single cell is higher than 50%.

Figure 4.4: Micrograph of cell trapping. The scale bar is $50 \mu\text{m}$.

Fig 4.5 Statistics of single cell trapping.

4.2.4 Experimental setup

The experimental setup for investigating single-cell membrane poration consists of a pulsed laser, mirrors and lenses, an inverted microscope and a high-speed camera. The Nd:YAG laser is used to generate a laser pulse with a wavelength of 532 nm and a pulse duration of 6 ns. The energy of the laser pulse is maintained at 100 μ J. The laser beam is expanded and directed into the back aperture of a microscope objective (20 $\times$, NA = 0.75, Olympus) housed in an inverted microscope (Olympus 71X). This allows a tight focusing of the laser pulse into the liquid within the microfluidic chip. Illumination for the high-speed photography is provided from the microscope condenser. The light propagates through the dichroic mirror of the microscope and the images are recorded by a high-speed CMOS-based camera (SA-1.1, Photron). A notch filter at 532 nm (NF02-532S-25, Semrock) protects the sensor of the camera from the intense laser light. Videos of the bubble dynamics without and with boundaries are recorded at

300,000 frames per second with an exposure time of 1 μ s. The dynamic deformation and poration processes of a single trapped myeloma cell have been recorded at 500,000 frames per second with an exposure time of 370 ns. The frames span an area of $64 \mu\text{m} \times 32 \mu\text{m}$.

In the experiments, the laser is also used as a trigger to turn on the high speed camera. As the camera and the laser are not synchronized, the time of bubble creation varies within one framing interval, i.e. at 300,000 frames/s, the jitter can span up to 3 μ s. Thus, the first frame may or may not show a bubble, and if the bubble is present, it is of variable size. Therefore, for consistency, $t = 0$ is chosen when the bubble reaches its maximum size or when the bubble is presented at the first time. In addition, the choice does not affect any of the results but only allows a simpler description of the experimental outcomes and the comparison with the simulations.

4.3 Microjet generation

This section presents the dynamics of the interaction between the cavitation bubble and the rigid boundary, the generation of microjet and the projection of particles by the microjet.

4.3.1 Bubble-boundary interaction

The growth and collapse of a single bubble without/with the boundary effects from the trapping structure are first studied in the absence of a cell with a high-speed video. Selected micrographs in Fig. 4.6 reveal that the bubble dynamics is strongly affected by the trapping structure. Here, time $t = 0$ is the time when a bubble is first seen in the recordings. In the absence of a boundary, the bubble expands radially to a diameter of approximately $100\text{ }\mu\text{m}$ within $3.3\text{ }\mu\text{s}$ and collapses completely at $13.3\text{ }\mu\text{s}$ as shown in Fig. 4.6(a). Both the expansion and the shrinkage do not show any translational motion of the bubble center. Yet, when the bubble expands in the vicinity of the trapping structure as shown in Fig. 4.6(b), the bubble

Figure 4.6: Selected frames from the bubble growth and collapse process (a) with boundary and (b) without boundary. The high speed video is recorded at 300,000 frames per second with an exposure time of $1\text{ }\mu\text{s}$. The scale bar is $100\text{ }\mu\text{m}$.

flattens at the side closer to the structure. During the collapse starting at $t = 6.7 \mu\text{s}$, the bubble takes a triangular shape and develops a fast and thin jetting flow at $t = 10 \mu\text{s}$. The flow is directed towards the gap of the trapping structure. This jetting flow is evident from the thin black stripe passing through the bubble center. Similar jetting has been observed for much larger bubbles close to a rigid boundary [269].

4.3.2 Particle projection

Figure 4.7: Selected frames of the oscillation process of one microbead (circled) driven by the expansion and collapse of the cavitation bubble. The scale bar is 15 μm .

Selected micrographs of the oscillation process of a microsphere in the microchannel are shown in Fig. 4.7. The video is recorded at 525,000 frames per second with an exposure time of 370 ns. The polymer microspheres with a diameter of 2 μm (Duke) suspended in the refill ink for inkjet printer (red, Maxtec) are pumped into the microfluidic chip. The width of the micro-nozzle is 10 μm . The cavitation bubble is created using a laser pulse with an energy 315 μJ . The standoff distance is 72 μm . The maximum of the radius of the bubble is 59 μm . The micrograph of the bubble with the maximum radius is defined as $t = 0$ as shown in Fig. 4.7(ba) and the upper boundary of the trapping structure is defined as $y = 0$. The location of the microsphere in the micro-nozzle moves up and down during the bubble expansion (see Fig. 4.7(a)-(c)) and collapse (d) and the distance between the microsphere and the upper boundary change from (a) 4 μm , (c) 11 μm to (d) 8 μm . The displacement of the microsphere at $t = 0$ (see Fig. 4.7(b)) should be between that at $t = -3.8$ (see Fig. 4.7(a)) and at $t = 5.7$ (see Fig. 4.7(c)). However, the microsphere is flowing with a high speed in Fig. 4.7(b), and the microsphere is too blurry in the

Figure 4.8: Selected frames of the movement of the microsphere (white circle line) driven by the bubble-induced microjet. The scale bar is 15 μm .

Figure 4.9: Displacements of the microsphere.

micrograph. The displacement of the microsphere is negative in Fig. 4.7(c) and (d), which is due to the collapse of the bubble.

Since the effect of the solid boundary, the bubble collapses asymmetrically as shown in Fig. 4.7 (c), which induces the formation of a microjet. The microjet makes the microsphere to move along the micro-nozzle and selected micrographs of the displacements of the microsphere are shown in Fig. 4.8. The displacements of

the microsphere (white circle line) are 22 μm , 26 μm and 29 μm at 7.6 μs , 11.4 μs and 19 μs , respectively.

The displacement of the microsphere as a function of time is plotted in Fig. 4.9. The error bars represent the measurement error of ± 1 pixel accuracy. The net displacement is up to 21 μm at $t = 19 \mu\text{s}$ and the mean velocity of the testing microbead is up to 1.2 m/s. The displacement of the microsphere is affected by several factors, including the strength of the microjet and the properties of the fluid and the microsphere. The strength of the microjet depends on two parameters, including the size of the bubble and the standoff distance between bubble and the trapping structure. When the standoff distance decreases and the size of bubble increases, the strength of the microjet is enhanced. The properties of the fluid and microsphere, such as viscosity and density, also affect the displacement of the microsphere. When the viscosity of the fluid is higher, the displacement of the microsphere is smaller.

4.3.3 Particle Tracking

4.3.3.1 Microjet measurement

For the visualization of the flow field of the microjet generation, polystyrene microspheres with a diameter of 2 μm (1×10^6 beads/ml, Duke Scientific) are used as tracers as shown in Fig. 4.10(a). The flow velocities are estimated by cross-correlating parts of consecutive frames taken at 250,000 frames per second with an exposure time of 370 ns. Estimated velocity field based on the flow pattern for the bubble-boundary interaction induced flow pattern is shown in Fig. 4.10(b). A microjet towards the micronozzle and two vortices are clearly observed in the flow pattern.

Figure 4.10: (a) Visualized flow pattern using 2- μm polystyrene microspheres. (b) Estimated velocity field based on the flow pattern. The scale bar is 25 μm .

The width of the micronozzle and the standoff distance strongly affect the velocity of the microjet. The diameter of the cavitation bubble in this section is approximately $70 \pm 5 \mu\text{m}$. The effect of the width of the micronozzle is investigated using three different widths: 5 μm , 7.5 μm and 10 μm . The velocity of the microjet as a function of the micronozzle width is plotted in Fig. 4.11. When the width of the

Figure 4.11: Velocity of cavitation induced jet as a function of the width of the nozzle with a standoff distance 20 μm .

Figure 4.12: Velocity of cavitation induced jet as a function of the standoff distance with the width of the nozzle 10 μm .

micronozzle is increased, the velocity of the microjet increases. The effect of the standoff distance is studied using three different standoff distances: 15 μm , 20 μm and 25 μm . The velocity of the microjet as a function of the standoff distance with a

micronozzle width 10 μm is plotted in Fig. 4.12. When the standoff distance is increased, the velocity of the microjet increases.

4.3.3.2 Vortex measurement

For the visualization of the flow field of the generated vortices and the microjet, polystyrene microspheres with a diameter of 15 μm are used to mimic the trapped cells (1×10^2 beads/ml, Invitrogen) and polystyrene microspheres with a diameter of 2 μm (1×10^6 beads/ml, Duke Scientific) are used as tracers. Selected micrographs of the flow field are shown in Fig. 4.13. The video is recorded at 250,000 frames per second with an exposure time 370 ns. When the cavitation bubble with the maximum diameter of 60 μm expands and collapses asymmetrically, a pair of vortices and a microjet is generated. The movements of the tracking particles are analyzed by using the particle tracking code of 10 consecutive frames of the high speed video and the velocity fields are generated.

Figure 4.13: Selected frames of the flow field of the microjet generation. The first micrograph with a bubble present is defined as time $t = 0$. The scale bar is 25 μm .

The estimated velocity fields of the cavitation bubble-induced vortices and the microjet are shown in Fig. 4.14. The standoff distances are (a) 15 μm , (b) 20 μm and (c) 25 μm , respectively. When the cavitation bubble collapses, one pairs of vortices are generated at the same time with the generation of the microjet. One

Figure 4.14: Asymmetrical collapse of the cavitation bubble-induced vortices and the microjet. The scale bar is 25 μm .

vortex rotates clockwise and the other rotates counterclockwise as shown in Fig. 4.14(a). The flow velocities are estimated by cross-correlating parts of consecutive frames. The maximum vorticity is up to $30,000 \text{ s}^{-1}$. It is comparable with the vortex with the maximum vorticity of $100,000 \text{ s}^{-1}$ generated by the single cavitation bubble with the diameter of $150 \mu\text{m}$ [269] and that with the maximum vorticity of $350,000 \text{ s}^{-1}$ generated by two tandem bubbles with a diameter of $50 \mu\text{m}$ [159]. The analysis of the flow patterns in this section shows the generation of the microjet towards the micronozzle and its high strength. Therefore, the microjet generated by the cavitation bubble will be employed for single-cell membrane poration.

4.4 Cell membrane poration

This section presents and discusses the experimental results of single-cell membrane deformation and poration using the cavitation bubble induced microjet, and the restoration and swelling of the porated cell in longer time scale.

4.4.1 Cell membrane deformation

The dynamic deformation and poration process of one trapped cell has been recorded at 500,000 frames per second with an exposure time of 370 ns. The frames span an area of $64 \mu\text{m} \times 32 \mu\text{m}$. Selected micrographs are shown in Fig. 4.15. A suspended myeloma cell is trapped by the converging structure as shown in Fig. 4.15(a). One cavitation bubble is created at the standoff distance of $25 \mu\text{m}$ at $t = 0 \mu\text{s}$ by focusing the green (532 nm) laser pulse in the trypan blue solution (see Fig. 4.15(b)). The maximum diameter of the bubble is $42 \mu\text{m}$. The cell is pushed by the expanding bubble towards the trapping structure at $t = 0 \mu\text{s}$, i.e. it flattens horizontally. When the bubble begins to shrink and collapse at $t = 2 \mu\text{s}$ (see Fig. 4.15(c)), the cell elongates vertically towards the collapsing bubble. Then, a high microjet is created between $t = 4 \mu\text{s}$ and $t = 6 \mu\text{s}$ due to the asymmetric growth and

Figure 4.15: Selected frames of the dynamic deformation and poration process of one trapped cell. The first micrograph with a bubble present is defined as time $t = 0$. The scale bar is $10 \mu\text{m}$.

Figure 4.16: Simulation results of the deformation process of one trapped cell by a cavitation bubble-induced microjet.

collapse of the cavitation bubble. It causes a pronounced indentation at the top of the cell as shown in the micrograph $t = 6 \mu\text{s}$ (see Fig. 4.15(e)). After the bubble collapses, the cell regains approximately its original shape, which takes several milliseconds as shown in frame $t = 8 \text{ ms}$ (see Fig. 4.15(f)). The dark point in the frames $t = 6 \mu\text{s}$ in Fig. 4.15(e) is the cavitation bubble residue after the bubble collapses. In this figure, any flattening of the bubble at $t = 0 \mu\text{s}$ is not observed. This is different with the previous figures. The possible reason is that the bubble in this figure is smaller. However, a clear flattening of the collapsing bubble is observed at $t = 2 \mu\text{s}$ (see Fig. 4.15(c)).

The bubble-cell interaction process is simulated numerically using a solver for potential flow. The boundary element solver [275, 280-283] has been adapted to simulate the bubble-cell interaction process with the assumption of the axisymmetric and inviscid flow. The cell boundary is modeled as free fluid particles, while the trapping structure is modeled as fixed in space. Detailed information of the implementation of the boundary element solver is provided in the Appendix A. The experimental and simulation results are compared in Fig. 4.15 and 4.16, respectively. The cell trapped in the converging structure is stretched both in the simulations and the experiments at $t = 0 \mu\text{s}$. The upper part of the cell is not

pushed as much in the experiments as predicted in the simulations. This discrepancy is due to the fact that the simulation neglects the membrane tension and viscoelasticity of the cytoplasm. The simulations are able to provide a closer look on the dynamics during the collapse. This event is too fast to be captured with the camera, yet can be revealed in the simulations. The bubble is shrinking with its major axis perpendicular to the cell at $t = 4.5 \mu\text{s}$ (see Fig. 4.16(e)). During the collapse, the bubble center accelerates towards the structure, which causes the formation of a fast microjet at $t = 5.2 \mu\text{s}$ (see Fig. 4.16(f)). This rapid jetting flow is the likely cause of the pronounced indentation seen in the experiment at $t = 6 \mu\text{s}$.

To determine when and where the highest strain is caused to the cell membrane, the extent, location and dynamics of the shear stress imposed on the cell membrane are presented in greater details in Fig. 4.17(a) to (c). The radial dynamics of the non-spherical bubble near the structure is obtained as an equivalent radius $R_b(t)/R(t=0)$ as shown in Fig. 4.17(b). Here, $R_b(t)$ is the equivalent radius of the axisymmetric cell obtained from the volume average, which is expressed as

$$R_b(t) = \left[\frac{3}{4} \int_0^z r^2(t, z) dz \right]^{\frac{1}{3}} \quad (4.2)$$

where $r(t, z)$ is the position of the bubble interface in cylindrical coordinates. The quick expansion and collapse of the bubble are observed in Fig. 4.17(b) with the timescale being enlarged from $t = 4.5 \mu\text{s}$.

Within the limits of this simplified cell model, the areal strain on the cell as a function of time and location can be determined. Therefore, the cell surface is discretized through 10 nodes, which are initially uniformly distributed on an upper quarter of the initial spherical cell as shown in Fig. 4.17(a). Node 1 is on the axis of symmetry and node 10 is at 9 o'clock position. The normalized areal strain $S^i(t)$ of

Figure 4.17: (a) Nodes distribution on the virtual cell membrane for areal strain calculation. (b) Normalized equivalent radius of the bubble, $R_b(t)/R(t=0)$ as a function of time. (c) Selected simulated areal strains vs. time for selected nodes. The time of the bubble with maximum size is defined as time $t = 0$.

the surface between node i and $i+1$ is defined as,

$$S^i(t) = (A^i(t) - A^i(ini)) / A^i(ini) \quad (4.3)$$

where $A^i(ini)$ is the area covered between the neighboring nodes i and $i+1$ at the initial time ($t = -5.1 \mu s$) and $A^i(t)$ is this area at the time t . For simplicity, all areas are assumed as planes. When the strain is below zero, the cell surface is compressed locally. While for $S^i(t) > 0$, the cell membrane is expanded relatively to the initial spherical configuration. Four selected areal strains $S^i(t)$ with $i = 1, 2, 4$ and 9 are plotted in Fig. 4.17(c) as a function of time. During bubble expansion and collapse, the upper part of the cell has a positive strain ($i = 1, 2$ and 4), while on the side, e. g. $i = 9$, compression occurs. The strain up to 300% is an over estimation. In the

experiment, a milder deformation during this time is observed as shown in Fig. 4.15. During the collapse, all strains relax, yet due to the formation of the jet, the strain $S'(t)$ increases sharply at $t = 5.2 \mu\text{s}$. Unfortunately, the simulation does not capture the later stage of the jet flow. It is likely that the strain may increase considerably above the value during the bubble expansion. All of the expansion, collapse and jetting lead to the largest strains at the node closest to the bubble. Therefore, from the numerical simulation, it can be deduced that when membrane poration occurs, it is most likely near the location of jet impact.

4.4.2 Cell membrane restoration

The deformed cell recovery process has been recorded at 500,000 frames per second with an exposure time of 370 ns. Selected frames from the movie are shown in Fig. 4.18. The shape of the cell membrane progressively returns to its initial shape over the course of 8 ms. A pronounced indentation at the top of the cell is induced by the high speed microjet at the time $t = 6 \mu\text{s}$ as shown Fig. 4.18(a). The whole cell is pushed more towards the trapping structure and flattens horizontally at $t = 8 \mu\text{s}$ (see Fig. 4.18(b)). From (a) to (b), the indentation starts to release. The dark

Figure 4.18: Selected frames from the cell restoration process from $6 \mu\text{s}$ to 8 ms. The scale bar is $10 \mu\text{m}$.

point in Fig. 4.18(a-c) is the cavitation bubble residue after the bubble collapses. It flows out of the view field at $t = 20 \mu\text{s}$ (see Fig. 4.18(d)). The bubble residue pushes the right side of the indentation smoother at $t = 8 \mu\text{s}$ (see Fig. 4.18(b)) and the right side of the indentation almost release completely at $t = 10 \mu\text{s}$ (see Fig. 4.18(c)). From the micrograph (d) to (l), the cell starts to elongate, which is normal to the trapping structure, until the cell almost restore its initial shape.

(a)

(b)

Figure 4.19: (a) Variation of cell area as the function of time. (b) Re-plotted part from 0 μs to 100 μs (dash line).

Based on the boundary location, the projected area of the cell with the varied membrane at different time can be calculated. The calculated projected area as a function of time is plotted in Fig. 4.19(a) and the part from 0 μs to 100 μs is replotted in Fig 4.19(b). After the microjet poration, the area decreases to its minimum at $t = 20 \mu\text{s}$ and then gradually increases back to the initial value. The

area of the cell at the $t = 8$ ms is approximately 99% of the initial value. Assuming that the volume of the cell keeps as a constant, the reduction in the projected area means that the cell is squeezed between the bubble and the trapping structure, i.e. more of the cell volume element now lies further from the symmetry axis.

4.4.3 Trypan blue diffusion

To prove the simulation prediction, the uptake process of a membrane impermeable dye following the bubble collapse is studied. The uptake of trypan blue is recorded with the high speed camera and selected micrographs of the process are shown in Fig. 4.20, which are recorded at 500,000 frames per second with an exposure time of 370 ns. Trypan blue enters only through the upper pole of the cell, from where it slowly darkens the cytoplasm. The change of the grayscale is used to track the uptake of the trypan blue. The dark front of trypan blue staining increases within $t = 2.5$ s to $6\text{ }\mu\text{m}$. This slow timescale suggests that only diffusive transport (not convection) is important for the staining of the cytoplasm.

Figure 4.20: Selected micrographs of the trypan blue uptake process for the porated cell in 2.5 second. The scale bar is $10\text{ }\mu\text{m}$. δ is defined as the distance between the diffusion frontier and the upper boundary of the cell. The diffusion frontier is defined as the 50% level between the background gray level value and the darkest gray level value.

The grayscale distribution of the trypan blue stained cell at the time $t = 0$, 5 s, and 10 s are plotted in Fig. 4.21. The grayscale distribution line is labeled as shown in the inset micrograph. Before the trypan blue uptake, the grayscale of the left part of the cell is higher than the right part ($t = 0$). When the uptake process starts, the grayscale of the right part decreases due to the fact that the trypan blue dye diffuses into the cell from the right pole of the cell ($t = 5$ s). At the time $t = 10$ s,

Figure 4.21: Grayscale distribution of the trypan blue stained cell along the straight line labeled in the inset micrograph at the time $t = 0$, 5 s, and 10 s, respectively.

the grayscale of the right part decreases further. However, the decrease gap between $t = 5$ s and $t = 10$ s is obviously smaller than that between $t = 0$ s and $t = 5$ s. That is due to the one-dimensional diffusion distance D proportional to the square root of time t . The grayscale gap between two same time intervals will decrease with the increase of the time.

For further validation, the uptake process is compared to a solution of the diffusion equation, which is expressed as

$$\frac{\partial c(x,t)}{\partial t} = D \nabla^2 c(x,t) \quad (4.5)$$

where $c(x, t)$ is the mass concentration of trypan blue solution (mol/m^3) and D is the diffusion constant. Due to the non-trivial geometry, the simulation tool (COMSOL Multiphysics v3.5a, Burlington, MA) is used for the modeling of the diffusion process. To simplify the simulation, several assumptions are made, including the diffusion coefficient inside and outside the cell is the same, the diffusion coefficient inside the cell is not time-dependent, and the pore size is a constant for the whole

Figure 4.22: Simulation results of the diffusion front.

diffusion process. The cell is approximated with a three-dimensional ellipsoid with a major axis $a = 12.5 \mu\text{m}$ and a minor axis $b = 10 \mu\text{m}$, approximately simulating the shape at time $t = 0$ as shown in Fig. 4.22(a). The cell is located at the center of a simulation domain with large lateral dimensions of $250 \mu\text{m} \times 200 \mu\text{m}$ and the same channel height as in the experiment, i.e. $27 \mu\text{m}$. The cell is isolated from the rectangle trypan blue surrounding with an isolation boundary condition but only connected at the location of the pore at the top pole of the cell. The whole model is meshed using total 123,494 tetrahedral elements. The diffusion coefficient in the simulation domain outside the cell is $D_{out} = 2.2 \times 10^{-10} \text{ m}^2/\text{s}$ [285] and that inside the cell is $D_{in} = 1.1 \times 10^{-12} \text{ m}^2/\text{s}$ [147, 286]. Only one circular hole of the constant diameter with radius r is located at the upper pole of the cell. Initially, the normalized dye concentration outside the cell is assumed to be $C_{out} = 1$ while inside the cell is $C_{in} = 0$. A fixed arbitrary threshold ($C_{threshold} = 2.94 \times 10^{-4}$) is used to define the frontier of the diffusional transport.

Figure 4.23 depicts the frontier from the simulation for $r = 250 \text{ nm}$. Keeping in mind that there are two adjustable parameters, $C_{threshold}$ and D , a reasonable agreement for a pore size between 100 nm and $1 \mu\text{m}$ is found. As expected, the position of the diffusion frontier shows the expected $t^{1/2}$ dependency for sufficiently short times as the solid line in Fig. 4.23. A smaller pore radius ($r_p = 62.5 \text{ nm}$, dotted line in Fig. 4.23) slows down the diffusion process while a larger pore ($r_p = 1 \mu\text{m}$,

Figure 4.23: Simulation of the diffusion front for three different pore sizes, i.e. $r = 62.5$ nm, 250 nm and 1000 nm, respectively.

Figure 4.24: Averaged uptake distance of trypan blue into porated cells 3 s after the treatment and for different stand-off distances of 15 μm, 20 μm, 25 μm and 30 μm. The error bars depicts the standard deviation for 10 repeated experiments.

dashed line in Fig. 4.23) accelerates the transport.

The results so far suggest that the bubble dynamics induced the microjet is straining the cell membrane, which leads to a membrane poration and dye uptake. Thus, the changing of the standoff distance may affect the quality of the pore. For sufficiently large distance, the strain may not be sufficiently strong to porate the cell membrane. The experimental findings are presented in Fig. 4.24 for 10 repeated experiments at each standoff distance. Here, the distance of the diffusion front travelled 3 s after the bubble generation is measured for four different standoff distances. Statistically significant (Student's t-test, $p < 0.05$) increase of the average diffusion distance from 6.9 μm , 8.9 μm to 10.1 μm is observed when the standoff distance decreases from 25 μm , 20 μm to 15 μm , respectively. At a standoff distance of 30 mm, no uptake is observed. Even 30 s after the bubble generation, not a single experiment shows any uptake.

4.4.4 Cell Swelling

Besides the uptake of the trypan blue and the restoration of the shape, the swelling process of the porated cell within the first 35 s is also observed. Selected micrographs from the recorded swelling process are shown in Fig. 4.25. The video is recorded at 625,000 frames per second with an exposure time of 370 ns. The micrographs show that the cell volume increases 2-fold within the first 35 second. In addition, it is shown that trypan blue stains more of the cytoplasm as it diffuses deeper into the cell.

Figure 4.25: Selected frames of the swelling process of the porated cell in 35 seconds. The scale bar is 10 μm .

The cell volume V is tracked and the ratio of the volume to its original volume V_0 as a function of time is plotted in Fig. 4.26. The cell volume grows continuously over the first 35 s. The cell volume at $t = 35$ s approximately twice its original volume V_0 is observed due to the swelling of the cell. The swelling is likely caused by the osmotic pressure difference, which drives the extracellular liquid into the cell at a much reduced membrane resistance. One may expect that the ability to repair the cell membrane is affected by the toxicity of the dye. In other words, the

Figure 4.26: Variation of cell volume ratio as a function of time. The error bars indicate the measurement error.

uptake of a nontoxic drug may not cause osmotic swelling.

4.4.5 Discussions

For the diffusion coefficient of trypan blue, it can be estimated using an adaptation of the Stokes–Einstein equation, which is usually applied for an oblate disc-shaped ellipsoid, as $2 \times 10^{-10} \text{ m}^2/\text{s}$ at 298 K [287]. It is also experimentally measured using a NMR pulsed-field gradient technique as $2.2 \times 10^{-10} \text{ m}^2/\text{s}$ at 303 K [285]. However, the diffusion constant in pore radius space is different from that in bulk liquid. This concept is firstly introduced for electroporation [288]. The diffusion coefficient of trypan blue in the pore radius space is estimated as $1.1 \times 10^{-12} \text{ m}^2/\text{s}$ [286, 289].

The cell viability after the membrane poration process is one of the key points of an efficient and reliable poration technique. Due to the toxicity of trypan blue, the porated cell viability is affected both by the poration and the trypan blue,

and the viability with this poration method is not probed, and only the permanent poration is analyzed. Previous cell works with laser-induced bubbles in microfluidics also suffered for the same limitations. The viability based on other laser-induced cavitation bubble has been detected and extensively discussed [178]. A viability rate of more than 90% can be achieved by choosing the appropriate experimental parameters.

To conduct a viability test, a non-toxic absorbing dye, a shorter pulsed laser creating smaller bubbles through dielectric breakdown, or a particle based absorbing mechanism may be used. A recent work [290] demonstrated the use of plasmonic absorption from gold dots to generate small cavitation bubbles with a nanosecond pulses. This approach may help to analyze the viability of cells.

Another important factor that affects the cell viability may be a potential heat shock from the laser pulse. Although about 80% of the energy of the laser pulse heats the liquid [233], the heated spot is limited to the size of the focal area and is quickly equilibrated by adiabatic cooling during expansion [233, 291]. Similar laser based techniques have concluded that a mechanical stress is dominant and that thermal induced stresses are of secondary importance [232, 292].

4.5 Summary

This chapter focuses on the development of the microfluidic system for single-cell membrane poration using laser-induced cavitation bubbles. Theoretical analysis, microfluidic chip design for cell trapping, fabrication process, experimental setup, and experimental results of the single-cell membrane poration have been presented. The theoretical analysis explores the mechanism of single-cell membrane poration using the microjet, which is induced by the asymmetrical collapse of laser-induced cavitation bubbles. A microfluidic chip with an array of cell trapping structures is designed and fabricated to trap single cells in the flowing liquid medium and the experimental results of single cell trapping are demonstrated. The experimental setup is built for cavitation bubble formation and high speed imaging. Experimental results of microjet formation, single particle displacement and flow field visualization using particle tracking are demonstrated. Using the formed microjet, experimental results of single-cell membrane poration is presented and discussed. The most possible location of the poration is predicted using the numerical simulation. The cell membrane poration is probed by the transport of trypan blue molecules through diffusion, which is further validated using the numerical simulation of the diffusion process. The processes of the cell membrane restoration and swelling are also demonstrated and discussed.

The innovation of the developed single suspension cell poration technique is summarized as follow,

- a) This is the first effort demonstrated to develop a highly controllable contact-free technique for single-cell membrane poration using the cavitation bubble induced microjet. Single-cell membrane poration is

realized, which is validated by the transport of trypan blue molecules through diffusion.

- b) The demonstrated cell poration method provides a fast and localized solution to control the membrane poration of a single suspension cell. It provides a feasible approach to integrate the technique into automated microfluidic total analysis systems and high throughput systems.

CHAPTER 5

TENSILE STRENGTH MEASUREMENT

This chapter presents the design, fabrication and experiment of the microfluidic system for the tensile strength measurement of liquids. The principle of the tensile strength measurement of liquids is theoretically analyzed. Subsequently, the experimental setup and the laser energy measurement are presented and discussed. Then, the experimental results of the homogenous nucleation of DI water and glycerol in microfluidic chips is presented and discussed, including the photography of the homogeneous nucleation, the shock peak pressure measurement with various standoff distances and the measurement of the tensile strength of DI water and glycerol. Finally, the effects of repetitive tensile waves, microparticles and surfactants on the nucleation bubbles are presented and discussed.

5.1 Principle of tensile strength measurement

The schematic of the principle of tensile strength measurement of liquids is illustrated in Fig. 5.1. A fairly flat air-liquid interface is formed when a microfluidic channel is partially filled with a liquid due to the hydrophobic PDMS walls (see Fig. 5.1(a)). When a pulsed laser beam is focused into the liquid medium, the liquid molecules are ionized within the focal volume and plasma is generated. With the ultrafast expansion of the plasma, a spherical shock wave is created and a spherical cavitation bubble is formed due to the plasma recombination (see Fig. 5.1(a)). With the bubble expansion, the shock wave reaches the air-liquid interface and is reflected as a tension wave by the air-liquid interface due to the acoustic impedance mismatch (see Fig. 5.1(b)).

Figure 5.1: Schematic illustration of the principle of tensile strength measurement of liquids.

For a compression wave propagation from one material with the acoustic impedance Z_1 to another material with the acoustic impedance Z_2 , the reflected fraction R can be expressed as

$$R = \left(\frac{Z_2 - Z_1}{Z_2 + Z_1} \right)^2 \quad (5.1)$$

where the acoustic impedance Z is equal to the product of the liquid density and the shock wave velocity. Since the density of air is just one thousandth of that of water, the reflected fraction R can be approximated as 1 and the compressive wave is totally reflected as a tension wave. When the magnitude of the tension wave is sufficiently large, the rupture of the liquid occurs and can be detected by the presence of the nucleation of vapor bubbles (see Fig. 5.1(b)).

When a shock wave is reflected by the air-liquid interface as a tension wave, the liquid medium in the region between the air-liquid interface and the reflected shock is projected, which is normal to the air-liquid interface, with a velocity v , which is exactly twice of the particle velocity u of the compression wave [293]. The particle velocity u is given by

$$u = \frac{P}{\rho U} \quad (5.2)$$

and the velocity of the projected air-liquid interface is twice of the particle velocity,

$$v = \frac{2P}{\rho U} \quad (5.3)$$

where P is the pressure of the compression wave, ρ is the density of the liquid medium and U is the propagation speed of the shock wave. The pressure P of the compression wave can be estimated as

$$P = \frac{\rho uv}{2} \quad (5.4)$$

by measuring the velocity v and the shock speed u . Here, the velocity v is given by

$$v = \frac{D}{t_r - t_c} = \frac{D}{t_n} \quad (5.5)$$

where D is the displacement of the deformed air-liquid interface, t_r is defined as the recording time that the micrograph is taken and t_c is the compression time of the

shock from the initial point to the air-liquid interface, whereby the shock is a compression wave. t_n , i.e. $t_r - t_s$, is defined as the tension time when the liquid is under tension. In the experiments, the recording time t_r is determined by measuring the time delay between the infrared laser pulse and the green laser pulse. The compression time t_c is determined by measuring the shock speed u and the standoff distance SD , which is the distance from the original point of the shock to the air-liquid interface. The shock speed u can be determined by measuring the radii of the shock at the different time points.

The tensile strength of the liquid can be determined by detecting the critical standoff distance. When the standoff distance is smaller than the critical distance, the reflected tensile wave is negative enough to rupture the liquid medium. When the standoff distance is larger than the critical distance, the reflected tensile wave is not sufficiently negative to rupture the liquid medium. The tensile strength TS of the liquid can be calculated using the following equation, which is expressed as

$$TS = \frac{1}{2} \rho u_c v_c \quad (5.6)$$

where u_c and v_c are the propagation speed of the shock wave and the velocity of the projected air-liquid interface at the critical standoff distance, respectively. In the experiments, the critical standoff distance is determined by increasing the standoff distance gradually until the rupture of the liquid medium cannot be observed.

5.2 Experimental setup and laser energy measurement

5.2.1 Experimental setup

The schematic of the experimental setup for liquids tensile strength measurement is illustrated in Fig. 5.2. An Nd:YAG laser (Orion, New Wave Research) is used to create an infrared laser pulse at the wavelength of 1064 nm with the duration of 7.5 ns and a green laser pulse at the wavelength of 532 nm with the duration of 6 ns duration simultaneously. The infrared laser pulse is introduced into a microscope (IX81, Olympus) by lenses and mirrors in free space. The infrared laser pulse energy is maintained at 1.84 ± 0.05 mJ. A dichroic mirror is used to reflect the infrared pulse into a 40 $\times$ objective (NA = 0.8, water immersion, Olympus) and focused into the liquid medium in a microfluidic channel to create a strong optical breakdown of liquid molecules to generate a hot plasma, a cavitation

Figure 5.2: Schematic illustration of the experimental setup for the tensile strength measurement of liquids.

bubble and a shock wave. A camera (Pixelfly, PCO imaging) controlled by external triggers is used to capture the micrographs. The infrared and green laser beams are prevented from entering the camera to protect it, by the dichroic mirror and the bandpass filter, respectively. The green laser pulse is guided by an optical fibre (FT600EMT, ThorLabs) used as the illumination light. The time delay between the infrared and green laser pulses can be controlled by adjusting the length of the optical fibre. The width and height of the microfluidic channel for the experiments are 400 μm and 85 μm , respectively. The microfluidic channel is fabricated by using the soft lithography technique as shown in Chapter 3.

5.2.2 Laser energy measurement

The energy of the infrared laser pulse is measured using a digital power meter (PD100M, ThorLabs) with a pyroelectric energy sensor (ES111C, ThorLabs). The measured laser wavelength is set as 1060 nm and the diameter of the measured beam is set as 8.8 mm, because it is best for the laser beam to fill ~80% of the aperture for the pyroelectric energy sensor. When measuring the laser energy, the sensor is directly put on the top of the objective lens. The energy values of the infrared laser pulses for the laser energy level setting from 25% to 50% at high voltage setting are measured and plotted in the Fig. 5.3. The error bars show 6 distinct experimental results. The measured results show that the energy values of the laser pulses are highly reproducible.

Figure 5.3: Infrared laser pulse energy vs. the laser energy level setting.

5.3 Shock pressure and tensile strength measurement

In this section, the bubble dynamics in a DI water droplet is presented and discussed. Subsequently, the homogeneous nucleation of DI water in the microfluidic channel is studied. Then, the shock pressures at various standoff distances and the tensile strength of DI water are measured and discussed. Finally, the tensile strength of the glycerol is measured and discussed as well.

5.3.1 Bubble dynamics in a DI water droplet

The evolution of the pulsed laser-induced cavitation bubble and shock are presented in this section. Both the shock and the cavitation bubble do not interact with the wall of microchannel or free surfaces. A DI water droplet is dropped on a microscope glass slide as the sample liquid. The symmetry of the spherical shock and cavitation bubble can be ensured by setting the focal point of the infrared laser pulse far away (200 μm) from the bottom of the testing droplet. The two micrographs of the plasma, shock wave and cavitation bubble in the DI water

Figure 5.4: Micrographs of the plasma, cavitation bubble and shock after the optical breakdown in a DI water droplet. The scale bar is 50 μm .

Figure 5.5: Comparison of the experimental and simulation results of the radii of the shock and bubble in the DI water droplet.

droplet with different recording times are shown in Fig. 5.4. The emission of the plasma forms the bright spot in the center of the cavitation bubble. The liquid water around the plasma is pushed outward by the expanding plasma, leading to form a circular shock (the dark fringe, see Fig. 5.4(a) and (b)). The radii of the cavitation bubble are $36.0 \mu\text{m}$ and $47.6 \mu\text{m}$ and that of the shock wave are $110.7 \mu\text{m}$ and $190.3 \mu\text{m}$ at $t = 48.0 \text{ ns}$ and 97.9 ns , respectively.

The temporal evolution of the shock and the bubble radii in the DI water droplet are plotted in Fig. 5.5. The error bars show the standard deviation of six different runs of the experimental results. The shock and bubble dynamics are simulated by solving the Euler equation of 2D azimuthal symmetry and the Tait equation for the thermodynamic state along an isentrope. More details can be referenced the supplementary material of Ref [241]. The initial conditions include $10\text{-}\mu\text{m}$ bubble radius and 5-GPa bubble pressure. The experimental results agree very well with the simulation results as shown in Fig. 5.5.

5.3.2 Homogeneous nucleation of DI water

Figure 5.6: Micrographs of the DI water rupture with the nucleation of a cloud of vapor bubbles. The scale bar is 50 μm .

Micrographs of the DI water rupture with the nucleation of vapor bubbles in a microfluidic chip are shown in Fig. 5.6. The standoff distance is 135.6 μm . The air-water interface (dark line) is formed in the partially filled microfluidic channel before the optical breakdown (see Fig. 5.6(a)). After the pulsed laser-induced optical breakdown, the shock wave (dark ring), plasma (white spot) and cavitation bubble (covered by the white spot) are generated at $t = 23.2$ ns (see Fig. 5.6(b)). The bubble and shock are still expanding at $t = 48$ ns (see Fig. 5.6 (c)) and the air-liquid interface is kept at the same location. When the shock reaches the interface at $t = 74.2$ ns (see Fig. 5.6 (d)), it is reflected as a tension wave at $t = 97.9$ ns due to the acoustic impedance mismatch between air and water (the white dash line in Fig. 5.6 (e)). In the small region close to the air-water interface, DI water is ruptured with the nucleation of a cloud of vapor bubbles (see Fig. 5.6 (e)). The region is defined as the nucleation region, which is the region between the deformed air-water interface and the reflected shock (the region between the white dash line and the white dot line in Fig. 5.6(e)). A single nucleus is difficult to be distinguished and only a few nuclei are distinct in the margin of the dark region. When the pressure decreases quickly from 0.1 MPa to -100 MPa, the visible bubbles grow to a visible size (approximated 1 mm) only in a few nanoseconds [290]. The nucleation region expands bigger at $t = 124.5$ ns and more nucleation nuclei are observed (see Fig. 5.6(f)).

5.3.3 Tensile strength measurement

5.3.3.1 Shock pressure measurement

Based on the theoretical analysis in the previous section, the shock pressure can be calculated using Eq. 5.4 by measuring the velocity of the projected air-water interface v and the shock wave propagation speed u . From Eq. 5.5, three parameters including the displacement of the interface D , the recording time t_r , and the compression time t_c have to be measured in order to calculate the velocity v . The displacement D is determined by comparing the micrographs before and after the optical breakdown. The recording time t_r is determined by measuring the time delay between the infrared and green laser pulses. Detailed information of the measurement of the time delay between the infrared and green laser pulses is provided in the Appendix B. The compression time t_c is determined by measuring the shock speed u and the standoff distance SD . The standoff distance SD is determined by image analysis of the micrograph before the optical breakdown. The shock speed u can be determined by measuring the radii of the shock at different time points, which are shown in Fig. 5.5. The standoff distance SD and the shock speed u as a function of time can be expressed as

$$SD = a_0 t_1 + b \times \ln((t_1 + c)/d) \quad (5.7(a))$$

$$u = a_0 + b/(t_1 + c) \quad (5.7(b))$$

where a_0 is the speed of sound in the liquid medium, and b , c and d are fitting parameters.

Figure 5.7: Comparison of the experimental and simulated results of the shock velocity as a function of time.

Figure 5.8: Comparison of the experimental and simulated results of the shock velocity as a function of the shock radius.

The comparisons of the experimental and simulated results of the shock velocity as functions of time and shock radius are shown in Fig. 5.7 and Fig. 5.8, respectively. When the recording time is longer than 30 ns or the standoff distance

is larger than 74 μm , the deviation of the experimental result from the simulation results is less than 6%. In the experiments, the recording time for all micrographs is longer than 30 ns and the standoff distance for all micrographs is larger than 74 μm . Therefore, the estimated results of the shock speed based on Fig. 5.5 are directly used for the estimation of the shock pressure. For a high viscous liquid, such as glycerol, the simulation for the shock velocity is extremely difficult. Therefore, the shock velocity is estimated based on the experimental results of the cavitation bubble and shock dynamics.

Micrographs of the rupture of the DI water for the shock pressure measurement after the optical breakdown are shown in Fig. 5.9. To keep the tension time to be approximately 40 ns, different lengths of the optical fibers are chosen for different standoff distances. The standoff distances are (a) 34.0 μm , (b) 51.1 μm , (c) 75.7 μm , (d) 117.8 μm , (e) 135.3 μm and (f) 159.9 μm , respectively. The elongation of the cavitation bubble towards the air-water interface is observed due to the effects from the reflected tension wave at $t = 48$ ns and $t = 58.8$ ns (see Fig. 5.9(a) and (b)). The cavitation bubble is not elongated with the increase distance increase after $t = 74.2$ ns (see Fig. 5.9(c)). The nucleation region becomes larger and larger at $t = 97.9$ ns, 108.1 ns and 124.5 ns (see Fig. 5.9(d), (e) and (f)). Based on the micrographs, the displacement of the deformed air-water interface is measured and the corresponding shock peak pressure is estimated. The comparison of the experimental and simulation results of the shock pressure with various standoff distances are plotted in Fig. 5.10. For the standoff distance ranging from 30 to 300 μm , the simulation results can be expressed as $P(r) = 5.0 \times 10^4 r^{-1.25}$, where r is the standoff distance in micrometer. The experimental results agree very well with the simulation results.

Figure 5.9: Micrographs of the DI water rupture with the nucleation of a cloud of vapor bubbles with different standoff distances. The scale bar is 50 μm .

Figure 5.10: Comparison of the experimental and simulation results of the shock pressures with different standoff distances.

The sources of the uncertainty of the measured shock pressure consist of the errors from the measurement of the displacement of the deformed air-water interface D , the measurement of the standoff distance SD and the measurement of the recording time t_r ; and the error of the parameter estimations for the shock speed u . When the standoff distance is small, the main source of error is from the parameter estimations for the shock speed, which decay quickly with the increase of the standoff distance. For a larger standoff distance, the main source of error is the measurement error of the displacement of the deformed air-water interface.

In the measurement of the displacement of the air-water interface, the vapor bubble next to the air-liquid interface may also affect the measurement of the displacement. The vapor bubble may induce an overestimation of the displacement of the deformed interface D . In the measurements, the velocity of

Figure 5.11: Measured velocity of the deformed air-water interface decreases with the increase of the time after the shock reflected.

the deformed interface is underestimated. The measured velocity decreases with the increase of the time period under the tension as shown in Fig.5.11. Under the same conditions, the tension time is longer, and consequently the deviation is larger. In Fig. 5.10, the tension time for all seven experimental results is controlled to be approximately 40 ns to minimize this effect by adjusting the length of the optical fiber for the guiding of the illumination green laser.

The effect of the curvature of the air-water interface is also studied experimentally. Figure 5.12 shows micrographs of water rupture for three different curvatures of air-water interfaces, including (a) concave, (b) flat and (c) convex. The standoff distance is 136.8 μm and the recording time is 97.9 ns. By comparing the vapor bubbles in the three micrographs with different curvatures, they are similar. Therefore, it is concluded that the difference in the curvature of the air-water interface may do not affect the rupture of water.

(a)

(b)

(c)

Figure 5.12: Micrographs of water rupture with three different curvatures of air-water interface. The scale bar is 50 μm .

5.3.3.2 Critical distance and tensile strength measurement

Figure 5.13: Micrographs of rupture of water with various standoff distances. The scale bar is 50 μm .

With the same experimental conditions, the shock pressure decreases with the increase of the standoff distance (see Fig. 5.10). When the standoff distance is decreased to the critical standoff distance, the shock pressure is decreased to the critical shock pressure and the reflected tensile wave is not sufficiently negative to rupture DI water. And nucleation bubbles cannot be observed. Micrographs of the rupture of water with various standoff distances are shown in Fig. 5.13. When the standoff distance increases from (a) 220.7 μm , (b) 231.0 μm , (c) 242.4 μm and (d) 252.0 μm to (e) 260.2 μm , the cavitation nucleation in the nucleation region decreases gradually and finally no cavitation nucleation when the standoff distance reaches 260.2 μm (see Fig. 5.13(e)). Based on the statistics of the nucleation as plotted in Fig 5.14, when the tensile pressure increases from -45.7 MPa to -33.3 MPa, the number of dark pixels (nucleation bubbles) in the nucleation region decreases from 13736 to 0. When the tensile pressure is more negative, more cavitation bubbles are observed. When the tensile pressure is larger than -33.3 MPa, no cavitation bubbles are observed

Figure 5.14: Statistics of the number of the dark pixels (nucleation bubbles) corresponding to the tensile pressure increasing from -45.7 MPa to -33.3 MPa.

(see Fig. 5.13(e)). Therefore, the critical distance for DI water is determined at 260.2 μm . Here, the critical shock speed u_{sc} is 1573.3 m/s, the critical velocity of the air-water interface v_c is 42.4 m/s and the density of water at room temperature is 998 kg/m^3 . Therefore, the tensile strength of DI water at room temperature is -33.3 ± 2.8 MPa.

The current measurement of the tensile strength of water is compared with other experimental measurements, which gives the most negative values, as shown in Table 5.1. The extent of water under tension $(V\tau)^{-1}$ is $1 \times 10^{-11} \text{ mm}^{-3}\text{s}^{-1}$ for the current measurement, where V is defined as the volume of the nucleation region and τ is defined as the characteristic time of DI water under the tension wave [181, 196]. This measurement value is comparable with that from Ref [196] with the similar $(V\tau)^{-1}$ value. The tensile strength for water is predicted at -60 ± 5 MPa from the similar method but based on the numerical simulation [241]. This value is more negative than the current measurement. This may

result from the substantially underestimated velocity of the deformed air-water interface is due to the fact that the tension time is much longer than that discussed in Fig 5.11.

Table 5.1 Comparison of different tensile strength measurement for DI water.

Method	TS (MPa)	$(V\tau)^{-1}$ ($\text{mm}^{-3}\text{s}^{-1}$)	T ($^{\circ}\text{C}$)	Ref
Berthelot	-18.5	4.3×10^{-3}	53	293
Centrifugation	-27.7	2.6×10^{-1}	10	187
Acoustic	-27	3.3×10^{-10}	25	294
Inclusions	-140	2.4×10^5	40	185
Shock refelction	-33.3	1×10^{-11}	20	This work

5.3.4 Tensile strength measurement of glycerol

In this section, the tensile strength of glycerol is measured based on the technique developed in the previous section. Due to the high viscosity, the simulation of the shock speed will be extremely difficult. Therefore, it is appropriate to estimate the shock speed in glycerol only based on the experimental results. This section presents the viscous effects on the bubble dynamics, the shock pressure and the tensile strength measurement of glycerol.

5.3.4.1 Viscous effects on bubble dynamics

Micrographs of the shock and cavitation bubble in DI water and glycerol are shown in Fig. 5.15. The recording time is 48 ns. It is observed that the radius of the shock in the glycerol droplet of 133.4 μm is obviously larger than that in the DI water droplet of 110.7 μm , which is because the shock speed in glycerol is larger than that in water. The comparison of the radii of the cavitation bubble in the DI water droplet and the glycerol droplet is plotted in Fig. 5.16. The radii of the

Figure 5.15: Micrographs of the plasma, shock and cavitation bubble in different liquids: (a) DI water and (b) glycerol, respectively. The scale bar is 50 μm .

Figure 5.16: Comparison of the radii of the cavitation bubble in water and glycerol.

Figure 5.17: Comparison of the radius of the cavitation bubble in liquids with different viscosities.

cavitation bubbles in the DI water droplet are always larger than that in the glycerol droplet. The comparison of the radius of the cavitation bubble in the liquids with different viscosities is plotted in Fig. 5.17. The recording time is 48 ns. When the viscosity of the liquid is increased, the radius of the cavitation bubble is decreased,

i.e., the bubble growth is hindered by the liquid viscosity. The viscosity effects are also studied by using the numerical simulation based on Rayleigh-Plesset equation and the optical beam detection experiments [299]. The viscosity dampens the mechanical power in both the expanding and collapsing processes of the cavitation bubble. Therefore, when the viscosity of the liquid is higher, the maximum radius of the cavitation bubble and its growth rate is smaller. But the life time of the cavitation bubbles are increased.

5.3.4.2 Tensile strength of measurement of glycerol

Micrographs of glycerol rupture with the nucleation of vapor bubbles are shown in Fig. 5.18. The standoff distance is 135.6 μm . The air-glycerol interface (dark line) is formed in the partially filled microfluidic channel before the optical breakdown (see Fig. 5.18(a)). After the pulsed laser-induced optical breakdown, the shock wave (dark ring), plasma (white spot) and cavitation bubble (covered by the white spot) are generated at $t = 23.2$ ns (see Fig. 5.18(b)). The bubble and shock are still expanding at $t = 48$ ns (see Fig. 5.18(c)) and the air-glycerol interface is kept at the same location. The shock reaches the interface at the time between $t = 48$ ns and $t = 74.2$ ns. It is reflected as a tension wave due to the acoustic impedance mismatch between air and glycerol at $t = 74.2$ ns (see Fig. 5.18(d)). In the nucleation region, glycerol is ruptured with the nucleation of a cloud of the vapor bubbles (see Fig. 5.18 (d) to (f)). A single nucleus is difficult to be distinguished and only a few nuclei are distinct in the margin of the dark region. The nucleation region expands bigger and more nucleation nuclei are observed at $t = 124.5$ ns (see Fig. 5.18(f)).

Figure 5.18: Micrographs of glycerol rupture with the nucleation of a cloud of vapor bubbles. The scale bar is 50 μm .

Figure 5.19: Micrographs of the rupture with a cloud of vapor bubbles in (a) DI water and (b) glycerol. The scale bar is 50 μm .

The micrographs of the rupture of DI water and glycerol with a cloud of vapor bubbles are compared as shown in Fig. 5.19, respectively. The standoff distance is 117.8 μm and the recording time is 97.9 ns. It is obvious that the size of the nucleated bubbles in glycerol is rather small (probably nano-sized) and looks fairly homogeneous. In water, the nucleation region is extending towards the laser-induced bubble. In glycerol, on the contrary, the nucleation is limited only in the narrower region in vicinity of the interface. One possible explanation is that the viscosity may suppress (retard) the initial growth of cavitation bubble [206], while the effects on the relaxation time for homogeneous nucleation may account for the phenomenon [209-210].

Micrographs of glycerol rupture for the shock pressure measurement after the optical breakdown are shown in Fig. 5.20. To keep the tension time to be approximately 40 ns, different lengths of optical fibers are chosen for different standoff distances. The standoff distances are (a) 36.4 μm , (b) 58.8 μm , (c) 89.5 μm , (d) 141.4 μm , (e) 164.3 μm and (f) 195.3 μm , respectively.

Figure 5.20: Micrographs of glycerol rupture with the nucleation of a cloud of vapor bubbles with different standoff distances. The scale bar is 50 μm .

Figure 5.21: Measured results of shock peak pressure with various standoff distances.

The elongation of the cavitation bubble towards the air-glycerol interface is observed due to the effects from the reflected tension wave at $t = 48$ ns (see Fig. 5.20(a)). The cavitation bubble is not elongated with the increased distance at $t = 58.8$ ns (see Fig. 5.20(b)). The nucleation region becomes larger and larger at the time $t = 74.2$ ns, 97.9 ns, 108.1 ns and 124.5 ns (see Fig. 5.20(c), (d), (e) and (f)). Based on micrographs, the displacement of the deformed air-glycerol interface is measured and its corresponding velocity is estimated. Then, the shock peak pressure is calculated using eq (5.6). The measurement results of the shock wave pressures of glycerol with different standoff distances are plotted in Fig. 5.21. The shock pressure decreases with the increase of the standoff distance.

When the standoff distance is 195.3 μm , no nucleation bubble is observed (see Fig 5.20(f)). Therefore, the tensile strength of glycerol at 20 °C is -59.8 ± 10.7 MPa. The current measurement is comparable with the values in earlier

researches [191-193]. The earliest measurement value is -6.3 MPa using the modified Bullet-piston method [191]. It have also been measured based on the reflected shock methods and estimated as -25 MPa [192], -60 MPa [194] and -81 MPa [193] at 20 °C.

5.4 Effects of impurities

The measured the tensile strength of water is much lower than the theoretical prediction as discussed in the previous section. This discrepancy may be attributed to the existence of the cavitation nuclei in water. Microparticles and surfactants in water both may affect the presence of the cavitation nuclei. In this section, the effects of repetitive tensile waves, microparticles and surfactants on the cavitation nucleation are studied.

5.4.1 Effects of repetitive tensile waves

In this section, the effect of repetitive tensile waves on the cavitation nucleation of DI water is investigated using the microfluidic chip. Micrographs of the cavitation nucleation with different time interval between two consecutive shots are shown in Fig. 5.22. The standoff distance is 136.8 μm and the recording time is 97.9 ns. Three group experiments with different time intervals from 5, 30 to 60 minutes are carried out. In one group experiment, 5 consecutive lasers shooting are carried out with the same liquid sample. It is found that the number of the cavitation nucleation increases per shot. But the growth rate decreases with the increase of the time interval between two shots.

The ratio of dark pixels (avitation nucleation) to the nucleation region (white dash line) is plotted in Fig. 5.23. For these three groups, the ratios are almost the same at the first shot. However, the ratio for the group with the time interval of 5 minutes at the second shot is obviously higher than that for the groups with the time interval of 30 and 60 minutes. It may be accounted for the life time of vapor bubbles. It is believed that the life time of the nucleation bubbles is larger than 5 minutes and they will be nucleated in the next shot. When the time interval is

Figure 5.22: Micrographs of the cavitation nucleation with different time interval.

The scale bar is 50 μm .

Figure 5.23: Statistics of the ratio of the dark pixel to the nucleation region for the corresponding micrographs in Fig. 5.22.

increased to 30 minutes, parts of nucleation bubbles dissolve in water and the number of nucleation bubbles, which is nucleated in the next shot, is decreased. When the time interval is increased to 60 minutes, most of the nucleation bubbles dissolve in water and only a few nucleation bubbles are nucleated in the next shot. Due to the increase of the cavitation nucleation with the number of shot, the liquid samples in the investigation of the microparticles and surfactant effects should be refreshed for each new shot.

5.4.2 Effects of microparticles

The effect of microparticles on the cavitation nucleation is investigated in this section. Micrographs of water rupture without/with microparticles are shown in Fig. 5.24. The standoff distance is $136.8\ \mu\text{m}$. The concentration of the microparticle solution with the polystyrene microparticles of $1\text{-}\mu\text{m}$ diameter(Fluoro-Max, Thermo

Figure 5.24: Micrographs of water rupture without/with polystyrene microparticles.

The scale bar is $50\ \mu\text{m}$.

Figure 5.25: Micrographs of the rupture of DI water with 1- μm polystyrene microspheres. The scale bar is 50 μm .

Scientific) is 1.8×10^{12} beads/L. At $t = 23.2$ ns, 48.0 ns and 74.2 ns, the shock is still expanding and does not reach the interface. The shock has been reflected as the tensile wave at $t = 97.9$ and 124.5 ns. Both water and the microparticle solution are ruptured but more nucleation vapor bubbles can be observed in the microparticle solution.

To enhance the difference, the standoff distance is enlarged to 260.2 μm and the recording time is 246.9 ns. DI water cannot be ruptured based on the previous study at this distance as shown in Fig. 5.25(a). For microparticle solutions with the

Figure 5.26: Statistics of the number of the dark pixels (nucleation bubbles) corresponding to the micrographs in Fig. 5.25.

concentrations of 1.8×10^{10} and 5.5×10^{10} beads/L, individual vapor bubbles can be distinguished (see Fig. 5.25(b) and (c)). With the concentration beyond 1.8×10^{11} beads/L, individual bubbles cannot be distinguished from each other (see Fig. 5.25(d), (e) and (f)).

To statistically study the cavitation nucleation, the number of dark pixels (nucleation bubbles) in the nucleation region is plotted in Fig. 5.26 as a function of the microparticle concentration. When the concentration is increased from 1.8×10^{10} , 5.5×10^{10} , 1.8×10^{11} , 5.5×10^{11} to 1.8×10^{12} beads/L, the number of dark pixels increases from 115, 1524, 2882, and 7962 to 10179, respectively. Therefore, more nucleation bubbles occur with more microparticles in the solution.

Figure 5.27: SEM photograph of one polystyrene microsphere with the diameter of 1 μm . The scale bar is 200 nm.

As discussed in the literature survey, polystyrene microparticles may bring more nuclei to the solution. A scanning electron microscope (SEM) photograph of one polystyrene microparticle is shown in Fig. 5.27. For the SEM sample preparation, the original solution with polystyrene microspheres (1% solid, Thermo Scientific) is diluted by 1000 times with DI water. Then, the diluted solution is dropped on the silicon wafer substrate and put into the dry cabinet (DHC-200, Digi-Cabi) for 24 hours. The photograph is obtained using a scanning electron microscope (JSM-6700F, JEOL). Even though the fine structure on the surface of the microsphere cannot be clearly identified in this photograph, it is believed that the rough-structure on the particle surface may bring more nuclei to increase cavitation nucleation in microparticle solutions with higher concentrations.

5.4.3 Effects of surfactant

To investigate the surfactant effects on water rupture, an anionic surfactant sodium dodecyl sulfate (SDS) is used in this section. It is an organic compound with the formula $\text{CH}_3(\text{CH}_2)^{11}\text{OSO}_3\text{Na}$. Its molecular structure is shown in Fig. 5.28, which consists of a hydrophobic head and a 12-C hydrophilic tail. Its molar mass and density are 288.372 g/mol and 1.01 g/cm³, respectively. In the experiments, the SDS should be properly dissolved in DI water. In this case, 9.15 mg SDS (Hoefer) is dissolved in 10 mL DI water to get the SDS solution with a concentration of 3.2×10^{-3} mol/L, which is smaller than the critical micelle concentration (CMC)

Figure 5.28: Molecular structure of sodium dodecyl sulfate. Note on the large hydrophilic head and the 12-C hydrophobic tail.

Figure 5.29: Comparison of the cavitation nucleation without/with the ultrasonic treatment. The scale bar is 50 μm .

Figure 5.30: Surface tension of the various concentrations of the aqueous SDS solutions.

of 8.2×10^{-3} mol/L for SDS. To enhance the dissolution process, a heating process at 50 °C for 20 minutes is applied.

Another method to enhance the SDS dissolution to water is the ultrasonic treatment process. Micrographs of the cavitation nucleation without/with the ultrasonic treatment are shown in the Fig. 5.28. The standoff distance is 136.8 μm and the recroding time is 97.9 ns. More nucleation bubbles are formed in the water with a 10-minute ultrasonic treatment (D200H, MRC). One possible reason is that the ultrasonic treatment may bring more tiny free gas bubbles into DI water. Therefore, the ultrasonic treatment process cannot be applied in the dissolving process.

Surface tensions of the aqueous SDS solutions for various concentrations are plotted in Fig. 5.30. When the concentration of the aqueous SDS solution is increased, the surface tension is decreased [300]. Based on the Church's

Figure 5.31: Micrographs of the SDS solution rupture with different concentrations. The scale bar is 50 μm .

homogeneous nucleation model [201], the probability of the cavitation nucleation events increases with the decrease of the surface tension of the solution. This means that with the decrease of the surface tension of the aqueous SDS solutions, more cavitation nucleation will be observed.

Micrographs of the rupture of SDS aqueous solutions with various concentrations are shown in Fig. 5.31. The standoff distance is 136.8 μm the recording time is 97.9 ns. The control case is corresponding to the DI water (see Fig. 5.31(a)). The concentrations of the SDS solutions are (b) 3.2×10^{-6} , (c) 1.0×10^{-5} , (d) 3.2×10^{-5} , (e) 1.0×10^{-4} , (f) 3.2×10^{-4} and (g) 1.0×10^{-3} mol/L, respectively. When the concentration of the SDS solution is increased, the formed nucleation vapor bubbles are increased as well. The ratio of the area of dark pixels (nucleation bubbles) for the nucleation region is plotted in Fig. 5.32. When the concentration of the SDS solution is increased, the ratio is also increased. It indicates that more cavitation nucleation bubbles occur for higher concentration aqueous solutions with lower surface tension.

To enhance the difference, micrographs of the rupture of SDS aqueous solutions with a larger standoff distance are shown in Fig. 5.33. The standoff

Figure 5.32: Ratio of the dark pixels (nucleation bubbles) to the area of the shock reflected region (shown by the white dash line in Fig. 5.31(a)) vs. the concentration of the aqueous SDS solutions.

distance is 260.2 μm and the recording time is 246.9 ns. DI water cannot be ruptured based on the previous study as shown in Fig. 5.33(a). When a small amount of SDS is added to the DI water, there are no nucleation bubbles at the concentration 3.2×10^{-6} mol/L (see Fig. 5.33(b)). When the SDS concentration is increased to 1.0×10^{-5} mol/L, one nucleation bubble is observed (see Fig. 5.33(c)). With the increase of the concentration of the SDS aqueous solution, more and more nucleation bubbles are observed (see Fig. 5.33(d), (e), (f) and (g)). It is also observed that from low to moderate concentration (1.0×10^{-5} mol/L to 1.0×10^{-4} mol/L), individual bubbles can be identified. Beyond 3.2×10^{-4} mol/L, individual bubbles cannot be identified from each other (see Fig. 5.33 (f) and (g)).

Figure 5.33: Micrographs of the rupture of SDS aqueous solutions. The scale bar is 10 μm .

Figure 5.34: Statistics of the number of the dark pixels (nucleation bubbles) vs. concentration of the aqueous SDS solutions.

To statistically study the cavitation nucleation, the number of dark pixels (nucleation bubbles) in the nucleation region is plotted in Fig 5.34. With the concentration increases from 1.0×10^{-5} mol/L to 1.0×10^{-3} mol/L, the number of the dark pixels increases from 75 to approximately 8000. It is concluded that when the concentration of SDS is increased, more cavitation nuclei may be introduced into the SDS solution.

5.5 Summary

This chapter focuses on shock pressure and tensile strength measurement of liquids using the microfluidic channel, including theoretical analysis, microchannel design, experimental setup and experimental results of shock pressure and tensile strength measurement of liquids. The theoretical analysis explores the principle of shock pressure and tensile strength measurement of liquids. Subsequently, the experimental setup and the laser energy measurement are presented and discussed. The experimental results of shock pressure and tensile strength measurement of DI water and glycerol are presented and discussed, including the photography of the homogeneous nucleation, the shock peak pressure measurement with various standoff distances and the tensile strength measurement of DI water and glycerol. Finally, the experimental results of the effects of repetitive of tensile waves, microparticle and SDS surfactant on the cavitation nucleation are presented and discussed.

The innovation of the developed controlled dynamic stressing in microfluidic chips is summarized as follow,

- a) The experimental approach to measure the shock pressure and the tensile strength of DI water and glycerol using the microfluidic channel is demonstrated for the first time. The shock pressure values for different standoff distances are experimentally measured and the tensile strength is determined using the critical standoff distance method. The tensile strengths of DI water and glycerol at 20 °C are -33.3 ± 2.8 MPa and -59.8 ± 10.7 MPa, respectively.

- b) The effect of repetitive tensile waves is explored. The experimental result indicates both the shot number and the time interval between two consecutive shots affect the cavitation nucleation. When the time interval is longer or the shot number is larger, the number of the cavitation nucleation is higher.
- c) The effects of microparticle and the SDS on the water rupture are investigated. More cavitation bubbles are observed with a higher concentration of the microparticle solution and microstructures on the surface of microparticles may bring more cavitation nuclei. SDS affects the liquid properties, such as the surface tension, which significantly affects the cavitation nucleation. With a higher SDS concentration, the surface tension is lower and more cavitation nucleation bubbles are observed.
- d) The developed tensile strength measurement method has several advantages, such as a small amount of liquid volume and the low probability of the heterogeneous nucleation. It can be applicable for the shock pressure and tensile strength measurement of high viscous liquids as well.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Three different microfluidic systems, i.e. microfluidic systems for droplet fusion, single-cell membrane poration, and tensile strength measurement of liquids, driven by pulsed laser induced cavitation bubbles have been innovated. Various aspects of this research, including theoretical studies, microfluidic designs, fabrication process, experimental results and discussion, have been presented. The contents of this thesis cover mainly the theoretical analyses and the experiments of the three successfully designed and integrated pulsed laser induced cavitation bubbles driven microfluidic systems. The major conclusions of the research drawn are listed here.

The microfluidic system for droplet fusion is studied theoretically, fabricated and experimented. The working principle of employing pulsed laser induced cavitation bubbles to actuate droplet fusion is discussed thoroughly. A microfluidic chip with a T-junction is designed and fabricated to generate droplets and an experimental setup is developed for cavitation bubble generation and high speed imaging. Experimental results of the bubble dynamics in bulk liquid, two parallel immiscible flows and a single droplet are demonstrated. Using the developed droplet fusion system, droplets under different conditions are fused and experimentally demonstrated, including the fusion of the static and moving droplets,

the fusion of the droplets of equal and unequal sizes and the fusion of multiple droplets in a hexagonal structure. In addition, the diffusion dominant mixing between two fusing droplets and the fusion of droplet with single cells are also presented and discussed.

- (i) It is demonstrated the first time to fuse droplets using laser-induced cavitation bubbles in microfluidic systems. Fast on-demand droplet fusion is realized via the transient and rapid flow from a short-lived laser-induced cavitation bubble inside one of the droplets. Droplet fusions under different conditions are demonstrated and the neck growth between two equal-sized fusing droplets is discussed.
- (ii) Unlike other fusion techniques in microfluidics, the developed technique can not only be employed in two equal-sized droplets fusion, but also be employed in the fusion of two unequal-sized droplets, droplets in a continuous stream and multiple droplets in a hexagonal structure. The potential applications are presented with the mixing of two reagents and the merging of droplets with an encapsulated cell.
- (iii) The developed microfluidic system for droplet fusion has several advantages, including a good controllability in time and space, simple fabrication process as only optical access is necessary and fast actuation leading to the rapid droplet fusion.

The microfluidic system for single-cell membrane poration is designed, and fabricated. The experiment for single-cell membrane poration is carried out. The theoretical analysis explores the microjet formation due to the asymmetrical collapse of laser-induced cavitation bubbles and the mechanism of single-cell membrane poration using the formed microjet. A microfluidic chip with an array of

cell trapping structures is designed and fabricated to trap single cells in the flowing liquid medium and the single cell trapping is demonstrated. An experimental setup is built for forming cavitation bubble and high speed imaging. Experimental results of the microjet formation, single particle projection and flow field visualization using particle tracking are demonstrated. Using the microjet, experimental results of the single trapped cell membrane is deformed and the most possible poration location with the highest strain is predicted using the numerical simulation. The cell membrane restoration is observed and demonstrated and the cell membrane poration is probed by the transport of trypan blue molecules through diffusion, which is further validated using the numerical simulation of the diffusion process.

- (i) A highly controllable contact-free technique for single-cell membrane poration using laser-induced cavitation bubbles is demonstrated for the first time. The membrane poration of single Myeloma cell is demonstrated, which is validated by the transport of trypan blue molecules through diffusion.
- (ii) The demonstrated cell poration method provides a fast and localized solution to control single-cell membrane poration. It provides a feasible approach to integrate the technique into automated microfluidic total analysis systems and high throughput systems.

The microfluidic system for shock pressure and tensile strength measurement of liquids is designed, fabricated and experimented. The theoretical analysis explores the principle of shock pressure and tensile strength measurement of liquids. An experimental setup is built for cavitation bubble generation and imaging. Experimental results of shock pressure and tensile strength measurement of DI water and glycerol is presented and discussed, including photography of

homogeneous nucleation, shock peak pressure measurement with different standoff distances and tensile strength measurement using the critical standoff distance method. Furthermore, experimental results of the effects of repetitive tensile waves, microparticle and SDS surfactant on the nucleation bubbles are presented and discussed.

The innovation of the developed microfluidic system for shock pressure and tensile strength measurement of liquids is summarized as follow,

- (i) An experimental approach to measure shock pressure and tensile strength of DI water and glycerol in a microfluidic chip is the firstly demonstrated. The shock pressure values for different standoff distances are experimentally measured. The tensile strength can be determined using the critical standoff distance method. The measured tensile strengths of DI water and glycerol at 20 °C are -33.3 ± 2.8 MPa and -59.8 ± 10.7 MPa, respectively.
- (ii) Effect of repetitive tensile waves on the nucleation bubble in nucleation region is demonstrated using a microfluidic chip. The experimental result indicates that both the shot number and the time interval between two consecutive shots affect cavitation nucleation bubbles. When the waiting time is longer, the ratio of increasing nucleation bubbles is lower.
- (iii) An experimental approach to investigate the controlled impurities effects using a microfluidic chip is demonstrated for the first time. The microparticle and SDS surfactant effects on cavitation nucleation are investigated. Microstructures on the surface of the microparticle may bring more cavitation nuclei, and more cavitation bubbles are observed with

higher concentration of microparticle solutions. The SDS surfactant affects the liquid properties, such as the surface tension, which significantly affect the cavitation nucleation. With a higher SDS concentration, surface tension is lower and more cavitation nucleation bubbles are observed.

- (iv) The developed shock pressure and tensile strength measurement method has several advantages, which include a small amount of liquid volume is needed, reducing the impurities effect and decreasing the probability of the heterogeneous nucleation, and it can be applicable for high viscous liquid.

To further improve the three developed microfluidic systems, several future works with relevant recommendations are described in the following section.

6.2 Recommendations

Recommendations for future research are summarized as follows

- a) In the development of the cavitation bubble-driven microfluidic system for droplet fusion, the viscosity of the liquid is one of the most important factors that affect the cavitation bubble dynamics. The viscosity of water (disperse phase) is lower than that of oil (continuous phase). If the viscosity of continuous phase is lower than that of disperse phase, such as hydrofluoroether (HFE) fluid, the dynamics of the cavitation bubble may be totally different.
- b) With the developed microfluidic systems for single-cell membrane poration, it can be extended to porate other types of cells. This system can also be improved by the integration with the images from the scanning electron microscope (SEM) for the porated cells. With the special treatment for the porated cell membrane, the porated holes on the membrane can be clearly observed. Another important factor is the cell viability. One can design a new cell trapping chip to conduct more membrane poration experiments and cell viability testing after membrane poration.
- c) In the development of the microfluidic system for shock pressure and tensile strength measurement of liquids, the tensile strength of high viscous liquids can be measured. If one can measure a series of liquids with increasing viscosity, it can give more insights about the relationship between the viscosity and the tensile strength.
- d) In this thesis, the developed microfluidic systems driven by pulsed laser-induced cavitation bubbles are mainly focused on droplet fusion, single-cell membrane poration and shock pressure and tensile strength measurement. It

is highly recommended to develop further more microfluidic systems for droplet and cell manipulation, such as droplet splitting, cell lysis etc. This will expand the usefulness of the microfluidic systems driven by pulsed laser-induced cavitation bubbles for different applications.

APPENDIX A

BOUNDARY ELEMENT METHOD

The cavitation bubble is usually oscillating very rapidly, such that the flow created by it can, as a good approximation, be considered as potential flow [275-277]. There exists then a velocity potential such that the Laplace equation is valid in the fluid domain, $\nabla^2\phi = 0$, and the velocity vector is given by $\underline{u} = \nabla\phi$. Since the Laplace equation is elliptic, the solution anywhere in the fluid domain is fully determined when the potential or its normal derivative $\partial\phi/\partial n = \underline{n} \cdot \nabla\phi$ are given on the boundaries (with $\underline{n}$ being the normal vector pointing out of the fluid). Then, a boundary integral formulation can be written as [278, 279]

$$c(\underline{x}_0)\phi(\underline{x}_0) + \int_S \phi(\underline{x}) \frac{\partial G(\underline{x}_0, \underline{x})}{\partial n} dS = \int_S \frac{\partial \phi(\underline{x})}{\partial n} G(\underline{x}_0, \underline{x}) dS \quad (\text{A.1})$$

where G is the free field Green function $G(\underline{x}_0, \underline{x}) = 1/|\underline{x} - \underline{x}_0|$, $\underline{x}_0$ is a fixed point (in practice one node of the numerical mesh) and $\underline{x}$ is the integration variable situated on S . The S consists of two surfaces, the bubble surface and the solid surface. An axisymmetrical implementation [276] is used with a linear representation of the potential and the normal velocity on S . If $\underline{x}_0$ is situated on the surface S , c represents the solid angle at that location. If $\underline{x}_0$ is located in the fluid domain, then $c = 4\pi$. After discretizing the surfaces of the bubble and the solid (51 nodes each), a matrix equation relating all the potentials and its normal derivative results in ($\underline{\phi}$ and $\underline{\partial\phi/\partial n}$ represent vectors with the potential and its normal derivative for each node)

$$\underline{G} \cdot \partial \phi / \partial n = \underline{H} \cdot \phi \quad (\text{A.2})$$

and if $\underline{x}$ is situated on the surface S , Eq. A.1 can be rewritten as [279]

$$\int_S [\phi(\underline{x}) - \phi(\underline{x}_0)] \frac{\partial G(\underline{x}_0, \underline{x})}{\partial n} dS = \int_S \frac{\partial \phi(\underline{x})}{\partial n} G(\underline{x}_0, \underline{x}) dS \quad (\text{A.3})$$

The above formulation is equivalent to stating that each diagonal element of the matrix $\underline{H}$, is equal to 4π minus the sum of the other elements of that row. This also eliminates the need to calculate the solid angle, c .

On the solid surface, $\partial \phi / \partial n = 0$ (non-penetrating condition or normal velocity is zero). On the bubble surface, the Bernoulli equation is used, which is expressed as

$$p = p_{ref} - \rho \frac{D\phi}{Dt} + \frac{1}{2} \rho |\underline{u}|^2 \quad (\text{A.4})$$

and an adiabatic function for the bubble pressure p as:

$$p = p_0 (V_b / V_{b0})^\gamma \quad (\text{A.5})$$

where ρ is the density of the liquid (1000 kg/m³), p_{ref} is the reference pressure (1 Bar), p_0 is the initial pressure of the bubble (100 Bar), V_b is the volume of the bubble, V_{b0} is the initial volume and γ is the ratio of specific heats for the bubble contents (here a value of 1.25 is chosen). The surface tension and gravity effect have been ignored. The material derivative D/Dt in Eq. A.4 is used to get the potential on the bubble surface for the next time step. In Eq. A.2, the potential on the bubble and the normal velocity on the solid surface are now known. The unknown normal velocity on the bubble and the potential on the solid surface can then be calculated by solving the system of equations Eq. A.2. From the normal velocity on the bubble surface and the potential distribution on that surface, the velocity vector $\underline{u}$ can be calculated. With this velocity, the nodes are being updated

on the bubble surface (the solid surface does not move). For more details on the numerical implementation, see for example in Ref [276] and [277]. The initial radius of the bubble is chosen to be 0.14851 times the maximum bubble radius. Since the bubble is oscillating near a solid structure, a jet will be generated towards it [275].

The cell is modeled as being part of the fluid (i.e. the cell is assumed not to have much influence on the behavior of the bubble; its stresses are supposed to be negligible to the ones generated by the bubble and its density is close to the surrounding fluid). The potential in any part of the fluid (and thus also at the location of the cell) can be obtained after the potentials and normal velocities on both bubble and solid structure have been calculated with Eq. A.1 by putting $\underline{x}_0$ on the location of the cell and setting $c = 4\pi$. The velocity at this point can easily be obtained with $\underline{u} = \nabla\phi$ (by taking ϕ at different locations very close to $\underline{x}_0$ and calculating the gradient numerically). The cell position is updated with $\underline{x}_0^{t+\Delta t} = \underline{x}_0^t + \Delta t \underline{u}$ (Δt is the time step) and thus follows the flow field created by the interaction of the oscillating bubble and the solid structure. The cell location was monitored with 20 nodes initially, which are evenly distributed along the cell surface.

APPENDIX B

TIME DELAY OF THE OPTICAL FIBER

The green laser pulse acting as the illumination light is guided by optical fibers to the experimental regime. The time delay between the infrared and green laser pulses is dependent on the length of the optical fiber. The material of the core of the employed multimode optical fibers (FT600EMT, ThorLabs) is silica and its refractive index is measured at 1.45846 for the wavelength of 587.56 nm and at 1.44920 for the wavelength of 1.10 μm , respectively [285]. Based on the estimation from the coefficients of the glass, the estimated refractive index of the core at 532 nm is approximate 1.4614. The diameter of the silica core is $600 \pm 10 \mu\text{m}$ with a TEQS hard cladding to increase the fiber strength. The numerical aperture is 0.39 ± 0.02 and the maximum power capability for pulsed laser is 9.0 MW. The high speed photo-detector (DET10A/M, ThorLabs) is used to detect and convert the infrared (1064 nm) and green (532 nm) laser pulses to electrical signals. The digital storage oscilloscope (600 MHz 10 GS/s, 64Xi-A, LeCory WaveRunner) acts as the electro-data collection system with the input impedance 50 ohms. The experimental setup is very similar with the dynamic stressing setup except the optical path of the infrared pulse is 0.12 m longer. Therefore, the time signal is corrected by subtracting 0.4 ns.

The time delay between the infrared and green laser pulses with 30 m optical length is detected as shown in Fig. B.1. The star symbol is the experimental results and the red line is the fitted line. The infrared laser pulse arrives at the

Figure B.1: Measurement of the time delay between infrared (1064 nm) and green (532nm) laser pulses with 50-meter illumination optical fiber.

Figure B.2: Measurement of the time delay for optical fibers with different lengths from 2 to 50 meters.

detector first. Then, the green pulse arrives at the detector with the time delay of $149.2 \text{ ns} \pm 0.2 \text{ ns}$ when the green pulse propagates through the 30-meter optical fiber. The time delay is determined using a MATLAB code based on the detected signal. The detected signal is fitted using a Gaussian function first. The time delay

is defined as the time difference of the two fitting maximum. The final result is based on the average of 20 times reproducible measurements. The measurement accuracy is 0.2 ns due to the limitation of the oscilloscope.

The time delays for different lengths of optical fibers from 2 to 50 meters are measured as shown in Fig. B.2. The measured results can be fitted using a linear line. Based on the fitting data, the refractive index of the optical fiber core is estimated as 1.4944, which is larger than the data from the ThorLabs's estimation [285]. The possible reason is that the length of the optical fiber cannot be measured with high accuracy, especially for a longer optical fiber.

APPENDIX C

PHYSICAL PROPERTIES OF LIQUID SAMPLES

The physical properties of water-glycerol combinations are listed in Table C.1, which include density, dynamic viscosity and surface tension for DI water, four different combinations of water-glycerol solutions at glycerol volume ratio of 20%, 40%, 60% and 80%, and pure glycerol at 20 °C. When the volume ratio of the glycerol increases, both density and viscosity of liquids increase but the surface tension decreases.

Photographs of the side view of DI water and glycerol droplets on PDMS substrate are shown in Fig. C.1. Contact angles for both DI water and glycerol on PDMS substrate are approximately 90°. Therefore, the air-water and air-glycerol interfaces in PDMS microchannels (400 $\mu\text{m} \times 85 \mu\text{m}$) both are fairly flat as shown in Fig. C.2.

Table C.1 Physical properties of experimental water-glycerol combinations [294]

Glycerin (% by vol.)	0 (DI Water)	20	40	60	80	100 (Glycerin)
Density (g cm^{-3})	0.99823	1.0469	1.0993	1.1538	1.2085	1.2597
Dynamic Viscosity ($10^{-3} \text{ mPa}\cdot\text{s}$)	1.005	1.775	3.840	9.450	60.10	1250.0
Kinetic Viscosity ($10^{-6} \text{ m}^2\text{s}^{-1}$)	1.005	1.695	3.494	8.189	49.71	1188.739
Surface Tension ($\text{g/s}^2 \text{ cm}^{-2}$)	71.68	70.94	69.61	67.82	65.50	62.39

Figure C.1: Photographs of (a) DI water and (b) glycerol droplets on PDMS substrate. The scale bar is 1 mm.

Figure C.2: Photographs of the interfaces of (a) the air/water and (b) air/glycerol in PDMS microchannels. The scale bar is 50 μm .

AUTHOR'S PUBLICATIONS

Journal Papers

1. X. C. Li, J. Wu, A. Q. Liu, Z. G. Li, Y. C. Seow, H. J. Huang, K. Xu and J. T. Lin, "A liquid waveguide based evanescent wave sensor integrated onto a microfluidic chip," *Applied Physics Letters*, **93**, 193901 (2008).
2. Z. G. Li, Y. Yang, X. M. Zhang, A. Q. Liu, J. B. Zhang, L. Cheng, and Z. H. Li, "Tunable visual color filter using microfluidic grating," *Biomicrofluidics*, **4**, 043013 (2010).
3. Z. G. Li, K. Ando, J. Q. Yu, A. Q. Liu, J. B. Zhang and C. D. Ohl, "Fast on-demand droplet fusion using transient cavitation bubbles," *Lab on a Chip*, **11**, pp. 1879-1885 (2011).
4. Z. G. Li, A. Q. Liu, E. Klaseboer, J. B. Zhang and C. D. Ohl, "Single cell membrane poration by bubble-induced microjets in a microfluidic chip," *Lab on a Chip*, **13**, pp. 1144-1150 (2013).
5. Z. G. Li, A. Q. Liu, K. Ando, M. Arora, J. B. Zhang, and C. D. Ohl, "Measurement of optical breakdown induced shock pressure using interface displacement in a microfluidic chip," *Applied Physics Letters* (submitted).
6. Z. G. Li, A. Q. Liu, M. Arora, J. B. Zhang, and C. D. Ohl, " Experimental Investigation of Repetitive Tensile Waves, Microparticle and Surfactant Effects using Microfluidic Chip," *Physical Review Letters* (submitted).

Conference Papers (Selected)

7. Z. G. Li, K. Ando, J. B. Zhang, A. Q. Liu and C. D. Ohl, "A study of liquid dynamic rupture in microfluidics," μ TAS2012, Okinawa, Japan (2012).
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