Trapping and Removal of Bubbles in a Microfluidic Format

by

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A thesis submitted in conformity with the requirements for the degree of Master of Applied Science
Institute of Biomaterials and Biomedical Engineering
University of Toronto

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2012

Abstract

Unwanted gas bubbles are a challenge for microfluidic-based systems, as adherence to channel networks can disrupt fluid delivery. This is especially true for devices with biological applications, as the presence of a single bubble creates thin fluid films with extremely high shear stresses, which can damage biological samples. Current strategies to remove bubbles require complicated fabrication or off-chip components. This thesis describes an on-chip microfluidic strategy utilizing permeation for in-plane trapping and removal of occasional gas bubbles. The trap was demonstrated with nitrogen bubbles, which were consistently removed at a rate of 0.14 μL/min for a single trap, and shown to have long-term operation capability by removing approximately 4,000 bubbles during one day without failure. The trap was integrated with a microfluidic system for the study of small blood vessels. Experiments were complemented with analytical and numerical models to characterize the bubble removal process.
Acknowledgements

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AcH</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AoC</td>
<td>Artery-on-a-Chip</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide gas</td>
</tr>
<tr>
<td>CYTOP</td>
<td>CTL-809-A amorphous fluoropolymer</td>
</tr>
<tr>
<td>EC</td>
<td>Endothelial cell</td>
</tr>
<tr>
<td>LED</td>
<td>Light-emitting diode</td>
</tr>
<tr>
<td>MOPS</td>
<td>3-(N-morpholino) propanesulfonic acid</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen gas</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen gas</td>
</tr>
<tr>
<td>PDMS</td>
<td>Poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>PE</td>
<td>Phenylephrine</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methylmethacrylate)</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SMA</td>
<td>Subminiature version A</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
</tr>
<tr>
<td>STP</td>
<td>Standard temperature and pressure</td>
</tr>
<tr>
<td>SU-8</td>
<td>Photolithography resist</td>
</tr>
<tr>
<td>Tween (20)</td>
<td>Poly(ethylene-glycol) (20)sorbitan monolaurate (surfactant)</td>
</tr>
</tbody>
</table>
List of Symbols

\( T_i \)  Temperature, at condition \( i \)
\( w \)  Channel width
\( h \)  Channel height
\( d_h \)  Hydraulic diameter
\( d_f \)  Film thickness
\( \mu \)  Dynamic viscosity
\( Q \)  Flow rate
\( \tau_{w,i} \)  Wall shear stress, at condition \( i \)
\( U \)  Fluid velocity
\( \gamma \)  Surface/interfacial tension
\( Ca_b \)  Capillary number
\( N_i \)  Molecular flux of species \( i \)
\( P_i \)  Permeability of species \( i \)
\( p \)  Pressure
\( b_i \)  Channel wall thickness (permeation distance), at condition \( i \)
\( f \)  Focal distance
\( \beta \)  Gap thickness
\( \lambda \)  Channel wall length
\( A \)  Bubble length contacting channel wall
\( r \)  Radius
\( \xi \)  Geometric correction factor
\( J_i \)  Volumetric flux, condition \( i \)

\( V \)  Volume

\( V_B \)  Bubble volume

\( V_{B,0} \)  Initial bubble volume

\( V_N \)  Normalized bubble volume

\( D_{ij} \)  Diffusivity of species \( i \) in material \( j \)

\( t \)  Time

\( S_{ij} \)  Solubility of species \( i \) in material \( j \)

\( A \)  Cross-sectional area

\( A_x \)  Channel cross-sectional area

\( A_B \)  Bubble cross-sectional area

\( \theta \)  Contact angle

\( t_R \)  Time require for complete bubble removal

\( t_N \)  Normalized time

\( C_i \)  Concentration at condition \( i \)

\( k_H \)  Henry’s Law constant

\( S^\infty \)  Infinite dilution solubility

\( M \)  Stiff spring constant

\( K \)  Partition Coefficient
Chapter 1

Introduction

1.1 Motivation

Microfluidic systems and devices have provided miniature, flow-based alternatives to established laboratory procedures in a diversity of areas that include chemical synthesis and (bio)molecular analysis. The growing adaptation of microfluidic devices has been fuelled by their significantly reduced footprint, enhanced molecular transport, more efficient use of reagents, and increased scalability.\textsuperscript{1, 2}

Microfluidic technologies are particularly useful to a wide range of biological applications, as they offer precise spatiotemporal control over fluids in microenvironments, making them suitable platforms for investigating biological samples, as well as for developing lab-on-a-chip diagnostic devices.\textsuperscript{3, 4} Furthermore, microfluidic devices intrinsically have geometries on the length scales of cells, making them ideal tools for research in cell-biology.\textsuperscript{5, 6}

However, the adaptation of microfluidic approaches for routine biological protocols is still hindered by several technical limitations, such as scaling effects related to large surface-to-volume ratios;\textsuperscript{7} challenges interfacing with conventional laboratory processes and analytical/imaging equipment;\textsuperscript{8} as well as limitations of poly(dimethylsiloxane) (PDMS), a widely used substrate material.\textsuperscript{3} One of the characteristic difficulties of PDMS is its high permeability to gas molecules. While this allows fluids in a PDMS-based device to rapidly equilibrate to ambient gas conditions, and is thus useful in allowing aerobic cell culture, this
also leaves the microfluidic device susceptible to the generation and growth of unwanted gas bubbles.

The ability to controllably form gas bubbles in liquid-perfused microchannel networks has been beneficial in contributing to the successful translation of several bench-scale laboratory processes to miniature, flow-based alternatives. This translation was achieved in part due to uniform bubble size distributions, improved mixing behaviour, and reduced sample dispersion associated with bubble flows through microchannels. Examples include chemical and materials synthesis in continuous-flow formats, studies of cellular decision processes, and the preparation of contrast agents for ultrasound imaging.

On the other hand, unwanted bubbles present a major practical challenge that prevents the routine use of microfluidic devices in certain biological applications, particularly long-term culture of cells, organs, or organisms. High interfacial forces associated with gas bubbles passing through microfluidic channel networks give rise to high shear stresses that adversely affect adherent cells, which may even rupture cell membranes and destroy the cells. Larger physiological structures and tissues may be functionally damaged during bubble passage, or bubbles may become lodged on the loaded sample, blocking liquid and nutrient access. Additionally, bubbles can disrupt on-chip experiments by blocking flows in parallel channel networks and perturbing system stability.

Possible sources of unwanted bubbles include gas trapped in dead volumes inside fluidic connections or within the microfluidic network itself, the unintentional introduction of gas through feed lines, interface disconnects requiring interrupting operation of the device, or nucleation and subsequent growth of bubbles in supersaturated liquids (see Figure 1-1A).
Nucleation is particularly relevant in cases where liquids were initially saturated at room temperature, prior to the temperature being raised (in biomedical applications often to physiological temperature) while passing through a microchannel network. The solubility of gasses in aqueous liquids decreases with increasing temperature, which promotes the formation of gas bubbles (see Figure 1-1B). Nucleation is particularly problematic in highly integrated microfluidic systems, which would ideally place heating components on-chip, causing nucleation to occur near critical regions of interest. Most current solutions separate gas bubbles off-chip using external infrastructure, and require very tedious and manual procedures that are time-consuming and inconsistent at trapping and removing all bubbles. This particular problem calls for a novel method that operates locally near the region of interest.

Figure 1-1: The problem of nucleation in microfluidic devices. (A) Standard microfluidic devices face many potential inputs for unwanted bubbles. (B) Localized heating on-chip near regions of interest, such as culture chambers, lead to localized changes in solubility and nucleation of unwanted gas bubbles.
An ideal solution should not only trap bubbles but also remove them from the targeted liquid stream. Trapping refers to the ability to prevent bubbles from continuing to travel in a flowing liquid solution; in the case of a biological application, trapping should be consistent as the presence of a single bubble may foul the entire experiment or sample. Bubble removal implies either the phase-separation of trapped gas bubbles from a microchannel, or the transport of gas molecules into either the surrounding liquid or through the microchannel walls. Bubble removal increases the useful volume of the bubble trap available over time, by freeing up volume taken by previously trapped bubbles.

In view of the advantages of microfluidics, it was chosen as the platform of choice to develop a system for investigating small-artery function, the artery-on-a-chip (AoC). Like all microdevices used for biological investigation, bubbles became a persistent problem. For the purposes of the AoC, a microfluidic-based bubble trap that utilizes vacuum to remove bubbles was designed and characterized, that is both single-layer and has a small footprint, and therefore can be easily integrated into any existing microchannel design. The trap was integrated and tested with current AoC devices.

1.2 Background and Literature Review

1.2.1 Artery-on-a-Chip

Cardiovascular disease is the number one cause of death amongst the North American population. High blood pressure (hypertension) is a primary risk factor for several
cardiovascular diseases. Small resistance arteries (diameters 60 – 250 µm), are small vessels prior to capillaries in the vascular network that play a key role in regulating peripheral vascular resistance by dynamically changing their diameter in response to extracellular signalling cues and changes in blood pressure. They are therefore hypothesized to be critical to the development of hypertension. Despite the clinical importance of cardiovascular disease and hypertension, there is a deficit in the current understanding of small artery structure and function caused by a lack of ideal experimental tools for probing them.

Forerunners to conventional setups for the manipulation and investigation of small murine blood vessels were developed by Duling et al. Mulvany et al. in the early 1980s.\textsuperscript{17,18} Current experimental setups use similar cannulation and pressure myograph methods, which are employed to study 1-2 mm excised segments of resistance arteries. These require the insertion of glass pipettes into the luminal ends of the vessel, and the tying of surgical sutures to seal the vessel to the pipettes. These protocols are conducted manually, and thus require highly specialized training; suturing can be damaging to endothelial cells (ECs) lining the inside lumen, and smooth muscle cells (SMCs) along the outside of the vessel; and the overall methods are expensive and time-consuming. These traditional setups also lack precise spatiotemporal control of the microenvironment around the loaded vessel, as well as the experimental versatility to probe vessel segments in multiple formats. To address these issues, the microfluidics-based, AoC platform was developed.
Figure 1-2: Artery loading and fixation. (A) Schematic of a resistance artery segment consisting of ECs lining the inner wall of the vessel and SMCs wrapped around the outer wall. (B) Schematic of the microfluidic chip containing a microchannel network that includes an artery loading well and the artery inspection area. (C-E) illustrate the reversible procedures for loading, fixation and inspection of a small artery segment.¹⁹

The AoC microfluidic device was fabricated using standard multilayer soft-lithography techniques in PDMS, and assessed by loading mesenteric (gut) arteries from mice (see Figure 1-2). Functional assays were performed to determine vessel viability after loading using phenylephrine (PE), a vaso-constrictor which acts on the SMCs of blood vessels, and acetylcholine (AcH), a vaso-dilator acting on the endothelium. Results were consistent with measurements obtained in conventional cannulation systems, suggesting the survival of vessel
segments during loading of samples onto the device, and that biological function remained intact.\textsuperscript{20} To further determine the effect of the design had on the physiological structure of loaded vessel segments, special devices fabricated on thin cover slides were used to observe the vessel shape within the microchannel on a confocal microscopy setup using fluorescently labelled ECs. Further designs were developed and tested that demonstrated the ability of the AoC format to be adapted for different flow-based formats, including devices with increased degrees of spatiotemporal control over drug application. These devices showed non-uniform vessel response based on precise spatiotemporal control of drug delivery.

In summary, the AoC provides a versatile platform that is adaptable and scalable to a variety of experimental formats, allows standardization in microvascular research due to potential for automation and precise microenvironmental control, and has the potential to accelerate the discovery of antihypertensive drugs if developed into a parallel drug discovery-based format.

One commonly encountered problem on the AoC, however, was the presence of unwanted gas bubbles on-chip. These would appear despite (1) pre-purging of gas bubbles from the chip, (2) care taken when connecting fluidic interfaces, and (3) flushing of the microchannels with bovine serum albumin (BSA). Bubbles would sometimes appear from dead volumes near punched interfaces, or from unpurged channels due to the symmetric nature of the microchannel network. These bubbles would enter the superfusion or perfusion channel; once the bubble made contact with a loaded vessel segment, the artery would lose functional response to both vasoconstrictors and dilators. This would require removing the loaded vessel, thus making bubbles costly in both wasted samples and time.
1.2.2 Shear Stress and Bubbles

The shear stresses caused by passing bubbles are a particular problem for biological applications. Bubbles flowing through a well wetting liquid and having a length exceeding the width of microchannels they are passing through, are typically separated from the channel walls by a thin liquid film. This film is a region of significantly increased shear stress. The resulting high wall shear stress levels experienced by cells adhering to a microchannel wall potentially affect cell function, cause cell detachment and, at very high shear rates, lead to the rupture of cell membranes. The influence of fluid shear on adherent cells has been investigated for several cell types. Wall shear stresses induced by passing bubbles are expected to be within the range of critical values for cell detachment for fibroblasts. ECs were found to exhibit reduced adhesion strength at wall shear stresses exceeding 100 dynes/cm². The highest shear rates that cells in the vascular system are exposed to are associated with areas of locally disturbed flow and correspond to 40-50 dynes/cm².

One can estimate the magnitude by which the wall shear stress increases when a gas bubble flows through a liquid-filled microchannel. We assume the microchannel to be 200 µm wide (w) and 150 µm deep (h), leading to hydraulic diameter \( d_h \),

\[
d_h = \frac{2hw}{h+w}
\]  

of 171 µm. We further assume an aqueous solution with a viscosity of \( \mu = 6.92 \times 10^{-4} \) Pa·s (37°C) and a volumetric flow rate of \( Q = 10 \) µL/min. In the absence of a bubble, the wall shear stress for fully-developed laminar flow is

\[
\tau_{w,0} = 32 Q \mu \pi^{-1} d_h^{-3} = 0.23 \text{ Pa} = 2.3 \text{ dynes/cm}^2
\]
However, if a bubble is present, a thin liquid film covers the microchannel side walls, except for the liquid filled corners (sometimes referred to as “gutters”). With the bubble velocity $U = Q w^{-1} h^{-1}$ and the interfacial tension $\gamma \sim 0.025 \text{ N/m}$ (as with most cell culture conditions, the presence of surfactants is assumed), the capillary number, which displays the relative effect of viscous forces to surface tension, can be calculated,

$$Ca_b = \mu U \gamma^{-1} = 1.5 \times 10^{-4}$$

(3)

The film thickness can then be estimated using an analytical expression that was first introduced by Bretherton and Taylor,$^{26, 27}$

$$d_f = 0.67 Ca_b^{2/3} d_h$$

(4)

For the assumed volumetric flow rate of 10 µL/min we obtain film thickness $d_f \sim 0.33$ µm, and $\tau_{w,B} = 118 \text{ dynes/cm}^2$. The wall shear stress therefore increases by a factor of 51 due to the presence of a bubble, and such shear stresses are typically beyond critical shear stresses found in fluid flows in the body.

1.2.3 Bubble Removal Techniques

Different strategies have been proposed to trap gas bubbles in well-defined regions of a microfluidic device based on geometric confinement or on locally varying wetting properties. Yang et al. $^{28}$ used microchannels with locally different wetting properties for bubble removal. A piezoelectric transducer operated at a frequency of 49 kHz and a peak-to-peak voltage of 100 V was employed to induce nucleation and bubble growth, reducing the dissolved oxygen
concentration in the liquid by up to 50% at a liquid flow rate of 120 µL/min. A number of strategies employed phase separation, often relying on microchannels or membranes with selectively different wetting properties. Hibara et al.\textsuperscript{29} used a configuration consisting of two parallel microchannels, one of which was hydrophobic, the other hydrophilic. This allowed bubbles to enter the hydrophobic channel while liquid continued to flow in the hydrophilic channel section. An off-chip solution by Zhu et al.\textsuperscript{30} utilized a micromachined configuration with polymer membranes. An off-chip compression seal using commercial filter membranes (made of polycarbonate, polypropylene, nylon and polytetrafluoroethylene) with different wetting characteristics, and pore sizes between 0.2-10 µm, was similar to a method previously proposed for liquid-liquid extraction by Kralj et al.\textsuperscript{31} When subjecting one side of the membrane to a segmented flow and applying a differential pressure of 68.9 kPa across the membrane, complete flow separation was achieved for flow rates of up to 22 µL/min. Selva et al.\textsuperscript{32} utilized a patterned resistive heater to establish an 11 K/m temperature gradient perpendicular to the flow direction. Due to a surface tension driven flow (Marangoni convection), bubbles moved at velocities of approximately 1 µL/min in the direction of the temperature gradient until they were trapped in alveolar structures (repeated circular arcs, 300 µm diameter). Switching the resistor off was sufficient to release the bubbles. Methods to promote bubble nucleation and coalescence, and to manipulate bubbles within microfabricated geometries by controlling the wetting behaviour, have also been suggested as possible bubble trapping mechanisms.\textsuperscript{33, 34}

Several trapping strategies take advantage of the large interfacial forces that are associated with microscale bubbles. Kohnle et al.\textsuperscript{39} presented a two-layer design that allowed bubble expansion into a wider, parallel channel on a top layer while permitting bubble-free flow in a bottom channel, essentially creating a T-shaped cross-section. These
two-layer microchannels prevented bubbles from clogging or impeding fluid flow, as channels with such cross-sections exhibited fluid flow rates up to four times higher and moving bubble velocities up to six times higher than microchannels with normal rectangular cross-sections. Eddington et al.\textsuperscript{40} manually added a chamber in-line with a fabricated microchannel network, which added additional height above the microchannels that allowed large bubbles to expand and rise due to buoyancy. Kang et al.\textsuperscript{41} designed a hemispherically-shaped bubble trap that better retains bubbles in liquid flow. The design was scaled out to a serial configuration of 35 hemispherically-shaped bubble traps in a PDMS substrate, and was used to prevent bubbles from flowing into a downstream cell culture area. Zheng et al.\textsuperscript{42} used a trap chamber with parabolic bypass tubing to connect to a separate channel ending in a porous filter. The position of the bypass tube at the top of the bubble trap chamber allowed selection of rising bubbles into the filtered channel. Sung et al.\textsuperscript{35} demonstrated a two-layer bubble trap with a trapping volume of up to 10 µL. The authors employed sieve-like barriers in the bottom layer that selectively trapped bubbles in the top layer while allowing uninterrupted liquid flow in the bottom layer. To remove trapped bubbles, the trap design was implemented on a separate device from cell-culture microchannel networks, so that the entire trap component could be detached and placed in vacuum. A maximum gas removal rate of approximately 0.6 µL/min was reported for an applied pressure of 78 kPa. Xu et al.\textsuperscript{38} employed a porous hydrophobic acrylic membrane (pore diameters: 0.2-10 µm) that was embedded in a poly-(methyl-methacrylate) (PMMA) device. Due to the high porosity, Darcy’s Law governs gas transport, which allows the device to achieve permeabilities between $7.8 \times 10^{-15}$ kmol/(Pa s m$^2$) and $1.3 \times 10^{-12}$ kmol/(Pa s m$^2$), significantly higher than the corresponding diffusion-based value for PDMS ($1.34 \times 10^{-16}$ kmol/(Pa s m$^2$) for $N_2$), and
removal rates of 26.6 mL/min (7.4 µL/s/mm$^2$ removal rate over a 60 mm$^2$ removal area).

Several solutions for gas removal rely on permeation through a membrane material. The molecular flux of a given gas molecule through a membrane of thickness $b$ can be expressed as

$$N_i = P_i \Delta p / b$$

(5)

where $P_i$ denotes the permeability of molecule $i$ and $\Delta p$ is the differential pressure applied across the membrane. PDMS, the substrate material of choice for microfluidic device fabrication using soft lithography, is highly gas permeable and therefore also widely used as a membrane material. Kang et al.$^{43}$ applied a positive pressure of 76 kPa to liquid-filled microchannels channels while sealing the outlets of the device, which discontinuously removed 80 nL of gas bubbles within five minutes. Skelley et al.$^{36}$ presented a combined solution that allowed simultaneous trapping and removal in a multilayer device with a trap volume of 25 µL. Vacuum was applied in a top layer above a 250 µm thick PDMS membrane which resulted in air bubbles being removed at a maximal rate of 0.14 µL/min. Johnson et al.$^{37}$ created a similar multilayer device that consisted of cylindrical reservoirs. Permeative fluxes of up to 0.35 µL/m$^2$s, equivalent to maximum gas removal rates of approximately 0.15 µL/min, were reported for air bubbles. Liu et al.$^{44}$ employed a hydrophobic membrane as a normally-closed valve. At a trans-membrane pressure of 25 kPa, the membrane removed bubbles at rate of 60 µL/s/mm$^2$ or 0.31 mL/min.
1.3 Research Objectives, Hypothesis, and Specific Aims

The AoC project required a method to remove unwanted bubbles for microfluidic devices, and to protect samples during operation of devices over long periods of time. The research objective was to develop a microfluidic bubble trap that was:

1. Simple and single-layer, allowing ease of fabrication with no additional alignment or bonding steps,
2. Small footprint, allowing easy insertion into already feature-dense designs,
3. Suitable for bubbles of sizes 100 pL to 500 nL, which was the range of bubbles sizes for infrequent bubbles; the trap was not built for gas purges which would lead to catastrophic device failure,
4. Consistent at trapping all bubbles, as a single bubble escaping the mechanism could damage the sample and impair overall vessel segment function,
5. Able to achieve removal rates equal or greater than those reported in literature,
6. Suitable for use over long periods of time (>24 hrs) and easy to operate, so as to be ideal for use during long-term culture.

The hypothesis behind this thesis is that a single-layer solution to bubble removal, and ultimately control of gas concentrations, is achievable in simple and robust fabrication, that permeation and overall removal rates in PDMS are sufficient for applications in the life sciences, and that the process of permeation can be understood in the simple single-layer format.

The presented solution is a scalable, single-layer microfluidic design, with a physical footprint of approximately 3 x 3 mm, for trapping and removal of gas bubbles that is comparable in performance to other solutions reported in literature. The method uses the permeability of PDMS to remove bubbles using an applied vacuum across a thin interchannel
wall (see Figure 1-3B), which is easily fabricated due to precision of photolithography, and robust due to the addition of buttresses in along the interchannel wall (see Figure 1-3). The trap is robust and can be consistently operated with maximum vacuum at high flow rates for long periods of time. For portability, the trap can be hooked up to a miniature vacuum pump, or if provided with a large dead volume, sealed after a vacuum has been established. To increase total removal rate and trapping volume, it can be easily scaled in a serial format (see Figure 1-3C).

Figure 1-3: Diagram demonstrating concept of bubble trap and removal process.45 (A) Inflowing bubbles are trapped and removed against an interchannel wall through the non-wetting surface. Dashed line indicates cross-section in (B) where bubble permeates through the trap wall due to difference in pressures between vacuum and liquid lines. (C) The trap design may be scaled. Scale bar is 1 mm in A.

To demonstrate achievement of the above criteria for the bubble trap, a single trap was characterized using the following:

1. To demonstrate consistent trapping and removal over long time periods (>24 hrs), integrated fibre optics and periodic bubble generation were used to detect passage of unwanted bubbles,
2. To demonstrate removal rates greater than those found in literature, consistent bubble generation, coupled with the imaging of the removal process in different liquids and vacuum conditions, was used to show evolution of bubbles during the removal process, and effects of liquid conditions similar to those found in most biological experiments,

3. To demonstrate understanding of the gas transport removal processes, a study of permeability in PDMS, backed by numerical analysis was conducted, and numerical analysis of removal rates and diffusive flux within the PDMS for the bubble trap were conducted in two dimensions,

4. To provide predictive models for users of the bubble trap, analytical models estimating overall time to remove a bubble based on initial size were generated,

5. To demonstrate scalability, parallel and serial formats of the bubble trap were designed, fabricated, and tested,

6. Demonstrating operation with a biological sample, the bubble trap was integrated into a specialized AoC device.

1.4 Thesis Organization

The following describes the thesis organization. In chapter two, basic methods used in generating bubbles, imaging and recording bubbles and their measurement over time, are described, as well as basic fabrication in soft lithography and photolithography of PDMS and SU-8, respectively. Chapter three covers investigation of permeability of PDMS to different gases, and the effect of geometry of microfluidic devices on diffusive transport in PDMS.
Chapter four covers characterization of a single trap, including bubble removal of different gases and in different liquids, at different applied vacuum levels, as well as numerical simulations and analytical models to predict removal rate and overall time for bubble removal. This chapter also covers methods investigating the effectiveness of trapping of bubbles in the device. Chapter five demonstrates integration of the bubble trap into a microfluidic device for AoC investigations, as well as scalability of the trap by fabricating eight traps in parallel and 100 traps in series. Chapter six discusses issues regarding liquid types common in biological testing and effects of diffusive transport on dissolved gas concentrations and bubble composition. Chapter seven summarizes the work completed, and discusses future applications of permeability in microfluidic devices, including the growth and shrinkage of bubbles, and manipulating dissolved gas concentrations in a controllable fashion.
Chapter 2

Research Methods

2.1 Device Fabrication

Microfluidic devices were designed using computer aided design software (AutoCAD, Autodesk Inc., San Rafael, CA, USA) and transferred to transparency masks with a spatial resolution of approximately 10 µm (CAD/ART Services, Bandon, OR, USA). Masters were fabricated using standard photolithographic techniques. Briefly, a layer of negative resist SU-8 25 (Microchem, Newton, MA, USA) was spun (2000 rpm, 30 s) on a pre-cleaned and dehydrated glass slide (75 mm × 50 mm × 1mm, Thermo Fischer Scientific, Waltham, MA, USA) to form a 25 µm thick seed layer that was subsequently pre-baked and flood-exposed. Two additional layers of SU-8 2050 were subsequently spun at 1750rpm and pre-baked in sequence, to define a feature layer height of 150 µm. The combined SU-8 layer was ultraviolet exposed (wavelength: 365 nm, total energy: 240 mJ/cm², Model 200 Mask Aligner, Optical Associates International Inc., San Jose, CA, USA) through a transparency mask, prior to a development step (SU-8 Developer, Microchem, Newton, MA, USA). The depth and uniformity of the feature layer was verified using an optical profilometer (Wyko NT1100, Veeco Instruments, Woodbury, NY, USA). PDMS (Sylgard 184 Elastomer Kit, Dow Corning Corp., Midland, MI, USA) was mixed at a 10:1 base-to-catalyst ratio and degassed both before and after pouring over the masters. Masters with PDMS were then cured in an oven at 80° C for 2 h. Upon peeling from the master, the PDMS was cut into rectangular sections with a footprint of 75 mm × 50 mm or 75 mm × 25 mm and 0.8 mm diameter holes were manually punched. The PDMS substrate was surface treated in oxygen plasma (Harrick, Ithaca, NY, USA) for 30 s and
bonded to a 1 mm thick glass slide (VWR, West Chester, PA, USA). Passivated stainless steel pins (length: 12.7 mm, size: 23 gauge, New England Small Tube Corp., Litchfield, NH, USA) were inserted into the holes of the microfluidic device, secured using epoxy, and connected to Tygon tubing (0.02” inner diameter x 0.06” outer diameter, Cole-Parmer, Vernon Hills, IL, USA) which provided the fluidic connections (Upchurch Scientific, Oak Harbor, WA, USA) to syringes, vacuum pumps and 20 mL vials.

2.2 On-Chip Valve Fabrication

The experimental device utilizes special valves for the creation of gas bubbles in liquid. While there is plenty of literature on the fabrication of on-chip valves for liquid-liquid droplet creation, no publications were found on the characterization of valves for generating gas bubbles in a liquid stream. The designs for the valves were inspired by Irimia et al., with the novelty being the method of fabrication to prevent bonding to the glass slide. For microchips with valve designs, prior to plasma treatment, glass slides were spincoated with CYTOP, an amorphous fluoropolymer (CTL-809-A, Bellex International Corp., Wilmington, DE, USA) at 600 rpm for 22 s. A stamp with posts of predefined sizes and distance separating them was fabricated by pouring PDMS onto a micromachined polycarbonate mold bonded to a glass slide. Thin layers of CYTOP were transferred via microcontact printing using this PDMS stamp to the valve region of the microdevice, to prevent bonding (see Figure 2-1). Precision in microcontact printing was enhanced by utilizing an alignment stage (Model 200 Mask Aligner, Optical Associates International Inc., San Jose, CA, USA). The stamp was sealed to a top glass layer and the device was placed feature-side up on a bottom layer. Using a 10x objective, the valve
features were aligned with the stamp, and then the top layer was lowered until contact was made. For two-layer devices with on-chip valves, PDMS was spincoated for the bottom fluidic layer at 450 rpm for 30 s, to obtain a thickness of 500 µm (350 µm thick membrane between layers). The separate layers were first partially cured at 80°C for approximately 15 min, then the upper control layer was peeled from its master before aligning and compressing on top of the bottom fluidic layer. This two layer structure was then left to cure overnight.

Figure 2-1: Process of valve fabrication. (A) A micromachined polycarbonate mold is used to create a PDMS stamp. (B) CYTOP is spincoated on a cleaned glass slide. (C) PDMS is manually stamped onto CYTOP to cover posts. (D) Posts are aligned with selected features and CYTOP is contact printed, preventing bonding at feature sites.

2.3 Experimental Setup

Liquid flow rates were controlled using syringe pumps (PHD 2000, Harvard Apparatus, Holliston, MA, USA). One of the following working liquids - de-ionized and filtered water, ethanol, or an aqueous solution containing 0.01M poly(ethylene-glycol) (20)sorbitan monolaurate (Tween 20, Sigma Aldrich, St. Louis, MO, USA), BSA (Sigma Aldrich, St. Louis, MO, USA), or 3-(N-morpholino) propanesulfonic acid (MOPS), a common solution used for in
vitro vascular studies - was filled into a syringe. A second syringe containing air, or a compressed gas cylinder containing purified N₂ (99.998%) or purified CO₂ (99.8%) (Linde Canada Ltd., Mississauga, ON, Canada), was connected via a combination pressure regulator (max. 15 psi, Condyne P/N: PR50A15Z1, Valco Instruments Comp. Inc., Houston, TX, USA) to a second inlet. Gas bubbles were added to the liquid stream at a T-junction on-demand, using two normally closed on-chip valves actuated by a miniature vacuum/open)/pressure(closed) combination pump (CTS series, Hargraves Technology Corp., Mooresville, NC, USA). Actuation of the on-chip valves was controlled using a manifold with two off-chip miniature pneumatic valves (Lee Comp., Westbrook, CT, USA) controlled via a custom Labview program and a 14-bit USB data acquisition interface (USB-6009, Labview version DS1, National Instruments, Austin, TX, USA). The necessary differential pressures were supplied using either a miniature vacuum pump (size: 51.8mm × 32.3mm × 20.3mm, weight: ~50g, pressures: 40.6 kPa or 74.5 kPa, CTS series, Hargraves Technology Corp., Mooresville, NC, USA) or a direct drive vacuum pump (pressure: 96.5 kPa, Welch model #: 8917A, Gardner Denver Welch Vacuum Technology Corp., Niles, IL, USA). Pressure measurements were manually conducted using a vacuum gauge and recorded with a piezoresistive transducer (140PC series, Honeywell, Morristown, NJ, USA). Poly(vinyl chloride) tubing (approx. 30 cm in total length, 1/8” ID, VWR, West Chester, PA, USA) was used to connect the vacuum pump with the microfluidic device via fittings (McMaster-Carr, Elmhurst, IL, USA). Separate devices were used to determine respective gas removal rates at different conditions to prevent any surface or material cross-contamination.
2.4 Image Acquisition and Analysis

Bubbles were imaged in the bright-field mode using a transmission configuration on a stereomicroscope (SMZ800, Nikon Instruments Inc., Tokyo, Japan) with a monochrome, charge-coupled device (CCD) camera (10bit, Retiga 2000R, QImaging, Surrey, BC, Canada), or a color CCD camera (3 × 8bit, Micropublisher 3.3, QImaging, Surrey, BC, Canada). The non-wetted surface area and bubble volume were evaluated from the captured images using the software program ImageJ (version 1.42q, National Institutes of Health, Bethesda, MD, USA). Further post-processing of image data was performed using a custom MatLab code (version 7.1, Mathworks, Natick, MA, USA).
Figure 2-2: Schematic of the experimental setup for bubble trap characterization using a two-layer device. (a) Syringe pumps supply a liquid (water, ethanol, or an aqueous surfactant solution) while gases are supplied via a mini-regulator from a compressed source. Uniform bubble sizes are generated using two on-chip valves operated sequentially in series (A) and introduced at a T-junction (B). Generated bubbles enter the trap chamber, where they are removed (C). A vacuum pump is connected to the vacuum channel. The reduction in bubble size is quantified from bright-field microscopic images. Captured images are post-processed and analyzed using ImageJ and MatLab. The number of bubbles entering and exiting the trap is measured using a fibre-optic based arrangement for excitation and detection fibres parallel to flow. (D) Arrow indicates flow direction, dashed line indicates optical fibre beam path in transmission mode. Scale bars are 5 mm (A), and 500 µm (B, C, D).45
Figure 2-3: Off-Chip fibre-optic setup. (A) Diagram showing theoretical setup. Bubbles passing through the liquid stream block emission from LED. A cladded detection fibre transmits intensity to a photodetector which passes a varying signal, recorded in LabView. (B) Experimental fibre-optic setup. Collimators were used to interface homemade bare-ended fibre-optic cables to photodetectors and the LED light source. Scale bar is 200 µm in (A).
2.5 Fibre Optic Measurements

Polymer optical fibres (diameter: 125 µm, NA: 0.22, Polymicro Technologies, Phoenix, AZ, USA) were prepared with one end bare, the other end constructed with a subminiature version A (SMA) connector (Thor Labs, Newton, NJ, USA). Bare ends were scored and inserted into special channels on the microfluidic device for emission and detection fibres, which were designed in-line with flow for transmission measurements. Emission fibres were connected to an LED (power: 7 mW, peak wavelength: 528 nm, Thor Labs, Newton, NJ, USA) coupled through a collimating lens. The detection fibre was connected via collimating lens into an amplified photodetector (PDA36A, Thor Labs, Newton, NJ, USA) (see Figure 2-3). Measurements were sampled via a custom LabView program at 1 kHz and analyzed in MatLab (see Appendix A for details).

2.6 Microfluidic Device Design

Single-layer soft lithography was used to design and fabricate a side wall with well-defined dimensions in a thin, gas-permeable substrate. The geometry of the interchannel wall was selected to minimize the gas permeation distance between gas bubbles that remained in a trapping chamber with a circular cross-section (radius $r = 1$ mm), and a neighbouring vacuum channel. An array of equidistantly spaced pillars retained the bubbles in the chamber from the perfusion liquid steam (see Figure 2-4A). The bubble trap has a uniform depth of $h = 150$ µm. A liquid stream enters the trapping chamber through a 200 µm wide channel. The array of pillars with elliptical cross-sections is placed at the outflow side of the trap, upstream of the 200 µm wide outflow channel. Pillars are separated by 50 µm gaps and prevent any bubble with a
diameter exceeding the gap size (corresponding to a minimum retainable bubble volume of \( \sim 0.5 \) nL) from leaving the chamber. The sum of all gaps is greater than the width of the inflow channel; the post array therefore only marginally contributes to the overall flow resistance in the microchannel. The vacuum channel lines three quarters of the trap chamber’s circumference and is separated by a 100 µm wide inter-channel wall. Cylindrical support elements (radius 100 µm) are placed equidistantly every 400 µm along the inter-channel wall’s circumference (see Figure 2-5C). The resultant arithmetic mean wall thickness is 137 µm, with a minimum thickness \( b_{\text{min}} = 100 \) µm, and a maximum thickness \( b_{\text{max}} = 200 \) µm. The design of the inter-channel wall serves to minimize the permeation distance, \( b \), while ensuring the mechanical stability of the wall for an applied pressure difference of approximately 1 atm. The added support elements significantly improved the yield of the fabrication process.

Figure 2-4: Detailed introduction of bubble trap design. Patterned channels are in white, while PDMS structures black. The locations of maximum and minimum wall thickness are indicated by arrows, (100 and 200 µm) in (C); the average wall thickness is 139 µm. Scale bars are 200 µm in (B) and 100 µm in (A, C) We chose a post spacing of 50 µm (\( \beta \)), radius of the trap of 1mm (\( r \)) and corresponding circumference of 2.37 mm (\( \lambda \)). For a chip with a depth of 150 µm, the total trap volume is 0.428 µL, and the surface area for bubble removal is 0.71 mm². Device footprint is less than 10 mm².
Chapter 3

Permeability

3.1 Overview

To better understand the mechanisms behind the operation of the designed bubble trap, the permeability of the material being used was tested. The permeability of different gases in PDMS, the substrate utilized for the AoC platform, is important in characterizing the bubble trap’s performance, and determining the maximum achievable removal rate. Specifically of interest is the PDMS chemistry kit commonly used for microfluidic devices, and used by the Lab-on-a-Chip group. At present, there is no known literature on permeation of gases through cured PDMS made from Sylgard 184 (Dow Corning Corp., Midland, MI), which differs from other PDMS chemistries. Previous literature has used permeation values derived from different chemistries and ambient conditions (most notably temperature), and encompasses a wide range of potential values. The permeability of PDMS to different gases of interest, including relevant solubilities and diffusivities, is given in Table 3.1.

Also of interest is the effect of geometry on diffusive transport within the PDMS. Previous designs of bubble removal devices assumed gas transport in a two-dimensional paradigm when evaluating gas transport, and calculating the overall removal rate. However, at the microscale, significant gas transport would be expected through the top of the PDMS channel (see Figure 3-1). Knowledge of the ratio of gas transport through the top channel ceiling, relative to the side walls of the microchannels, would allow calculation of a correction
factor which could be applied to analytical solutions, as well as numerical simulations done in the two-dimensional plane.

<table>
<thead>
<tr>
<th>Gas</th>
<th>Permeability (1 Barrer = $10^{-10}$ (cm$^3$ O$_2$ ) cm cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$)</th>
<th>Diffusivity (cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_2$</td>
<td>245 (20C) $^{51}$, 280 (20C) $^{52}$, 256 (25C), 395 (25C) $^{53}$, 180 ± 20 (28C) $^{54}$, 400 ± 10 (35C) $^{55}$, 450 (35C) $^{56}$, 525 (35C) $^{57}$, 220 (40C) $^{58}$</td>
<td>130 ± 4 (28C) $^{54}$, 34 ± 1 (35C) $^{55}$</td>
</tr>
<tr>
<td>O$_2$</td>
<td>825 (25C) $^{53}$, 800 ± 20 (28C) $^{55}$, 360, 1000 (35C) $^{56}$</td>
<td>34 ± 1 (35C) $^{55}$</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>1300 ± 200 (28C) $^{54}$, 3800 ± 70 (35C) $^{55}$, 4200 (35C) $^{56}$, 5600 (35C) $^{57}$</td>
<td>110 ± 10 (28C) $^{54}$, 22 ± 1 (35C) $^{55}$</td>
</tr>
<tr>
<td>Air</td>
<td>256 $^{59}$</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-1: Permeability and diffusivity data for gasses in PDMS from the literature. Temperatures are listed in brackets.

Figure 3-1: Cross-sectional image of fluid-filled channel, PDMS inter-channel wall (width $b$, height $h$) and glass cover slide, demonstrating the effect of geometry on gas transport in a single-layer device. The background shows the numerical simulation of gas transport between the fluid-filled channel and the vacuum channel with contours representing the concentration profile, calculated using a numerical simulation in Comsol. Arrows demonstrate diffusive flux through the PDMS.
3.2 Numerical Simulations

For the numerical simulations, we assume a gas bubble with an average length $\Lambda$ and a contact angle of 90° (i.e. excluding the cap regions) and neglect any gas transport through the liquid. The gas removal rate can therefore be estimated as

$$\frac{dV}{dt} = AP(\Delta p)b^{-1}$$

where $P$ is the permeability, $A (= \Lambda h)$ is the cross-sectional through which permeation occurs, and $(\Delta p)$ is the applied pressure difference. Figure 3-1 shows a cross-section of the bubble-containing channel and the neighbouring vacuum channel. As indicated by the arrows, permeation occurs not only through the side wall, area $\Lambda h$, but also through the top walls. In order to quantitatively assess permeation rates, we numerically estimate the permeation rate in a two-dimensional domain. The distribution of the dissolved gas (pure nitrogen, $T = 298$K, $D_{N2, PDMS} = 3.39 \times 10^{-9}$ m$^2$s$^{-1}$) concentration in the cross section is also shown in Figure 3-1. Parameters used for the numerical simulations are listed in Table 3-2. Geometries used were to scale with the PDMS devices, with boundary conditions at the periphery of the PDMS set at ambient atmospheric conditions, and assuming no transport occurs through the glass surface of the microfluidic channels. Due to the sharp corners at the top of the PDMS channel in the simulated geometry, anomalies were present at these locations in the numerical simulations. Therefore, simulations were conducted for cross-section geometries beginning with large fillets at the corners of the channels (approx. 50 µm), progressing towards smaller fillets (approx. 10 µm), and eventually a right-angled corner. The effect of these changes in cross-sectional geometry on the diffusive flux profiles are shown in Figure 3-2. The Umfpack solver was used for solving the diffusive transport equation, and diffusive fluxes through the channel boundaries
were calculated using boundary integration. To demonstrate grid independence, meshes with varying degrees of coarseness were generated and compared using boundary integration for permeative flux (see Table 3-3), for each geometry simulated. We found that as we approached a perfect right-angled corner, the solution for the correction factor

\[
\xi = \left( \frac{J_{\text{numerical}}}{J_{\text{analytical, side}}} \right) \rightarrow 1.23
\]  

(7)

Figure 3-2: Numerical simulations showing surface plots of diffusive flux for different corner geometries. Top geometry has 50μm fillets, while the bottom geometry has right-angled corners. Corrective factor trends from 1.10 to 1.23.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diffusivity</th>
<th>Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$3.39 \times 10^{-9}$</td>
<td>3.87</td>
<td>1.02 (74.5 kPa)</td>
</tr>
</tbody>
</table>

Table 3-2: Parameters used in numerical simulations for permeability.

<table>
<thead>
<tr>
<th>Mesh</th>
<th>50 µm Fillet</th>
<th>10 µm Fillet</th>
<th>No Fillet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Coarse)</td>
<td>$1.09 \times 10^{-8}$ (Mol)m$^{-1}$s$^{-1}$</td>
<td>$1.20 \times 10^{-8}$</td>
<td>$1.20 \times 10^{-8}$</td>
</tr>
<tr>
<td>2 (Fine)</td>
<td>$1.09 \times 10^{-8}$</td>
<td>$1.20 \times 10^{-8}$</td>
<td>$1.21 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

Table 3-3: Permeative fluxes for identical geometric model with different meshes, demonstrating grid independence.

### 3.3 Experimental Results

To measure permeabilities of different gases in the PDMS chemistry we used (Sylgard 184), we designed a microfluidic device (see Figure 3-3) that consists of a long meandering, liquid-filled channel and a vacuum channel, arranged in parallel, and separated by an inter-channel wall of known thickness $b = 200 \, \mu m$, with a feature depth of approximately 150 µm as verified by profilometer. A liquid flow containing equally-sized gas bubbles was initially guided through the meandering channel. After the perfusion flow was stopped, a known subatmospheric pressure was applied at the vacuum channel. A series of bright-field images of gas bubbles was acquired at a location close to the inter-channel wall, from which changes in gas bubble size could be measured, allowing the rate of gas removal to be quantified in a very well-defined
geometry, as opposed to the bubble trap design, which has a non-linear geometry and varying interchannel wall thickness in the feature plane.

We experimentally determined the approximate permeability of the relevant pure gases and the gas mixture of air in PDMS using the chip design shown in Figure 3-3, and adjusting for the numerically estimated value of $\xi = 1.23$. Figure 3-4 shows the experimental results for $n=5$ measurements, as well as literature data for comparison. Nitrogen and air show similar permeabilities in PDMS, while CO$_2$ is highly soluble in PDMS and therefore more readily gas-permeable in silicon-based devices.

**Figure 3-3:** Design of device for measuring permeability in PDMS. Sections of the device are labelled, with flow direction indicated by the arrow. Gas removal measured in the upper right-hand portion of the chip near the vacuum channel. Scale bar is 1 mm.
It should be noted that the rate of gas removal is strongly affected by the type of gas molecule contained within the bubble, since each gas molecule type may have a different permeability in PDMS. Multiple studies have been conducted on the effect of secondary gases on permeation rates of primary gases in mixture. In the case of the air sample, CO$_2$, O$_2$ and N$_2$ have significantly different permeabilities in PDMS. This is especially true for CO$_2$, as it has been shown that organic vapors are highly soluble in PDMS and other rubbery polymers. In the case of a mixture such as air, gas removal is a function of the different species in the mixture, and the relative mix of gases in the bubble will also change over time, becoming heavily saturated with the slowest removing species. The overall removal rate will be dominated by nitrogen as it has the highest percentage volume in the mixture, and is the slowest removing
species. What is expected is a rapid removal of the small amounts of carbon dioxide in early stages, followed by oxygen, leaving the remaining bubble removal to be a case of nitrogen removal.
Chapter 4

Single Trap Characterization

4.1 Removal Performance

In order to test the bubble trapping and removal strategy under well-defined conditions, a microfluidic device was fabricated that allowed generation of uniformly sized bubbles within a liquid-perfused microchannel. Gas and liquid phases were separately introduced through microfluidic resistor channels that were 20 cm and 10 cm long, respectively. Pneumatic actuation channels and valves that were incorporated onto the gas-filled microchannel allowed the formation of bubbles with well-defined sizes. Bubble sizes could be tuned by varying the overpressure, liquid flow rate, and actuation time. The bubbles entered the liquid-filled main channel and passed through a 200 µm wide (w), 150 µm deep (h) and 50 cm long microchannel that was situated upstream of the bubble trap.

Single bubbles were produced, and the total time required for removal was determined from a sequence of images captured in the bright-field mode. Figure 4-1 shows such a sequence of micrographs obtained during the removal of a nitrogen bubble that was retained within the trap. A pressure of 96.5 kPa was consistently applied at the vacuum channel and a constant flow rate of de-ionized and filtered water at 20 µL/min was applied. The cross-sectional area occupied by the bubble and the non-wetted fraction \( \lambda \) of the total length of the inter-channel wall, \( \lambda \), can be evaluated from a time-series of bright-field images every 15 s. At a given instant in time, the product of the cross-sectional area \( A \) and \( \mu \) provides us with an estimate of the
bubble volume, $V_B$, and $A_B = A \times h \times \zeta$ is an estimate of the non-wetted area of the inter-channel wall.

In line with results from our numerical simulations, gas molecules are primarily transported through the non-wetted section of the inter-channel wall. The gas removal rate therefore linearly scales with $A_B$. This trend is displayed in Figures 4-2 – 4-5, which show the temporal evolution of $V_B(t)$ and $A_B(t)$, for the working fluids water, an aqueous surfactant solution, ethanol, MOPS, and BSA, and at different vacuum levels (see Appendix B for details). The maximum obtained removal rate of approximately 0.0024 $\mu$L/s (or 0.14 $\mu$L/min) for nitrogen in water was found at the beginning of the process. These rates are comparable to those found in literature for similar multilayer solutions.$^{36, 37}$ The removal rate $dV_B / dt$ decreased over time, a relationship that can be attributed to shape evolution of the gas bubble during removal, specifically the decrease in non-wetted surface area through which permeation occurs.
The mean rate of gas removal, $V_{B,0}/t_R$, was approximately 0.065 µL/min for nitrogen bubbles at a differential pressure of 96.5 kPa. Reduced removal rates were measured in cases where the working fluid contained a surfactant.

In addition to the volumetric gas removal rate, the permeative flux was calculated by dividing the gas removal rate by the average of the non-wetted surface area, $A_B(t)$, which was obtained from the sequence of consecutive bright-field images:

$$J = \frac{dV_B}{dt}/A_B$$

(8)

While the removal rate decreased over time, the permeative flux remained relatively constant with a small increase towards the end of the removal process, for all wetting conditions (see Figure 4-8 and 4-9).

For Figures 4-2 thru 4-9, all bubbles are in nitrogen, and applied vacuum was 96.5 kPa.
Figure 4-2: Evolution in bubble volume for non-biological liquids. Squares represent data from deionized H₂O (■), triangles ethanol (▲), and circles surfactant (●). Data is for n=5 bubble measurements, and error bars represent one standard deviation. Bubbles in deionized water remove quickest, while those in surfactant remove slowest and take over twice as long to remove, for bubbles of similar initial volume.

Figure 4-3: Evolution in bubble volume for common biological media. Squares represent data from MOPS (■), and circles BSA (●). Data is for n=5 bubble measurements, and error bars represent one standard deviation. Bubbles in BSA, a surfactant, have similar evolutions to the surfactant-containing liquid (Tween), while bubbles in MOPS show closer similarities to ethanol and water.
Figure 4-4: Evolution in surface-areas contacting the trap wall (non-wetted) for non-biological liquids. Squares represent data from deionized H₂O (■), triangles ethanol (▲), and circles surfactant (●). Data is for n=5 bubble measurements, and error bars represent one standard deviation. Change in surface area for deionized water is almost linear.

Figure 4-5: Evolution in surface-areas contacting the trap wall (non-wetted) for common biological media. Squares represent data from MOPS (■), and circles BSA (●). Data is for n=5 bubble measurements, and error bars represent one standard deviation.
Figure 4-6: Evolution of removal rate for non-biological liquids. Squares represent data from deionized H₂O (■), triangles ethanol (▲), and circles surfactant (●), and data is for n=5 measurements. Error bars represent one standard deviation.

Figure 4-7: Evolution of removal rate for biological media. Squares represent data from MOPS (■), circles BSA (●), and data is for n=5 measurements. Error bars represent one standard deviation.
Figure 4-8: Evolution of permeative flux over time. Squares represent data from deionized H$_2$O (■), triangles ethanol (▲), and circles surfactant(●), and data is for n=5 measurements. Ranked from highest to lowest in terms of permeative flux are ethanol, deionized H$_2$O, and surfactant solution. The extremely large measured permeative fluxes for ethanol are probably partially attributable to greater solubilities for gases in ethanol.

Figure 4-9: Evolution of permeative flux over time for biological media. Squares represent data from MOPS (■), circles BSA (●), and data is for n=5 measurements. Permeative fluxes for biological media were comparable to that for deionized H$_2$O.
4.2 Liquid Characteristic Effect on Removal

The effect of surfactants on the bubble removal rate was investigated for de-ionized and filtered water and aqueous surfactant solution (0.01M Tween 20). The contact angle ($\theta$) and surface tension ($\gamma$) for gas bubbles varies greatly for different liquid-PDMS systems; this data is summarized in Table 4-1. Gas bubbles in surfactant were experimentally found to have small contact angles, the same as preferentially wetting liquids (see Figure 4-10). Lower gas removal rates were measured for preferentially wetting liquids (i.e., ethanol and an aqueous surfactant solution, and BSA) than for de-ionized and filtered water. The difference can be explained by $A_B$ being smaller in the wetting case, for a given bubble volume, $V_B$. Contact angles measured during the initial stages of the removal process (see Figure 4-10) and non-wetted surface area to volume ratios (see Figure 4-11) indicate that bubbles in pure water maintain a greater proportion of their surface area along the trap wall.

The bright-field micrographs shown in Figure 4-12 also suggested that bubbles in the preferentially wetting case assumed a more circular cross-sectional shape than for the case of de-ionized and filtered water. When the same differential pressures were applied at the vacuum channel, the permeative flux found for bubbles immersed in a preferentially wetting liquid was on average only 2.1-2.7 nLs$^{-1}$mm$^{-2}$ as compared to approximately 2.8-3.5 nLs$^{-1}$mm$^{-2}$ in the case of de-ionized and filtered water. The reduced gas removal rate is attributed to the presence of surfactant molecules at the gas-liquid and gas-solid interfaces, which improves wetting and increases liquid films, as well as directly reducing mass transport.
Bubbles were also formed in ethanol, which has a lower surface tension (22.27 mN/m) than water and a smaller contact angle against PDMS ($\theta = 31\pm5^\circ$, similar to the aqueous surfactant solution). While bubbles in ethanol had similar morphologies to those obtained in an aqueous surfactant solution (see Figure 4-12), removal rates were only slightly lower, $V_b(t)$ displayed a different behaviour, and permeative fluxes were higher and more similar to those achieved for bubbles in de-ionized and filtered water.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Surface Tension (mN/m)</th>
<th>Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>71.97 (25°C)</td>
<td>111 $^{32}$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>22.27 (20°C)</td>
<td>31 $^{32}$</td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>51.1 (0.5%wt, 20°C) $^{31}$</td>
<td>100 (1 mg/mL) $^{32}$</td>
</tr>
<tr>
<td>Tween 20</td>
<td>35.0 (CMC, 20°C) $^{31}$</td>
<td>49.3 (2% wt)</td>
</tr>
</tbody>
</table>

Table 4-1: Surface tension and contact angle with PDMS data for liquids from the literature. Temperatures and concentrations are listed in brackets.
Figure 4-10: Contact angles at bubble interfaces between liquid and bubble trap wall, for eight different bubbles. Bubbles in surfactant are in squares (filled and hollow), while those in water are in triangles (also filled and hollow). As can be seen from the above, the surfactant helps liquid contact and spreading on the PDMS surface.
Figure 4-11: Surface-area-to-volume ratios of bubbles, for deionized water (▲), ethanol (●), and surfactant solution (■). While water bubbles change in shape, bubbles in surfactant solution and ethanol maintain their morphology and surface-area-to-volume ratios.

Figure 4-12: Images showing example morphologies of bubbles in different liquids (right). Bubble surface contours for different bubbles were overlaid.
4.3 Analytical Modeling

In this section we introduce a simple analytical model that allows us to estimate the total time, \( t_R \), that is required to remove a gas bubble with the initial volume \( V_{B,0} \), for bubbles in water. As the bubble volume decreases over time, we assume that bubbles maintain a constant shape during the removal process as their volume decreases. The size-dependent relationship between the bubble volume and the portion of the interfacial area that is in contact with the trap wall - which therefore determines the removal rate - can be established. Integration of the equation for bubble removal (equation 6), following substitution of the interfacial area

\[
A = 2\sqrt{2V_{B,0}h\pi}
\]

leads to:

\[
t_R = \left(\frac{b}{P(\Delta p)}\right)\sqrt{\frac{V_{B,0}\pi}{2h}}
\]

which can be used to calculate the time required to remove a bubble of given size with initial volume \( V_{B,0} \). The analytical model also considers correction factor \( \xi = 1.23 \) to account for transport through the top of the PDMS channels.

This analytical model is plotted against experimental data for initial bubble volumes and total removal times (see Figure 4-13). The analytical model suggests a logarithmic relationship between total time required for removal and initial bubble volume, and shows good agreement when compared with experimental data for de-ionized water, at different applied vacuums. This model has applications in liquids which do not wet well on PDMS, including biological media such as MOPS solutions.
Figure 4-13: Comparison of actual required removal times for individual bubbles in deionized water, as compared to the analytical model. Bubbles removed at higher differential pressure (94.5 kPa, ▲) and lower pressure (74.5 kPa, ■) are plotted as points, while the analytical models for each case are overlaid as lines. An analytical model can also be attempted for fluids with surfactants in them, which changes the geometry of the bubble. We can continue to assume that a gas bubble maintains a particular geometry (i.e. surface area to volume ratio) in the bubble trap, but this geometry is different from the non-surfactant solution.

Fig 4-14: Bubble geometries for analytical model. On left is a semi-circular bubble in water. On the right is a bubble in surfactant containing liquid with a circular geometry.
For the previous equations, the solution assumes a semi-circular shape, which is reasonable given that the contact angle of water on PDMS is approximately 90°. For aqueous solutions containing surfactants, this angle can shrink, depending on which surfactants are used. Furthermore, the contact angle varies with concentration. For the utilized surfactant (Tween), reported literature data and experimental observations put the contact angle between 30-50°. Taking these angles, and assuming a circular shape for the bubble (which was observed experimentally), the bubble may be modeled as the larger portion of a circular segment, with surface area contacting the trap wall being the line segmenting the circle (the chord). Using this, we get

$$V_B = r^2 h(\pi - \theta + \sin 2\theta / 2)$$  \hspace{1cm} (11)

And

$$A_B = rh\sqrt{2 - 2 \cos 2\theta}$$  \hspace{1cm} (12)

Substituting for $r$ in equation 11 with $r = \sqrt{V_{B,0} / h(\pi - (\theta - (\sin 2\theta / 2)))}$ we get a separable which yields:

$$t_R = 2(b / P \Delta p) \sqrt{V(\pi - (\theta - (\sin(2\theta) / 2))) / \sqrt{h(2 - 2 \cos(2\theta))}}$$  \hspace{1cm} (13)

The results for a 50° contact angle are plotted against experimental data. There is less consistency within experimental data, possibly due to varying deposited surfactant concentrations on the device, or other variations in morphology, however percentage difference is within 15% in all but two of the 10 plotted data points.
Progression of bubble volume and surface area during removal is dependent on the initial bubble size and wetting behaviour. We normalize bubble volume and time as

\[ V_N = \frac{V}{V_{B,0}} \]  \hspace{1cm} (14)

\[ t_N = \frac{t}{t_R} \]  \hspace{1cm} (15)

where \( V_{B,0} \) is initial bubble volume, and \( t_R \) is the total time required for bubble removal. The graph (Fig. 4-14) shows no differences due to pressure applied or liquid conditions, but displays minor variations due to bubble geometry. This normalization removes all differences caused by global effects (applied vacuum, liquid conditions, bubble size). The only effects this normalization would not remove are those due to abnormal morphologies and hence gas-polymer interfacial surface areas.
4.4 Numerical Modeling

Numerical modeling was utilized to better understand how the trap operated, particularly with respect to fluid flow through the trap, and specifically how transport occurred through the PDMS membrane and in the fluid. The fluid velocity and dissolved gas concentrations within the fluid-filled trap were modeled using a multiphysics software program based on the finite element method (version 3.4, Comsol, Burlington, MA, USA). Gas removal rates were estimated as two-dimensional steady simulations using a range of bubble sizes and shapes, and applied vacuum levels. Trap and bubble geometries were imported into Comsol as boundaries from AutoCAD design files traced from experimental bubble images. The respective bubble shapes were obtained from the bright-field microscopic images, and the liquid-gas interfaces of
the bubbles were traced and represented as stepwise linear functions. Boundary conditions (concentrations, fluid velocities) for Navier-Stokes and diffusive and convective mass transport, were taken from experimental conditions, and determined from known solubilities in experimental liquids and PDMS. Solutions were obtained sequentially for stationary conditions using the direct linear Umfpack solver, and were checked for grid independence. Removal rates were calculated using boundary integration in two dimensions and multiplied by the known channel heights.

First, numerical models were used to calculate the effect of adding the support elements to the trap walls. These elements increased average thickness $b$ which reduced removal rate, but significantly improved the yield of the fabrication process. Simulations assuming surfactant-free bubbles found that at most, bubble removal rate was reduced by only 13%, compared to a channel wall with uniform thickness of 100 µm (see Figure 4-16).

![Figure 4-17: Effect of buttresses on bubble on theoretical bubble removal rates. The shaded portion indicates the additional removal rate from removing the buttresses in the design.](image-url)
Next, experimental results were compared to numerical simulations. Bubble geometries were traced from micrographs, and modeled as the removal of N₂ from a trapped bubble through the inter-channel wall. The models were also assumed to be steady state conditions. A temperature of 298 K and a pure gas were assumed. Boundary concentrations were determined in the liquid stream, at the PDMS-bubble and the PDMS-vacuum interfaces, at the PDMS-liquid interfaces, and at the gas bubble-liquid interface.

Gas concentrations in liquids were determined using Henry’s Law:

\[ C = \frac{p}{k_H} \]  

showing the dependence of concentration/solubility of gas in liquid on pressure. For N₂, \( k_H = 1639.34 \text{ L-atm/mol at 298K} \). The Henry Law constant \( k_H \) can be determined for a specific temperature using the modified van’t Hoff equation:

\[ k_{H,\text{pc}}(T) = k_H(T^\theta) \exp\left[-C\left(1/T - 1/T^\theta\right)\right] \]  

where \( T^\theta \) is the reference temperature (298 K), and C is a constant (1300 for N₂). For 1 atm pressure (negligible pressure drop from trap to outlet), the concentration of N₂ in the liquid phase and at the bubble-liquid interface (wetted perimeter) is approximately 0.63 mol/m³.

Concentrations at the PDMS-bubble and PDMS-vacuum interfaces were determined using the definition of solubility, and recognizing that the gas species in air mixtures are low-sorbing penetrants, thus solubility is independent of pressure. The gas concentrations at the polymer-bubble interface can be determined using solubility and values obtained from literature. Thus we use the infinite dilution solubility (\( S^\infty \)) and adjust for changing solubility due to penetrant pressure,
\[ S^\infty = \lim_{p \to 0} \frac{C}{p} \]  
\[
S = S^\infty (1 + np)
\]

\[ S = 0.093 \text{cm}^3(\text{STP})/\text{cm}^3\text{-atm} \text{ for N}_2 \text{ at 308 K. At 1 atm, the polymer interface has a concentration of approximately 3.87 mol/m}^3 \text{ at the gas bubble side, and approximately 1.02 mol/m}^3 \text{ at the vacuum side for 74.5 kPa, and 0.18 for 94.5 kPa.}^{55}
\]

Concentrations at the PDMS-liquid interface were determined using a partition coefficient based upon solubility differences between the polymer membrane and liquid domains which led to discontinuities in concentration values at the interfaces \((K = C_m/C_1 = 6.34)\). To model the discontinuity at the polymer interfaces, the stiff spring method using a large spring constant \(M\) and partition coefficient \(K\) was used to derive the following mass transport equations:

\[
(-D \nabla C_i + C_i \vec{u}) \cdot \vec{n} = M(C_m - KC_i)
\]
\[
(-D \nabla C_m) \cdot \vec{n} = M(KC_i - C_m)
\]

Diffusivities were determined for the liquid (H\(_2\)O) and solid (PDMS) subdomains. In water, nitrogen has a diffusivity of approximately \(1.88 \times 10^{-9} \text{ m}^2\text{s}^{-1}\) at 298 K, and is comparable to \(\text{O}_2\) \((1.60 \times 10^{-9} \text{ m}^2\text{s}^{-1})\) and \(\text{CO}_2\) \((2.00 \times 10^{-9} \text{ m}^2\text{s}^{-1})\) diffusivities.\(^7\) For PDMS, diffusivity is pressure dependent due to the effect of hydrostatic compression, which is minimal due to the gases being low-sorbing.

\[
\bar{D} = D_0 (1 + q\Delta p)
\]
Using Merkel et al., \textsuperscript{55} we account for temperature and pressure to obtain a diffusivity for nitrogen in PDMS of \(3.39 \times 10^{-9} \text{ m}^2\text{s}^{-1}\) which is similar to data obtained from other sources.\textsuperscript{71} A full list of subdomain and boundary conditions is given in the table below.

<table>
<thead>
<tr>
<th></th>
<th>Gas-Liquid</th>
<th>Gas-PDMS</th>
<th>Vacuum-PDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_0 \text{ (mol/m}^3)</td>
<td>0.63</td>
<td>3.87</td>
<td>0.18 (94.5 kPa)</td>
</tr>
<tr>
<td>(D \text{ (m}^2/\text{s}))</td>
<td>(3.39 \times 10^{-9})</td>
<td>(1.88 \times 10^{-9})</td>
<td></td>
</tr>
<tr>
<td>(N_0 \text{ (mol/m}^2\text{-s}))</td>
<td>(M(C_3-KCl))</td>
<td>(M(KCl-C_4))</td>
<td></td>
</tr>
</tbody>
</table>

Table 4-2: Subdomain and boundary conditions for numerical simulations.

Geometries copied from actual experimental bubble figures were modeled for gas flux through liquid and polymer interfaces and compared against the associated experimental data (Fig. 4-17A). Obtained permeative fluxes were multiplied by channel heights and the correction factor determined in chapter 3. Good agreement was found between a variety of different bubble sizes and geometries (Fig. 4-17C). Time evolution of select bubbles was also imported and compared, and similar correlations between the different simulation models and experimental results were also found.
Figure 4-18: Results from numerical simulations for bubble removal. (A) Bubbles were individually traced and modeled as a two-dimensional geometry. Surface plot in PDMS shows diffusive flux within the polymer (mol/m²s⁻¹), while the contour plot shows dissolved concentrations within the gas (mol/m³). (B) Plotted streamlines show flow profile around the trap with a bubble present. (C) Numerical simulations (■) were plotted against experimental data (□) for select time points, for bubbles in deionized water, for n=5 bubbles, showing good agreement.

Flow during bubble removal increased removal rate through the liquid interface by up to twofold, but the majority of flux out of the bubble occurred through the PDMS-gas interface, with flux through the bubble-liquid interface relatively insignificant (>2% total flux). However, as bubble sizes decreased during removal, and corresponding wetted surface area ratios increased, the proportion of flux through gas-liquid interface increased, up to 10% of total removal.

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Coarse)</td>
<td>5.25 × 10⁻⁷ (Mol)m⁻¹s⁻¹</td>
<td>5.19 × 10⁻⁷</td>
<td>5.03 × 10⁻⁷</td>
</tr>
<tr>
<td>2 (Fine)</td>
<td>5.25 × 10⁻⁷</td>
<td>5.20 × 10⁻⁷</td>
<td>5.02 × 10⁻⁷</td>
</tr>
</tbody>
</table>

Table 4-3: Permeative fluxes for different quality meshes and for the same numerical models, demonstrating grid independence.

Grid independence of the numerical simulations was determined by comparing boundary integral results between a coarser (mesh 1) and finer (mesh 2) mesh. Mesh 1 was generated with a maximum element size of 50 µm, an element growth rate of 1.2, a maximum scaling size factor of 0.2, and a resolution of the narrowest region of 5. Mesh 1 had an average of 27700 points, 55000 elements, and an element quality of 0.652. Mesh 2 was generated with a maximum element size of 20 µm, an element growth rate of 1.2, a maximum scaling size factor
or 0.2, and a resolution of the narrowest region of 2. Mesh 2 had an average of 88200 points, 175500 elements, and an element quality of 0.641. As shown in table 4-3, permeative flux rates were practically identical for multiple models.

The effect of bubbles in the trap on flow was also simulated, as well as the effect of flow on bubble removal. As long as the trap was not fully blocked, flow and flow profiles in channels up and downstream were not affected. The effect of the bubble trap on dissolved gas concentrations was also simulated at the experimental flow rates and shown to be insignificant. Simulations of the effect of an activated trap on the dissolved gas concentration in the liquid indicated that gas concentrations are reduced by approximately 5% or less at the highest considered vacuum levels and liquid flow rates of 1 mL/hr \( (2.5 \times 10^{-10} \text{ m}^3/\text{s}) \) (see Fig. 4-18).

![Figure 4-19](image-url)

**Figure 4-19:** Simulations showing changes in liquid gas concentrations at different flow rates. The presence of a bubble reduces changes in liquid-phase dissolved gas concentrations. At extremely low flow rates in empty traps, gas concentrations may be significantly reduced, however they can return to equilibrium with ambient conditions rapidly due to the permeability of PDMS.
4.5 Trapping Performance

Long-term culture requires that an integrated bubble trap successfully prevent even a single bubble from reaching critical chip components or loaded biological samples. To test our trap’s robustness over extended periods (≥24 hrs), an automated LabView code was used to periodically inject bubbles every 20 seconds into a liquid stream entering the trap. Average bubble size was approximately 0.022 ± 0.02 µL, though some bubbles coalesced during passage through the fluid resistor to the trap, creating occasionally larger bubbles, but maintaining overall gas mass transport. MOPS was used as the liquid to simulate typical cell-culture wetting characteristics. Fibre-optic detectors were run at 1 kHz, such that sampling was two orders of magnitude faster than calculated residence times (approximately 0.2 s). After 24 hours passed, the on-chip valves were opened to purge the device to provide a calibration measurement. Signal to noise ratio was calculated as the change in amplitude during bubble passage over the standard deviation of the signal (Δ/σ), and was found to be > 60 for all measurements. A total of n = 5 measurements were conducted which showed successful prevention of any bubbles escaping the trap chamber (see sample measurement, Figure 4-19).
Figure 4-20: Plot showing signal from fibre-optic at bubble trap outlet over period >24hrs. After 24 hrs, a long gas segment was injected into the stream to demonstrate signal-to-noise ratio and generate a known positive.45
Chapter 5

Applications and Scalability

5.1 Scalability

To demonstrate the ability to increase the gas removal rate and total trapping volume, a design was fabricated, which allows traps to fill with bubbles, and still permits uninterrupted fluid flow through alternative parallel trap routes. This design utilizes eight traps in parallel, with equal lengths for routes of flow into and out of the trap so that flow resistances are equal between the eight traps. Figure 5-1 displays the ability to minimize the footprint of a bubble trap and arrange single traps in a feature-dense design. Bubbles first merge, and then subsequently fill each trap in succession through four separate flow pathways, each leading to a pair of two traps, until all eight trapping chambers are filled. A train of bubbles was generated in a segmented flow with gas flow rates that exceeded 1.0 µL/min for a period >1 hr. As bubbles passed into the trap configuration, they were captured by a CCD camera and measured using ImageJ. By measuring the initial size of bubbles prior to entering the eight-parallel trap configuration, we quantitatively verified that the parallel traps consistently removed up to 0.9 µL/min of gas flow at 74.5 kPa applied differential pressure. This is near the calculated theoretical maximum for eight traps, and is significantly higher than previously recorded removal rates involving uninterrupted flow (see Figure. 5-2).
Figure 5-1: Scaled-out design consisting of eight parallel traps. Micrograph showing the superposition of 3 bright-field images that were captured every 60 s (inverted intensity). The arrow indicates the inflow direction.\textsuperscript{45}

Figure 5-2: Measured gas volume and respective gas flow rates (♦) entering the parallel scaled-out trap over a 30 minute interval during which all bubbles were continuously removed at an applied differential pressure of 74.5 kPa. Horizontal lines show maximum removal rates for the single and multi-trap configurations (at applied differential pressures of 94.6 kPa) and for literature data.\textsuperscript{45}
Additionally, to demonstrate removal capability in a different configuration, a design containing 100 traps in series was also successfully fabricated and tested. By placing 100 traps across the width and length of a 3” x 2” soft lithographic master, we demonstrated robustness of the trap design in fabrication during exposure and PDMS lift-off (see Figure 5-3). To achieve the large field-of-view in the image, fluorescent dyes were used to capture fluids and bubbles being removed in the device design. While the design has little practical bubble-removal application, the device demonstrates significantly demonstrates the ability to fabricate the bubble trap design in complicated arrangements, with sufficient fabrication effectiveness.

Figure 5-3: Micrograph of serial arrangement of 100 bubble traps. Color is inverted to show liquid filled channels as black, PDMS and gas filled channels as white. Magnified section is indicated on inset.

5.2 Integration into AoC Device

We integrated the presented bubble trap in a device that was used to study small artery structure and function (see Appendix C for device design). Liquid streams flow through the inside lumen of a loaded vessel, described as the perfusing stream, and around the outsides of
the vessel, described as the superfusing streams (Figure. 5-4). Unwanted bubbles arise from heating superfusing MOPS solution from room to physiological temperatures (22-37°C) on-chip, and from occluded regions on-chip which were not completely wetted when initially priming the device with liquid. Such bubbles have previously disrupted experiments by clogging parts of the microchannel network, causing asymmetric flow around the artery, and affecting artery function and viability. Individual bubble traps were added in the superfusion and perfusion lines prior to the region of interest in the presented design to protect any loaded samples from unwanted bubbles. To test the device, resistance arteries were isolated by microdissection from wild type CD1 mice. The device was initially flushed with 1% BSA while the gas inlet channel was plugged to limit bubble adhesion on-chip. The device was then purged with MOPS to remove any remaining bubbles. The chip was connected to syringe pumps and outlets as described, with the gas inlet channel and control valves connected to micropumps and a gas cylinder source. The artery segment was loaded and held in place on the device using hydrostatic pressure. The vessel was superfused with mixed MOPS and PE, at combined flow rates of 17 µL/min. Once a dose regime with PE had been completed to verify vessel viability, nitrogen gas bubbles were injected at set intervals into the superfusing streams. During bubble injection, the artery segment was imaged using a CCD camera. Any bubbles (approx. sizes 0.1-0.2 µL) that infrequently appeared during the experiment, which lasted for 1 hour, were all consistently trapped and removed (see Figure. 5-4). These traps were successfully used in preventing rarely occurring bubbles and in priming the device prior to loading the vessel segment.
Figure 5-4: Application of the bubble trap for the investigation of small artery function and long term culture. Nitrogen gas bubbles were injected into the microfluidic device. Mouse mesenteric arteries (diameter ~230 µm) were fixed on a microfluidic device, kept at physiological temperature and transmural pressure.45

Figure 5-5: Sample measurement of change in inner and outer diameter of loaded small blood vessel in response to PE doses, after device was perfused with nitrogen bubbles for at least 1 hr.
Chapter 6

Discussion

The bubble trap is designed to protect on-chip components downstream via trapping, while increasing the effective trap volume via removal. We refer to this combined removal technique as discontinuous, as it requires stopping movement of bubbles in flow. The trap is implemented in a single-plane, and due to its minimal footprint (~3 mm × 3 mm), it can be easily inserted into any flow path of a standard microfluidic device while not adversely complicating microchip design and fabrication (e.g. alignment between multiple layers). The small footprint allows the trap to be arranged in any number of configurations in serial or parallel, permitting greater trapping volumes and removal rates.

Bubble traps have potential use beyond

Shear stresses induced by passing bubbles, as predicted in Chapter 1, can be over 50 times larger than shear stress in bubble-free liquid with similar fluidic velocities. The film thickness was estimated using equations by Bretherton and Taylor\textsuperscript{26,27}. However, this prediction is smaller than has been measured experimentally by Fries et al.\textsuperscript{72}. This is most likely due to the rectangular-cross section of the microchannels used in their experiments and our devices, as opposed to a circular cross-section. However, we can use the Bretherton equation as an upper-limit prediction of shear stress.

Before analyzing the trap, the permeability of PDMS to different gases needs to be evaluated. A complication is that values for permeation of gases in PDMS have been experimentally determined for common mixtures such as air and pure gases, but for different
PDMS chemistries (see Table 3-1). To our knowledge there is no study that has attempted to determine permeability values for PDMS kits commonly used in the fabrication of microdevices (such as Sylgard 184, from Dow Corning). Additionally the effects of preferential permeation have not been strongly discussed, nor the effect of other non-trivial parameters such as mix ratios for PDMS or the age of PDMS devices, on permeability. Lastly, discrepancies in theoretical vs. experimental data for bubble removal reported in previous literature may be due to unreliable values for permeability of PDMS, especially in the case of gas mixtures such as air. The devices used in this thesis helped in determining different permeability values for this particular PDMS chemistry, which were incorporated in numerical and analytical models.

A further conclusion from the study of permeability is the effect of the three-dimensional geometry on permeation in PDMS. Previous bubble trap solutions discussed the permeation process in a 2D paradigm, which led to discrepancies between expected and experimental results. The numerical simulations allowed derivation of a corrective factor accounting for any non-direct/linear transport. The calculated factor 1.23 appears reasonable based on comparison of analytical and numerical results using this factor to compare with experimental data.

The singular trap was evaluated for performance against benchmarks in the literature. The presented trap’s theoretical maximum removal rate, based on experimentally obtained permeability data for air (~ 2.7 nL/mm²-s flux) and geometry (h = 150 μm, surface area maximally 0.71 mm²), and using the miniature pump (94.5 kPa), is approximately 0.0027 μL/s; rates upwards of 0.0024 μL/s were observed experimentally. These are comparable to what has been observed in the literature. In terms of actual volume, a single iteration of the presented design (h = 150 μm) has a maximum volume of 0.429 μL. While this volume is lower than for other reported bubble traps (e.g. Sung et al., >10 μL), our aim is to prevent occasional bubbles,
as opposed to continuous streams of bubbles which would only appear during initial purging or during catastrophic failure of the device. The bubble trap volume scales linearly with height; therefore assuming a single layer design with uniform height, and microchannels 150 \( \mu \text{m} \) wide, the design can trap a bubble of approximately 1.9 cm in length in the microchannel for any given height. The multiple trap arrangements have increased effective trapping volumes and removal rates. The serial trap arrangement has over 100 traps in series, allowing trapping and removal of up to 42 \( \mu \text{L} \). This trap arrangement, however, is not ideal as a bubble solution that prevents channel blockage, as it still allows a pressure buildup to occur when bubbles are trapped. Conversely, the parallel arrangement has four separate flow pathways, each with two traps in parallel, for a total of eight parallel traps. These traps simultaneously were able to remove at 0.62 \( \mu \text{L/min} \), or approx. 0.01 \( \mu \text{L/s} \). The effective trapping volume is also higher due to connecting flow channels and adequate alternative flow pathways limiting resistance in channels due to bubbles. Due to the configuration of the parallel bubble trap, bubbles have multiple pathways to pass through the trap region. Bubbles under most experimental conditions in microfluidic channels will not experience breakup. Thus bubbles will flow in fluid based on the “path of least resistance”. The parallel bubble trap not only increases overall trapping volume and removal rate, but also takes advantage of fluid dynamics to optimally allocate a passing bubble into the least filled trap.

A number of factors need to be considered when discussing trap removal rate, particularly the non-wetted surface area, the effect of liquid type on bubble morphology and hence surface area, channel wall thickness in the design, unaccounted for changes in pressure, and the non-linear nature of gas removal in a bubble containing a mixture such as air.
Changes in the non-wetted surface area significantly impact the removal rate. The equation for change in bubble volume due to permeation through the PDMS membrane is:

$$\frac{dV}{dt} = \frac{AP(\Delta p)b^{1}}{76}(T/273)(76/P_{\text{atm}})$$

where $A$ is surface area exposed to the gas bubble through which permeation occurs, $P$ is the permeability for the pure gas or mixture in the bubble, $p_2-p_1$ is the difference in pressure between the fluid and vacuum lines, $b$ is the thickness of the wall through which permeation occurs, $T$ is the ambient temperature (K), and $P_{\text{atm}}$ is the atmospheric pressure (cmHg). The first bracketed term is essentially the definition for permeation flux (see equation 5) multiplied by area to get overall permeation rate. The following two bracketed terms are corrective factors for the permeability value, which is scaled to permeation of pure oxygen at STP. From this equation we see that the overall removal rate scales to the surface area involved in permeation, and so as the bubble shrinks in volume and its surface areas concomitantly decrease, removal rate also decreases. Thus contact with the trap wall, and bubble shape, is critical to determining the rate of removal.

It is therefore difficult to compare individual bubbles based on removal rate, because of the different experimental conditions and unique morphologies. We can use permeative flux, which is per unit area, eliminating the effect of differing exposed surface areas (or morphological differences), both at gas-liquid and gas-polymer interfaces. Average permeative flux values support data from overall removal, showing that increased differential pressure increases removal rate. It was observed that measured permeative fluxes for all bubbles increases as they decrease in size. This is likely due partially to the fact that as bubbles decrease in size, their gas-liquid interface relative to their total surface area increases, leading to a larger...
component of gas removal through the gas-liquid interface of the bubble into the surrounding, gas-desaturated liquid, which would skew upwards the values for permeative flux.

Comparing permeative flux also shows that bubbles in surfactant have lower removal rates than their surfactant-free counterparts. Surfactants affect the bubble removal process through two dominant mechanisms: surface and morphology effects. Surface effects refer to how surfactant changes properties of the bubble interface which may affect mass transport. This is evidenced by the decreased values for permeative flux for bubbles in surfactant-containing liquid, as permeative flux discounts any of the effects that changes in morphology may incur due to reduced surface area. Surfactants affect mass transport by changing interfacial tension forces and inducing Marangoni effects\textsuperscript{73} and have been shown to reduce the mass transport of \textit{O}_2 out of bubbles in an aqueous solution\textsuperscript{65}. Morphology effects refer to how surfactant increases the wettability of PDMS, which can increase how much of the trap wall is wetted, and conversely decrease the non-wetted surface area of the bubble for a given bubble volume, including changing the contact angle of the contact points of the bubble along the trap wall.

Furthermore, the presence of surfactant in the liquid causes instability at the bubble interfaces, particularly with respect to the contact point along the trap wall. The contact point of the bubble surface along the trap wall was observed to move in a non-continuous fashion, and thus the actual bubble length (and bubble surface area) along the trap wall decreased in a stepwise pattern; where the contact point would either move very rapidly or be very stable for long periods of time. This result is not evident in the data as it has been smoothed over 15 second intervals and averaged for 5 different bubbles. Bubbles in surfactant, however, had continuously moving contact points, and thus length along the trap wall decreased at a constant rate.
Our design takes advantage of the high permeability of PDMS to gases. Permeation occurs when a pressure differential across a thin membrane creates a gas concentration gradient, causing diffusion. This gradient is generated since the solubility of gases in polymers is proportional to pressure. Considering the definition of permeative flux, our design takes advantage of the ease of precise fabrication in two dimensions (the feature plane) to minimize membrane thickness $b$, improving the permeation rate. This design is modifiable, and has been implemented with different circular chamber sizes and membrane wall thicknesses.

The effect of pressure in the channels due to resistance to flow needs to be considered for the experimental data. For our designs, the trap was placed relatively close to the outlet, minimizing pressure drop from the trap to the outlet. Using simple calculations for hydraulic diameter and Poiseuille flow, the approximate pressure drop is $<100$ Pa (<1% of applied pressure), and thus does not significantly affect permeation rate. It should be noted that the pressure difference between feed and vacuum can affect permeation rate (infusion vs. withdraw of fluid, flow rate and position along flow channel system) and should be factored when inserting traps into designs.

The removal rate is also strongly affected by the gas composition of the bubble. PDMS is much less permeable to oxygen than CO$_2$ and even less so to nitrogen. Air bubbles that form consequently become nitrogen rich during the removal process. This is further complicated by the fact that interactions between gases in mixtures affect permeation, causing deviation from ideal gas behavior. The aforementioned affects of mixtures, such as concentration polarization, can cause deviation from this ideal system and PDMS can exhibit preferential permeation to different gases.$^{74}$ Supporting experimental evidence from literature for air-like gas mixtures suggests mixtures with similar compositions to air act similarly to pure nitrogen, suggesting that
using nitrogen as an analogue is a sufficient assumption for the PDMS membrane for the modeling efforts. A further non-steady state investigation of changes in bubble composition over time would be useful in determining further applications of permeation technology for microfluidics.

It is important to note that the bubble trap does not significantly affect the gas concentrations in-flow in an adverse way, as culture media is primed for desired O₂ and CO₂ concentrations to maintain pH balance and provide essential nutrients. Simulations of the effect of an activated trap on gas concentrations in the liquid stream indicated that gas concentrations are reduced by approximately 5% or less at maximum applied vacuums for 1 mL/hr (2.5×10⁻¹⁰ m³/s).

Trapping experiments showed successful ability to trap bubbles at flow rates consistent with cell culture, and flow rates significantly above those usually found in cell culture. What is significant from the experiments is the consistency at which bubbles were prevented from continuing in flow – not one bubble in over 24 hrs of experiment, conducted n=5 times. While the bubble trap is not capable of trapping large trains of bubbles, or preventing catastrophic failure, trapping experiments suggest it is highly effective at preventing occasional bubbles.

Simulations of fluid flow with trapped bubbles in a near maximally filled bubble trap show restricted access from some gaps in the trap, leading to higher flow rates in less blocked gaps. However overall resistance is still minimal given that if even half the gaps were fully plugged, the combined width of the remaining gaps would still be 150 µm, which is near the 200 µm inlet width in this design. Variations on the design could be made such that the total gap
width is larger than the inlet/outlet channel widths, both to make resistance negligible when unplugged, and minimize flow resistance when blockage in a filled trap occurs.
Chapter 7

Conclusion

7.1 Thesis Contributions

After repeatedly encountering problems with bubbles in microfluidic devices such as the AoC device, a method for the simultaneous trapping and removal of bubbles, which could be implemented in a single layer, was designed. Custom devices were then fabricated to characterize the trap and demonstrate scalability, and the trap was functionally integrated into other devices.

To better understand the transport mechanisms affecting bubble removal, the permeability of different gases was researched and studied in a custom microfluidic device. Numerical simulations of the cross-sectional area were conducted to determine the amount of diffusive transport that occurred over the top of the channel, as opposed to the side channel, as would be assumed using the conventional analytical solution for gas removal. A small but significant contribution to flux was measured in the top channel, allowing calculation of a corrective factor that was used in subsequent analytical and numerical models.

To characterize the trap, experiments were conducted that addressed both the trapping and removal components of the device. To generate bubbles, a novel dual-valved, two-layer system was designed that could controllably inject bubbles into an intersecting liquid stream, allowing generation of bubbles on demand, or a programmed periodic injection of bubbles. To test trapping, photodetectors interfaced into the microfluidic device using fibre optics in a parallel, transmission-based scheme, were used to measure any incoming or outgoing bubbles.
from the trap. These trapping experiments were conducted over 24 hours and showed trapping efficacy. The trap was then tested to characterize bubble removal. Bubbles of different sizes, of different gas conditions, in different liquid conditions, and subjected to different differential pressures, were repeatedly injected into the trap to test for the effect of varying conditions on bubble removal. Using CCD camera images, bubble volumes and surface areas were measured, and removal rates and permeative fluxes were calculated for comparison. The maximum achieved removal rates were comparable to standards demonstrated in literature. Experimental data showed reduced removal rates and permeative fluxes in cases involving surfactants in the aqueous solution. To support the experimental data, two-dimensional numerical simulations were conducted using bubble morphologies from actual experiments imported into the numerical solver. Numerical simulations were also used to demonstrate uninterrupted flow in the trap, and estimate removal rates through the liquid interface. Simple analytical solutions for estimating the time required to completely remove a bubble, based on the bubble’s initial volume and removal conditions, were also presented.

To demonstrate application, the device was tested in a variety of configurations, and also implemented in a device for biological experiments, the AoC. The design was used in serial and parallel configurations, to demonstrate the scaling up of total trapping volume and removal rate. The serial configuration was tested with 100 traps in series, which demonstrated fabrication and operational robustness of the traps. A parallel configuration involving eight traps showed increased trapping volumes and total removal rates significantly higher than those reported in literature, up to 0.6 µL/min. To test trap integration, valves for bubble generation were integrated into an AoC device with bubble traps on the superfusing and perfusing channels. A blood vessel was inserted into the microfluidic device, and bubbles were periodically generated,
trapped and removed in the superfusing channels. To demonstrate success of the trap in protecting the loaded biological sample, tests for vessel viability were conducted before and after bubble generation, and showed vessel functionality was maintained.

The developed bubble trap has been characterized, and demonstrated to successfully trap and remove bubbles, and is suitable for protection against infrequent, unwanted bubbles in microfluidic devices where a single bubble can ruin an experiment, such as devices with biological samples. As a single-layer based design with a small footprint, it can be readily integrated into any previous microchip design.

While the bubble trap design has obvious applications for research-centred microfluidic platforms, it also has applications in clinical situations. One current application would be in dialysis. Bubbles that form in contact with blood cause coagulation, which can be a critical problem in hemodialysis. Bubble removal and prevention technologies could be applied to prevent such situations occurring in practice.

**7.2 Future Work**

A modified bubble trap with thinner interchannel walls between the vacuum line and trap chamber, as well as smaller buttresses, has been designed and fabricated (see Appendix C). The reduced permeation distance \( b \) should increase removal rates. Using previous data from numerical simulations, it is also likely that buttress shape and placement could be further improved to increase bubble removal rate while maintaining trap robustness.

While the bubble trap has been separately (1) integrated on biological microfluidic device, and (2) has been used on devices for extended periods of time, there has been no
experiment conducted to specifically demonstrate that the trap succeeds in protecting biological samples over extended periods (> 1hr, ideally > 24hrs) in combination. Designs have been created integrating the bubble trap, and valved bubble generation, with a variety of designs (see Appendix C) for long term cell culture, which if fabricated, could further demonstrate the trap’s application to biological cell culture.

The majority of current and proposed microfluidic platforms with biological purposes are not made of silicone-based materials, and thus do not have the inherent gas-permeable properties necessary for this type of bubble trap. For devices of this type, use of polymer portions or inserts that may be gas permeable (such as a PDMS insert on a polypropylene device, see Xu et al.\textsuperscript{38}) would be a potential solution. The possibility of adding or improving gas permeability may also be achieved by increasing porosity in a hardened polymer. With suitable porosity, gas transport would become governed by Darcy’s Law, and removal may be achieved.

One potential use for permeation in microfluidics is the precise control of dissolved gas concentrations in liquid streams. Controlling on-chip gas concentrations is crucial to biological studies, from devices for cell-culture and organism-based platforms to bioreactors.\textsuperscript{75-81} Furthermore, dissolved gases play critical physiological roles in tissues. Some dissolved gases – such as nitrous oxide, carbon monoxide\textsuperscript{82}, and hydrogen sulfide\textsuperscript{83} – act as signalling molecules in the vascular system. Tissues respond differently to varying oxygen levels – hyperoxia (above normal oxygen), hypoxic (lox oxygen), and anoxic (no oxygen) conditions – including changes in metabolic pathways and gene expression\textsuperscript{84}. The vascular physiology responds to local oxygen concentrations and needs – normally dilating in oxygen starved regions to increase bloodflow and delivery of oxygen, while constricting via hypoxic pulmonary vasoconstriction during hypoxic conditions in the lungs to improve the efficiency of oxygen uptake. Tissue zonation\textsuperscript{84},
stem cell differentiation\textsuperscript{85}, and angiogenesis\textsuperscript{86} are examples of longer-range, large scale physiological processes that also respond to oxygen conditions. The ability to control the delivery of dissolved gas concentrations on microfluidic chips to loaded cells, organs, or vessels, allows better investigation of these biological phenomena. Controlling dissolved gas concentrations in microfluidic devices is also of critical importance in microfluidic chemistry for reactions that involve dissolved gas components, or on precise variations in pH of fluid streams (where dissolved CO\textsubscript{2} is used as a modulator of pH).

The control of gas concentrations is related to the formation and removal of bubbles in liquid streams as bubbles may form based in supersaturation and changing microenvironment conditions (such as temperature or pressure). For gas-impermeable polymers, liquid streams may be pre-primed to required dissolved gas concentration levels. For gas-permeable polymers, it suffices to place the microfluidic device and setup in an atmospherically controlled environment (e.g. incubator). However, microscale precision and sub-second dynamic control cannot be achieved using conventional methods. Previous work by other groups has been towards creating concentration gradients in liquid streams, mostly using passive diffusion through PDMS, though also using other novel methods such as gas-liquid contacting. These methods have relied on multilayer fabrication, and typically operate on the order of 1-10 seconds.\textsuperscript{84, 87-89} We designed a method that uses permeation, as in the bubble removal case, that operates in a single-layer and can achieve better precision and temporal control of gas concentrations, and has the potential to control gas bubble size by driving growth or removal of gas bubbles in liquid streams (see Figure 7-A,B).

In cases where dissolved gas concentrations are not a concern, this method is an alternative for bubble removal whose capability is limited only by flow rate, and as it does not
require trapping of a bubble; thus we define it as “continuous” removal (see Figure 7-1a). This design uses separate vacuum channels interdigitating a resistor through which the fluid needing to be degassed is perfused. The method thus maximizes the surface area of the side walls in a single layer design through which the bubble is removed, and by also taking advantage of thin PDMS walls, higher removal rates are achieved. This system can be tuned to remove bubbles such that the length required for complete bubble removal is dependent on the fluid flow rate, and the device can achieve complete removal of all bubbles, with nearly no limit to the size of bubble or frequency within the fluid stream. Images showing complete bubble removal (Figure 7-1C) in a working device were obtained.

Figure 7-1: Continuous permeation method. (A) Design showing interdigitation of vacuum and fluid stream lines. (B) Concept for continuous bubble removal. Interdigitating vacuum channels along a microfluidic resistor increase the residence time of fluid under applied vacuum, allowing manipulation of dissolved gas concentrations and bubble sizes. (C) Fabricated functional device for continuous removal of bubble from liquid flow. As can be seen in contrast, all bubbles are removed approximately halfway down the length of the device (total resistor length = 1 m). Scale bars are approximately 1 cm in both A and C.
Extending the concept of continuous permeation, additional devices were fabricated for (a) first applying vacuum, then overpressure, to control gas concentrations and sizes of gas bubbles; and (b) selectively applying vacuum and overpressure on opposing interdigitating channels, with the potential to create concentration profiles within the microchannel. Working devices were created showing first shrinking, and then growth of bubbles, on the same microfluidic device (see Figure 7-2).

Figure 7-2: Continuous removal and growth of bubbles on the same microfluidic device. (A) Device showing combined removal and growth in two different stages. (B) removal via vacuum (~30 kPa) and (C) growth via overpressure (~80 kPa) of bubbles on the same liquid stream in a single-layer microfluidic device. Scale bars are approximately 1 cm in A, and 500 µm in B and C. Downstream is to the right, as indicated by the arrow.

Initial modeling of the phenomena, based on previous simulations of gas transport in the bubble trap devices, allowed prediction and tuning of different variables on the device to
achieve controlled removal and growth of bubbles (Figure 7-3). Due to the defined geometry of the microchannel, and assuming limited transport through the caps, volume based on interdigitated removal can be calculated using:

$$V(t) = V_{B,0} / e^{ct}$$

(24)

Where

$$c_1 = 2hP(\Delta p)/bA_x$$

(25)

Figure 7-3: Modeling of change in bubble length in interdigitating device for applied differential pressure of 74.5 kPa (solid line). Additional solid line is for a bubble of half the initial length (5 cm, 74.5 kPa), while the gray line is for a bubble removed under reduced differential pressure (50.8 kPa), and the dashed line is for bubble where removal occurs through only half of the interdigitation.
This design and method can be used for biological experiments requiring dynamic control of dissolved gas concentrations, such as hypoxia (O₂) or pH (CO₂) studies. In the case of the AoC project, capability for controlled gas concentrations could be integrated onto the device. Such methods could be used to achieve: (1) more rapid removal of bubbles during preparation of the AoC prior to loading; (2) additional protection against unwanted bubbles once the vessel is loaded; (3) the ability to condition liquid streams with an aim towards longer on-chip culture of vessels; and (4) the use of controlled gas concentrations as an additional tool to probe vessels, such as a hypoxia-based study.
References


Appendix

A. Labview Programs

Figure A-1: Sample dialog from custom LabView code for measuring the light intensity signal from the photodetector.

Figure A-2: (Following page) Block diagram of LabView code for photodetector.
Figure A-3: Front Panel on periodic valve trigger program.

Figure A-4: Block diagram for periodic valve program.
B. Additional Experimental Results

Figure B-1: Comparison of bubble volume and surface area for bubbles of different initial sizes. Experimental liquid was deionized water.

Figure B-2: Comparison of bubbles removed under different vacuum conditions, 74.5 kPa (●) vs 96.5 kPa (■). Experimental liquid was deionized water.
Figure B-3: Removal rates and permeative fluxes for bubbles removed under different vacuum conditions. Circles (●) represent 74.5 kPa, while squares represent 96.5 kPa (■). Solid squares show removal rate, while hollow squares are permeative flux. Error bars are one standard deviation.

Figure B-4: Removal rates for different bubble examples, comparing removal through PDMS interface and liquid interface. Shaded gray is for decreasing liquid flow rates, while solid black is removal rate through PDMS.
C. Additional Device Designs

Figure C-1: Design for single-bubble characterization. Control layer is indicated by dark grey shading. Scale bar is 5mm.

Figure C-2: Design for parallel trap configuration. Control layer is indicated in dark grey shading. Scale bar is 5mm.
Figure C-3: Design for AoC application. Control layer is indicated with dark grey shading. Scale bar is 5mm.

Figure C-4: Modified trap with smaller buttresses and reduced channel thickness \( b \).
Figure C-5: Design for cell culture applications, with added bubble trap prior to inflow.