Controlled Interfaces as Lab-on-a-chip Components

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Abstract

This thesis is devoted to the study and application of controlled two-phase interfaces in the context of microfluidic and lab-on-a-chip systems. Spontaneous emergence and presence of unwanted gas-liquid interfaces (i.e. bubbles) in microfluidic devices are often problematic. In this thesis it is shown through experimentation, numerical, and analytical modeling that precisely controlled gas-liquid interfaces may be exploited as effective microfluidic components to perform functions such as valving, sampling and liquid routing within microfluidic devices or even manipulate layers of pulmonary cells to recapitulate alveolar function. Flow control strategies presented in this thesis include the bubble gate for valving and sampling and the bubble pump for liquid routing in microchannels. Flow control and actuation in the devised techniques are done by controlled gas bubbles and therefore, their function is independent of the elasticity of substrate material and requires only a single layer fabrication unlike the majority of available microfluidic flow control techniques. In this thesis, the application of controlled two-phase interfaces is further extended by introduction and characterization of a microfluidic tensiometer that can readily measure interfacial tension between pairs of immiscible fluids both gas-liquid and liquid-liquid. Additionally, it is shown that controlled gas-liquid interfaces within a surface modified capillary plate may be used as cell culture platforms for in vitro modeling of
the alveolar epithelium function. In order to do so, it is investigated in this thesis whether pulmonary epithelial cells can be cultured atop a controlled air-liquid interface and form a confluent layer with functional tight junctions that can serve as a model for alveolar epithelium. Finally a number of further extensions and applications for the techniques developed in this thesis are presented in form of preliminary designs and calculation as groundwork for future continuation of this thesis.
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Chapter 2

Fig. 1 (a) Schematic illustration of different flow control strategies reported in the literature in terms of working liquids, substrate materials and number of masking layers required for device fabrication: (1, green colour) corresponds to electrical techniques, (2, blue colour) summarizes techniques based on the deflection of an elastomeric membrane, (3, yellow colour) summarizes conditions for flow control based on stimulus responsive materials and (4, light blue) corresponds to techniques employing flow obstruction via bubbles (see Appendix Table A1 for detailed references). Red region highlights “ideal” flow control strategy for microfluidic devices that is compliant with different working liquids and substrate materials and only requires a single masking layer. (b) Schematic illustration of “ideal” flow control strategy integrated in a device.

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Chapter 3

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Chapter 4

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**Chapter 5**

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Fig. 16 Fluorescent micrographs for N=3 experiment showing distribution of junctional protein ZO-1 and the cell nuclei following double stain of NCI-H441 cells at day 4 in culture atop a
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Chapter 6

Fig. 1 Schematic illustration showing different steps of a lung transplant surgery from left to right: Harvesting the donor lung, donor lung storage under cold ischemia condition, and finally transplantation and post-transplantation steps.

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Fig. 7 The proposed comb exchanger gas delivery strategy. A series of quasi-bubble gates in a comb configuration are opened and closed in an oscillatory manner to promote convective dissolution of the working gas in the culture media.

Fig. 8 Lung transplant-on-chip device and the temperature control unit assembled. Temperature control is achieved using thermoelectric elements attached to a sapphire disc that will sit on the microdevice. The sapphire disc with a relatively high conduction coefficient helps transfer heat to and from the TE element for cooling and heating respectively. An aluminum made cooling jacket will be attached to the top surface of the TE element as a heat sink. The cooling jacket has four tubular channels inside. Water at room temperature will be flown inside the cooling jacket using a syringe pump. (c) The cooling jacket designed to be placed on top of a thermoelectric element as a heat sink. The thermoelectric element will heat and cool the culture channel accurately and in case of cooling the absorbed heat by the TE element will be removed by the proposed cooling jacket heat sink. The maximum power of a TE element is 4.4W applied to the bottom surface in the model.

Fig. 9 Process flow for deep reactive ion etching of PMMA (or similar thermoplastics such as polycarbonate) (a) raw PMMA sheet (white) (b) a thin layer of titanium (yellow) sputtered on PMMA (c) A layer of photoresist (red) coated on top of the titanium layer (d) The photoresist layer is patterned by UV exposure and rinsing with photoresist solvent (e) Reactive ion etching (RIE) patterning of the titanium layer using CF4 (f) O2 RIE of PMMA using the patterned titanium as a mask (g) stripping the patterned PMMA sheet of the titanium layer using 1% HF solution.

Fig. 10 Process flow for KOH wet-etching of a through-hole in silicon (a) clean silicon wafer (dark grey) (b) a thin layer of silicon nitride (light grey) is deposited on the wafer using chemical vapor deposition (LPCVD or PECVD). Alternatively, silicon wafers with silicon nitride may be purchased to save fabrication time 12. (c) A layer of positive photoresist (red) coated on top of the nitride layer (d) The photoresist layer is patterned by UV exposure and rinsing with
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Fig. 11 Process flow for DRIE etching of a through-hole in silicon (a) clean silicon wafer (dark grey) (b) a thin layer of silicon oxide (light grey) is deposited on the wafer using chemical vapor deposition (LPCVD). Alternatively, silicon wafers with silicon oxide layer may be purchased to save fabrication time. (c) A layer of positive photoresist (red) coated on top of the nitride layer (d) The photoresist layer is patterned by UV exposure and rinsing with photoresist solvent (e) Buffered oxide etch (BOE) patterning of the silicon oxide layer. (f) DRIE etching of the silicon until the etched hole from top and bottom sides of the silicon wafer meet. The hole has vertical walls using this fabrication technique. Photoresist layer can be removed using either piranha cleaning or using acetone. The oxide layer can be removed using BOE.
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Table A1. Examples of the flow control techniques developed to date with details on their fabrication and operation

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Section A1. Solid mechanics considerations in bubble gate design

Section A2. Investigation of transient pressure disturbances on bubble gates
Chapter 1 Introduction

1.1 Introduction

Microfluidics which has emerged in the past two decades as a vibrant field of research across many disciplines is defined as the study and application of fluid flow in micro scale structures and conduits\textsuperscript{1-3}. Microscale pertains to characteristic dimensions from 1μm to 1mm\textsuperscript{4, 5}. Fluid flow in structures and passages with characteristic dimensions smaller than 1μm is referred to as nanofluidics\textsuperscript{6-9} while fluid flow in structures with characteristic dimensions larger than 1 mm are considered macro-scale. Microscale fluid flows occur widely in nature and have been focus of research for many decades as part of colloid science, soil science, plant biology, and biomedical science\textsuperscript{1, 5, 10}. In the past two decades, the new research area of microfluidics and lab-on-a-chip devices have emerged and grown rapidly following advancements in micro-engineering and microfabrication techniques\textsuperscript{1, 11-13}. Additionally, the increasing need for micro-devices in analytical chemistry, chemical synthesis, biology, and biotechnology have contributed to the rapid growth in the field of microfluidics and lab-on-a-chip devices\textsuperscript{2, 14, 15}.

Microscale fluid flow is characterized by laminar flow and low Reynolds\textsuperscript{16} numbers on the order of unity or lower\textsuperscript{2, 17}. Therefore, in microscale flows viscous forces dominate inertial forces. In single-phase microscale flow diffusion is the dominant means of transport and the fluid flow is instantaneous due to the negligible effects of inertial forces\textsuperscript{18, 19} meaning the flow behavior at any given moment is the result of forces applied on fluid elements at the very same moment. This instantaneous flow behavior makes microscale flows more controllable than fluid flows in macro-scale\textsuperscript{19}.

The unique characteristics of microfluidic systems, including their small size and portability as well as the minute sample volume requirements have led some recent healthcare research to focus on the development of point-of-care medical diagnosis systems. Microfluidic systems have shown promise in improving accuracy and speed and reducing the cost of medical diagnosis in the developed world while demonstrating a potential to provide the developing world quality
diagnostics without the costs associated with maintenance and operation of centralized lab facilities. Another major area of application for microfluidic technologies is energy. With the rapidly growing popularity of various portable electronic devices, there is growing demand for reliable and efficient power sources for portable applications. This demand has motivated a great amount of research on developing microfluidic power generation systems such as membraneless microfluidic fuel cells. These fuel cells take advantage of a colaminar flow of fuel and oxidant within a microfluidic channel for generating power. Additionally, due to their precisely tunable microscale features microfluidic devices have been employed to study oil extraction from porous rocks in oil reservoirs. Microfluidics technologies have found application in carbon management via studies of carbon sequestration and CO2 solubility using multiphase flow microfluidics.

The application of microfluidics-based platforms in implementation, control and facilitation of chemical and biological reactions is a well-established and growing field. Microreactors are advantageous compared to their macro-scale counterparts due to their small size, enhanced transport and their ability to integrate multiple functions within the same platform. Microreactors can be inexpensive; they can be mass-produced and scaled-up to achieve high throughputs comparable with macroscale reactors. In chemical synthesis and production, microreactors have been advantageous in a number of ways. The high heat and mass transfer rates associated with the characteristic high surface area to volume ratio in microfluidic channels allow microreactors to operate in more aggressive conditions and deliver higher process yields compared to the macroscale reactors. Microreactors also offer safety benefits through process intensification resulting from their small size and minute volume of reactant they process. Due to their unique safety characteristics microreactors can be employed as point-of-use chemical production units for materials with storage and shipment restrictions such as highly reactive or toxic chemicals.

Enhancing heat and mass transfer within microreactors has been one of the main areas of focus for research in the microprocess engineering field. Structuring and texturing the microreactor surface and thereby increasing the surface area has been shown to result in an order
of magnitude enhancement in heat transport within micoreactors\textsuperscript{37, 42-44}. Developing patterned liquid flow in microreactors has been shown to be an effective means of enhancing transport and mixing efficiency\textsuperscript{43, 44}. Multiphase flow patterns where a continuous flow of reactants is segmented by an immiscible disperse phase have been developed and extensively used as a powerful transport enhancing method in microfluidic reactors\textsuperscript{45-49}. Mass transfer enhancement in multiphase flow reactors is an outcome of segmentation which generates recirculating flow within both phases and reduces mass transfer in the axial direction\textsuperscript{1, 39, 41, 50}. Both gas-liquid and liquid-liquid segmented flows have been applied to a range of different applications where enhanced mixing or low axial dispersion is crucial, for instance, in chemistry, biology, chemical synthesis and catalytic chemical reactions\textsuperscript{41, 51}. Gas-liquid segmented flow microreactors have been shown to operate in a broader range of flow velocities and temperatures compared to liquid-liquid multiphase reactors\textsuperscript{48, 52}. Gas-liquid two phase flow microreactors have been widely applied to particle synthesis and catalytic monolith reactors\textsuperscript{48, 52-54}.

Unlike in multiphase flow microreactors, multiphase flows in microchannels are not always desirable. Unwanted emergence of gas-liquid interfaces or bubbles within microfluidic devices have been reported as a source of problem for a number of microfluidic applications as the gas bubbles may adversely affect living cells or tissues under study within the microfluidic device or even alter or block fluid flow in microchannels and render the microfluidic device dysfunctional\textsuperscript{55}. As a result, there have been a number of efforts directed at tackling the issue of unwanted bubbles and maintaining microfluidic channels bubble-free\textsuperscript{55-59}.

Following on the footsteps of the previous works on multiphase flow in microfluidics and lab-on-a-chip devices, the main motivation of this thesis is to verify whether precisely controlled gas-liquid two phase interfaces in microchannels can be exploited to perform desirable on-chip operations such as valving, sampling, and liquid routing. As a result, the first specific aim of the current thesis is allocated to developing and characterizing “the bubble gate” as the building block for our flow control and handling strategy using two phase interfaces. The second aim of the thesis is to use precisely controlled dynamic gas-liquid interfaces for driving liquid samples in microchannels. We intend to achieve low flow rates for applications where long term low flowrates of a large number of samples are required such as microfluidic cell culture or drug screening applications. The third aim of this thesis is to develop a scalable in-situ technique for
interfacial tensiometry in integrated microfluidic devices based on our ability to automatically and precisely control interfaces in microchannels. The forth specific aim focuses on applying our ability to precisely control a gas-liquid interface to recapitulating alveolar physiology by culturing a layer of pulmonary epithelial cell on top of a capillary plate with capillary diameters comparable to the diameter of the alveolus.

1.2 Microfluidic Transport Phenomena

Microfluidics is the study and application of fluid flow in microstructures and flow passages with characteristic dimensions between 1µm and 1mm. Microscale fluid flow is significantly different than flow in macroscale. In microscale, viscous and surface forces are the dominant forces while the effects of body inertia forces are negligible. The dominance of surface forces over body forces is a result of high surface area to volume ratio in microchannels.Viscous and surface tension forces are inversely proportional to the characteristic length of the system, and as a result for small length scales encountered in microfluidic applications these forces dominate inertia and gravity forces which are directly proportional with volume, or the cube of characteristic length. Microscale fluid flow can be characterized by dimensionless numbers such as Reynolds number, $Re$, which is defined as the ratio of inertia forces to viscous forces or in mathematical terms:

$$Re = \frac{\rho Ud}{\mu}$$

(1)

where $\rho$ is the density of fluid, $U$ is the characteristic velocity of the flow, $d_H$ is characteristic length of the flow, and $\mu$ is dynamic viscosity of the fluid. Microscale fluid flows are characterized by very small dimensions and characteristic velocity and therefore, in most cases have Reynolds numbers on the order of unity or lower and are characterized as laminar flows. In laminar flow, fluid elements move along parallel orderly streamlines and fluid flow is characterized by high diffusion and low convection of momentum.

As mentioned before, due to lack of inertia microfluidic flows are instantaneous. Instantaneous means the flow cannot store inertia; instead elements of fluid respond to instantaneous forces.
affecting them at each moment in time and are uninfluenced by flow history\textsuperscript{18}. In other words, fluid motion a certain point in time is a result of forces acting on fluid elements at the very same instant. This characteristic of microscale flows is a result of diffusion dominated momentum transport\textsuperscript{5}.

An extensive body of work has been devoted to the study of transport phenomena in microscale covering topics such as fluid flow\textsuperscript{2, 61, 62}, diffusion\textsuperscript{19, 63} and multiphase flows\textsuperscript{64, 65}. In the following sections we review the fundamentals of microscale fluid flow and transport phenomena relevant to the scope of this thesis.

1.2.1 Equations of Transport

1.2.1.1 Equations of Continuity and Momentum

In fluid mechanics a flow field can be fully described with the knowledge of the three velocity components in three spatial directions (e.g., Vx, Vy, Vz) and the three thermodynamic variables: pressure, P, density, ρ, and the temperature T everywhere in the flow field. Theoretically, this can be achieved by solving the conservation equations for mass, momentum, and energy and the thermodynamic equation of state\textsuperscript{60, 66, 67}. Liquids are normally considered as incompressible and their density ρ is can be assumed to be a constant scalar. In most instances incompressibility assumption also holds for gases flowing at low speeds (Mach number <0.3). In this section we introduce conservation laws for incompressible flow in the context of microscale fluid flows.

In any fluidic system three fundamental conservation laws laws namely, conservation of mass, momentum, and energy, must be satisfied at every point in the flow field. These fundamental laws are formulated by applying each of the three laws to differential fluid elements as shown in a schematic in Fig. 1\textsuperscript{60, 66, 67}. By doing this, each law will be described by a partial differential equation. Here we adopt a Cartesian system of coordinates to explain, derive, and present the differential equations.
Conservation of mass or continuity in a fluidic system states that the mass entering every and each fluid element minus the mass exiting that element must be equal to the change of mass in the element. For a fluid with velocity vector of \((v, u, w)\) and density of \(\rho\) conservation of mass can be written as:

\[
\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x} (\rho v) + \frac{\partial}{\partial y} (\rho w) + \frac{\partial}{\partial z} (\rho w) = 0
\]

For incompressible flow, \(\rho\) is a constant and Eqn. 2 is reduced to:

\[
\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0
\]

Equations of conservation of momentum are derived from Newton’s second law of motion which relates the acting forces to the acceleration of the system. Acceleration is a vector quantity and therefore, there are three equations of conservation of momentum for three different components of acceleration in x, y and z directions. Applying Newton’s second law of motion to the fluid element shown in Fig. 1 we obtain the famous Navier-Stokes equations of fluid motion for a Newtonian fluid:
\[ \rho \left( \frac{\partial \mathbf{V}}{\partial t} + (\mathbf{V} \cdot \nabla) \mathbf{V} \right) = \rho \mathbf{g} - \nabla p + \mu \nabla^2 \mathbf{V} \]  

(4)

where \( \mathbf{g} \) denotes the gravitational acceleration, \( p \) is pressure, \( \mathbf{V} \) is the velocity vector. The term on the left hand side of Eqn. 4 is the fluid acceleration and the term on the right hand side each represent a force acting on the infinitesimal fluid element. The first time on the right is the gravity force, the second term represents pressure forces and the last term on the right represents the viscous forces acting on the surface of the fluid element \(^{60, 66, 67}\).

A nondimensionalized form of Navier-Stokes equations (Eqn. 4) can be obtained from substitution of the following dimensionless terms in Eqn. 4.

\[
\mathbf{V} = \mathbf{V}^* \mathbf{U}, \quad \nabla = \nabla^* \left( \frac{I}{D} \right), \quad t = t^* \left( \frac{D}{U} \right), \quad p = p^* \left( \frac{\mu U}{D} \right), \quad g = g^* \left( \frac{U^2}{D} \right)
\]

(5)

The resulting dimensionless Navier-Stokes equation is shown in Eq. 6:

\[
Re \left( \frac{\partial \mathbf{V}^*}{\partial t^*} + (\mathbf{V}^* \cdot \nabla^*) \mathbf{V}^* - \rho^* g^* \right) = -\nabla^* p^* + \nabla^2 \mathbf{V}^*
\]

(6)

All variables in Eqn. 6 are in dimensionless format with \( Re \) number appearing as factor on the right hand side. For many microscale flows including those studied in this thesis \( Re \) number is very small and as such the left hand side of Eqn. 6 including inertia and gravity terms are negligible in comparison with the right hand side of Eqn. 6 \(^{67, 68}\) and as a result, the Navier-Stokes equation is reduced to a linear partial differential equation also known as Stokes equation:

\[
-\nabla^2 p^* + \mu \nabla^2 \mathbf{V}^* = 0
\]

(7)

For laminar flow in rectangular microchannels of constant cross-section, similar to the microchannels used in this thesis, Stokes equation can be written as:

\[
\frac{\partial p}{\partial x} = \mu \left( \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right)
\]

(8)
Eq. (8) has been solved analytically \(^{68}\) with the two-dimensional velocity profile given by the following equation:

\[
u(y, z) = \frac{16a^2}{\mu \pi^2} \left( -\frac{dp}{dx} \right) \sum_{i=1,2,...}^{\infty} (-1)^{(i-1)/2} \left[ 1 - \frac{\cosh(i \pi z / 2a)}{\cosh(i \pi b / 2a)} \right] \frac{\cos(i \pi y / 2a)}{i^3} \]

(9)

where \(a\) and \(b\) are the dimensions of the rectangular cross-section. For microchannels with high aspect ratio (width/height) the flow field can be considered one-dimensional and approximated by the flow between two infinite parallel plates where velocity profile only varies in one direction (y) and is given by the following equation:

\[
u(y) = \frac{1}{2 \mu} \frac{dp}{dx} \left( y^2 - \left( \frac{b}{2} \right)^2 \right) \]

(10)

where, \(b\) is the channel height \(^5, 60, 66, 67\).

The schematic illustration in Fig. 2 describes the velocity profiles across the channel width and height for rectangular microchannels with a finite aspect ratio, similar to those employed in experiments conducted as part of this thesis. The aspect ratio for microchannels employed in this thesis is relatively high (width/height ~ 6.67) but not high enough for the Eqn.10 to be applicable with high accuracy. Therefore the one dimensional flow assumption in Eqn 10 does not apply to flow field in devices tested in this thesis and velocity is a function of both \(y\) and \(z\). Velocity gradients are inversely proportional to characteristic length \((dU/dy, dU/dz)\), and therefore, are larger along the smaller dimension (height) than the larger dimension (width) of the microchannel as shown in Fig. 2.
Fig. 2 Velocity profile across the channel width and channel height of a rectangular microchannel with a finite aspect ratio similar to channels employed in this work.

1.2.1.2 Transport of Species

In previous section hydrodynamics of a homogeneous fluid flow was discussed. In many microfluidic applications, however, the working fluids contain dissolved substances and are therefore solutions or mixtures. The composition of solutions is characterized by the mass density of each constituent (i.e., the mass of dissolved substance per unit volume) or the dimensionless mass concentration \( C \) (i.e., the ratio of the mass density of a substance to the total density of the mixture) \(^5,67,69,70\). The concentration of each component in a solution or a mixture, at each point of the flow field, depends on the diffusive mass transfer, convective mass transfer, and the intensity of physical or chemical transformations or reactions present in the system \(^5,67,69,70\).

With the assumption of constant fluid density and viscosity regardless of the concentration of constituents, the concentration distribution does not influence the flow field\(^5\). With this
The assumption the hydrodynamic problem can be studied separately from the species transport problem\textsuperscript{5,67,69,70}. The equation of transport for individual species is given as

\[
\frac{\partial C}{\partial t} + V \cdot \nabla C = D \nabla^2 C
\]

(11)

The right hand side in Eqn. 11 is the diffusive transport term and the second term on the left hand side is the convective transport term. is the convective mass transfer term and the right hand side is the diffusive mass transfer term. Equation 11 only takes into account ordinary diffusion and convection described by Fick’s Law and neglects other forms of transport such as electromigration or thermodiffusion\textsuperscript{5,69,70}.

The species transport equation can be nondimensionalized by substituting the following dimensionless variables in Eq. (11)

\[
V^* = V^*U, \quad \nabla^* = \nabla^*\left(\frac{l}{l}\right), \quad t^* = \frac{Dt}{l^2}, \quad c = \frac{C_i - C}{C_i - C_s}
\]

(12)

In Eqn. 12 $U$ is the characteristic velocity, $l$ is the characteristic length, $D$ is the diffusion coefficient, and in case of simple dissolution of a solute in the flow, $C_i$ is the unperturbed concentration of the fluid and $C_s$ is the concentration of solute. Following nondimensionalization the dimensionless species transport equation is obtained as:

\[
\frac{\partial c^*}{\partial t^*} + Pe(V^*, \nabla^* c^*) = \nabla^{*2} c^*
\]

(13)

In Eqn. 13 the dimensionless group, $Pe$, is the Peclet number (defined as $Pe = \frac{Ul}{D}$), which appears as a factor behind the convective transport term.

Peclet number is the ratio of convective and diffusive mass transport. From Eqn. 13 it is evident that at very low Peclet numbers the convective term becomes negligible and molecular diffusion is the dominant means of transport in the system while at very large $Pe$ the right hand side becomes negligible compared to the left hand side and therefore convective transport becomes the prevailing means of species transport in the system. One can note the similarity between the
momentum and species transport equation where in the equation of momentum $Re$ number is similar to $Pe$ number in species transport equation with the difference that the molecular diffusion coefficient has been replaced by the kinematic viscosity. For typical aqueous solutions, for instance, the diffusion coefficient is nearly three orders of magnitude smaller than the kinematic viscosity coefficient and as a result in the momentum equation diffusion plays a more important role than it does in the species transport where both convective and diffusive components are important.67

1.2.2 Multiphase Microfluidics

When two or more immiscible moving fluids come into contact a multiphase flow is created. Multiphase flow in microchannels can present themselves in various forms such as a suspension of droplets or bubbles within a continuous phase, large liquid slugs or gas bubbles spanning across channel width or annular liquid films deposited on the walls of the microchannel. A large number of different flow patterns can occur when two or more immiscible phases meet depending on interactions among dominant forces in the system such as surface tension, viscosity, inertia and gravity forces. In static conditions when there is no movement, with negligible gravitational or body forces two phases are in equilibrium if the pressure in each of the immiscible phases is uniform and a pressure jump occurs at the location of the two-phase interface which is given by Young-Laplace equation.

$$\Delta P = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$  (14)

Where $\Delta P$ is the capillary pressure, $\gamma$ is the interfacial tension coefficient and $R_1$, $R_2$ are the radii of curvature of the interface. The schematic illustrations in Fig. 3 show two possible scenarios for static contact between two immiscible phases and the solid wall of a microchannel. The two equilibrium configurations are achievable depending upon the properties of the wall surface and the two immiscible fluids. In the first scenario shown in Fig. 3a the fluid-fluid interface does come in contact with the wall and there exists a contact line between the three phases. In the second scenario of Fig. 3b one fluid wets the wall surface entirely in form of an adsorbed liquid film, preventing contact between the second fluid and the channel wall.51 In the
first scenario, a contact angle ($\theta$) exists which is the angle at which the three phases coincide. The contact angle, $\theta$, is related to interfacial energies of the solid phase, ‘s’ and two fluid phases ‘1’ and ‘2’ by Young’s equation given below\textsuperscript{5, 51, 67, 72}.

$$\gamma_{12} \cos(\theta) = \gamma_{1s} - \gamma_{2s},$$

(15)

Fig. 3 Schematic illustrating a two phase flow interface in microchannels when: (a) Immiscible fluids and the microchannel wall form a three phase contact line. The contact angle $\Theta$ is the angle at which the three phases meet (b) One phase (1) is engulfed by the more wetting fluid (2). The more wetting fluid forms a thin adsorbed film of the thickness, $\varphi$, on the solid surface and therefore unlike (a), the interface is “lubricated”\textsuperscript{71} meaning there is no three phase contact line.

In dynamic conditions (i.e. where a multiphase flow occurs) however, the continuous and dispersed phase velocities (i.e., flowrates) bring viscous forces into play\textsuperscript{71} and add complexity to interactions of governing forces which is in turn responsible for possibility of achieving many different flow patterns inside microchannels depending on parameters such as: the liquid and gas flow rates, fluid properties and surface properties of the channel wall\textsuperscript{41, 51, 71}.

There has been a large body of literature that have described many flow patterns for multiphase flow in capillaries and microchannels\textsuperscript{41, 51, 73, 74}. Most flow regimes reported were based on experimentation including microscopy, observation, imaging, and image analysis to distinguish
different flow patterns. Achievable two-phase gas-liquid flow patterns in a capillary with wetting channel walls (i.e., where liquid wets the wall surface) have been studied extensively and can be found elsewhere.

All multiphase flows encountered and studied in this thesis involve lubricated gas-liquid or liquid-liquid interfaces similar to flow pattern in Fig. 3b where the disperse phase is separated from the channel wall by an adsorbed film of thickness, $\varphi$. The flow of a long lubricated bubble inside a circular capillary is a classical fluid mechanics problem. Bretherton first studied hydrodynamics of bubble flow inside capillaries and therefore it is known as the Bretherton problem. Prior to Bretherton’s analysis of bubble dynamics, bubbles in capillaries were used as tracers for measuring liquid velocity inside capillaries. Fairbrother and Stubbs first noticed the deposition of a liquid film around a moving bubble in a capillary and that bubbles move faster than the liquid due to presence of the film on capillary walls. From continuity standpoint, presence of a liquid film means that the bubbles travel at a higher velocity than the surrounding liquid. Fairbrother and Stubbs showed that the relative velocity of the bubble and the surrounding liquid phase is a function of capillary number, a dimensionless term. Later, Bretherton employed a lubrication analysis for the transition region between the spherical bubble cap and the flat liquid film and determined a relationship for the film thickness and pressure drop across a bubble as a function of capillary number, a dimensionless group relating viscous and surface tension forces given by

$$Ca = \frac{\mu U}{\gamma}$$

He also validated his analytical solution experimentally by measuring the film thickness through measurement of the reduction in the volume of a moving liquid slug as it left a thin film behind.

Capillary number for microfluidic devices studied in this thesis is on the order of $10^{-04}$, and is therefore considered low which according to Bretherton, corresponds to small film thicknesses. In other words, the gas bubble covers almost the entire channel cross-section, if circular. An important consequence of low capillary number and low film thickness in gas-liquid two phase flow is that the bubbles and liquid in front of the bubbles move at essentially the same
velocity\textsuperscript{84, 90}. However, microfluidic channels fabricated using the photolithography and soft lithography processes described earlier are rectangular in cross-section. Liquid film thickness in rectangular channels varies across the channel cross section with the thickest portion being at the corners of the channel\textsuperscript{89}.

Another unique characteristic of a moving bubble in a capillary or a microchannel is the circulating flow pattern induced by the shear forces from the channel walls in the liquid at the front of the bubbles. Taylor\textsuperscript{91} for the first time proposed three different streamline patterns for liquid phase in front of a moving bubble. He proposed three different streamline patterns for three different ranges of relative velocity (i.e., film thickness) of the bubbles and the surrounding liquid. Later his suggested circulating streamlines were verified experimentally, numerically and analytically by others\textsuperscript{84-86, 92, 93}.

1.3 Microfabrication

Soft lithography which was the main microfabrication technique used in this thesis consists of two steps 1) Rapid prototyping and 2) Replica molding. In this section we review these two steps in detail.

1.3.1 Rapid Prototyping

Rapid prototyping is the step where a replica is made by fabricating desired patterns and features on a substrate through photolithography\textsuperscript{5, 94}, and is shown in the first four steps of Fig. 4 (4a-d). A layer of photosensitive material\textsuperscript{5, 94}, photoresist, is spin-coated and baked on a substrate that is usually made of highly polished glass (e.g. microscope glass slides) or silicon (silicon wafers). A photomask with the desired device patterns is designed using a computer aided design (CAD) software package, usually, AutoCAD. The designed photomask is printed on a transparency sheet. The substrate already coated with a photoresist layer is exposed to collimated UV light with the printed photomask on top. Areas of the substrate that are exposed to UV light will polymerize and harden after a post-exposure baking step. Following post exposure bake the unexposed photoresist is washed away to leave the desired patterns and features as combination of raised rectangular ridges and grooves. The entire rapid prototyping process can be completed in a few hours depending on the photoresist thickness and number of masking layers required to
achieve the desired design. A photoresist template fabricated for the microreactor studied in this thesis is shown in Figure 1.5a.

1.3.2 Replica Molding

Following the fabrication of the replica as described in the previous section, a prepolymer is added on top of the replica inside a petri dish, as shown in Fig. 4e. The prepolymer is then cured and removed from the substrate. Of the polymers available, polydimethylsiloxane (PDMS) has become popular with the microfluidics and lab-on-a-chip community due to its many attributes including high-accuracy replication of patterns, low cost and ease of use and rapid curing. Another unique characteristic of PDMS is easy removal from the replica without damaging either the replica or the surface of the cured PDMS layer. Additionally, PDMS is transparent and as such compatible with various visualization and microscopy techniques.

Once molded and removed, as shown in Fig. 4f and 4g, the PDMS devices are cut and appropriate inlet and outlet holes are created. The PDMS device can then be sealed onto a second surface either reversibly or irreversibly. Reversible sealing takes advantage of the elasticity of PDMS by creating a conformal seal between the device and a second substrate. Reversible sealing is only suitable for applications involving low pressures as leakage may occur at high pressures. For high pressure applications irreversible sealing is appropriate. Irreversible sealing of PDMS devices can be achieved by exposure of the two surfaces to oxygen plasma before they are brought into direct contact to form an irreversible seal as a result of chemical reactions taking place at the interface of the layers being sealed. A PDMS device fabricated and sealed onto a glass slide and tested as part of this thesis is shown in Fig. 5b.

Plasma treatment of the PDMS surface works by creating by oxidizing the surface and creating Si – OH silanol groups will react with by Si – OH groups present on the second substrate and form strong permanent Si – O – Si bonds and as a result will permanently bond the two layers. Additionally plasma treatment of the PDMS surface has been shown to render the natively hydrophobic PDMS hydrophilic. This treatment has been extensively used to achieve hydrophilic surface for experiments where a wetting surface is required. The treated surface however will return to the native hydrophobic state with time.
Fig. 4 Fabrication process flow for photolithography (a-d) and soft lithography (e-g) (a) A substrate (e.g. glass slide or silicon wafer) is cleaned using isopropyl alcohol and acetone followed by oxygen plasma treatment (b) A layer of negative photoresist (red) is spin-coated on the surface of the substrate and the substrate is then baked at 65°C and 95°C (c) The baked and hardened photoresist layer is exposed to a collimated UV source in presence of a photomask which transfers the desired features to the photoresist layer (d) the substrate is baked at 65°C and 95°C to accelerate polymerization of the exposed photoresist. The unexposed photoresist is then washed away using the photoresist developer solution. The substrate is dried next and is ready to be used for PDMS molding (e) the replica is placed in a container such as a Petri dish and PDMS and curing agent mix is poured onto the replica and degassed in a vacuum oven. Once the PDMS mix is degassed, the Petri dish will be placed in an oven at 80°C for 30 to 60 minutes to cure PDMS. (f) Cured PDMS layer is peeled away from the replica and cut in appropriate size and inlet and outlet holes are punched when applicable. (g) The cured and cut PDMS slab is cleaned and placed in plasma cleaner along with a glass slide or another slab of PDMS for 30
seconds to oxidize the surfaces. The oxidized surfaces are brought in contact and an irreversible seal is formed.

Fig. 5 Images for (a) a photoresist master replica fabricated on a glass slide substrate through rapid prototyping and (b) A PDMS device made from PDMS by replica molding of uncured PDMS onto the template, and subsequent sealing to a PDMS-coated glass slide. Microchannels are filled with red dye to make the device design visible. Scale bar 15mm.
1.4 Experimental Methods

1.4.1 Fluorescence Microscopy

Visualization techniques are critical in studying microfluidic and lab-on-a-chip systems and optical access plays a major role in data acquisition within small inaccessible microchannels. Different visualization techniques are have been developed and applied for studying various aspects of microfluidic devices. A critical feature of microfluidic systems is fluid flow. Flow visualization techniques, specifically for liquid flow in microchannels can be classified in two major categories 1) particle based flow visualization methods and 2) qualitative flow visualization methods\(^5,98\). In particle based methods fluid velocity is determined by tracking the motion of tracer particles, often by capturing image series and extracting velocity data through image processing and calculating flow velocity from the distance traveled by particles in the time period between captured images. Particle image velocimetry (PIV) is a well-established technique and has been calibrated and applied for use in microscale flow visualization where it is often referred to as µPIV\(^99\). On the other hand, in qualitative visualization, a small amount of a tracer dye, is introduced in the flow and the motion of the fluid is approximated by imaging of the tracer motion\(^98\).

Fluorescent microscopy which was first used in biology to visualize structure of cells has been a critical tool for visualization including flow visualization in microfluidic devices particularly in applications involving mixing or dispersion within microchannels\(^39,94,100-102\). Fluorescence microscopy has been used in microfluidic applications to label various liquid samples and to visualize their interactions or to quantify their mixing, dispensing, or dispersion inside microchannels\(^48,54,89,102-104\).

Fluorescence microscopy rests upon selective visualization of fluorescent molecules\(^98\). Fluorescent molecules emit a photon when excited by an electromagnetic radiation. When a fluorescent molecule absorbs a photon it enters an excited state. The molecule remains excited for a brief period of time (1ns to 1ms) called the fluorescent lifetime\(^98\). The molecule then releases a photon and returns to its ground state. During the interim period the fluorescent molecule loses some of the absorbed energy and as a result the emitted photon is of lower energy.
and longer wavelength (i.e. different color). In this way fluorescence microscopy enables microscale flow visualization both using fluorescent dye and fluorescently-labelled particles.  

1.4.2 Confocal Laser Scanning Microscopy

Conventional fluorescence suffers from a major drawback which is out of focus blur. This is caused by illumination of the entire field of view with light of excitatory wavelength. The wide field illumination of the specimen results in excitement of the entire specimen including parts that are not in focus resulting in out of focus blur and background noise. Confocal microscopy offers a solution to the issue of out of focus blur. Confocal microscopy involves point illumination through a pinhole and a lens combined with limited field of view using a second pinhole placed at the image plane. To acquire an image the sample is scanned by a point of light in a raster pattern while the exit pinhole eliminates all lights coming from part of sample other than the illuminated point. Confocal microscopy when compared with conventional fluorescence microscopy results in reduced blurring, increased resolution, and enhanced signal to noise ratio. It also allows imaging of thick light scattering objects and conducting a z-scan through which a 3D reconstruction of the sample can be obtained. These characteristics make confocal microscopy a powerful tool for imaging biological samples as it allows penetrating thick light-diffracting samples, viewing sections within the sample or a plane oblique to the horizontal plane or even parallel to the line of sight.

1.4.3 Working fluids and materials

Ethanol (100%, Commercial Alcohols, ON, Canada) was used as a representative for organic solvents in bubble gate, bubble pump and tensiometry experiments. Mineral oil light grade (Bioshop Canada, Brulington, ON, Canada) was used as a representative for oils. A 1% by volume solution of micro particles (Flow Sphere, Invitrogen Inc., ON, Canada) in ethanol was used in micro particle image velocimetry (µPIV) experiments. An aqueous biological buffer containing 3-(N-morpholino) propanesulfonic acid (MOPS) was used to represent aqueous solutions in bubble gate experiments. 1mM solution of fluorescein (Invitrogen Inc., ON) in ethanol was prepared and used in sampling experiments. Octadecyltrichlorosilane (Sigma Aldrich, Oakville, ON, Canada) was vapor-deposited on glass capillary plates to render them hydrophobic for air-liquid cell culture experiments. Culture medium (RPMI 1640, ATCC,
Manassas, VA, USA) supplemented with fetal bovine serum (Sigma Aldrich, Oakville, ON, Canada) and Penicillin-Streptomycin (Life Technologies, Burlington, ON, Canada) was used in bubble pump and pulmonary cell culture experiments. Deionized water was used wherever water was needed in experiments. Air was used as the gas phase in all experiments.

The majority of microfluidic devices used in this thesis were fabricated by replica molding of poly (dimethylsiloxane) PDMS (SYLGARD 184, Dow Corning, Midland, MI, USA). Master replica features were made using a negative photoresist (SU8-2050, Microchem, Newton, MA, USA) and SU-8 developer solution (4-hydroxyl-4-methyl-2-pentanone) (Microchem, Newton, MA, USA).

1.4.4 Experimental Apparatus

An inverted microscope (Eclipse TE-2000-S, Nikon, Japan), shown in Fig. 6, was used for the majority of visualization and imaging performed during various experiments. Images and videos were captured using a CCD camera (QICam Fast 1394, QImaging, Surrey, BC, Canada) installed on the microscope. An inverted microscope (Nikon T1, Nikon, Japan) equipped with a confocal imaging system (Nikon A1, Nikon, Japan) was used to perform confocal imaging.

Computer-controlled digital servo pressure controllers (Type 3110, Marsh Bellofram, Newell, WV, USA) with various pressure range and resolution were employed in conjunction with LabView (V12.0.1, National Instruments, Austin TX, USA) codes to produce and monitor different gas pressure levels. Electromagnetic valves (The Lee Company USA, Westbrook, CT, USA) controlled via a LabView software were used to alternate between two different pressure levels when needed.

Teflon Tubing and fittings of 1/16 inch outer diameter (Upchurch Scientific, WA, USA) were used to connect fluid sources to microfluidic devices wherever needed. The tubing was inserted in the punched inlets and outlet holes on the microfluidic device and was sealed using epoxy (LePage Speed Set, Henkel Canada, Mississauga, Canada). Majority of characterization experiments were conducted using the setup in Fig. 6. The inverted microscope platform is convenient for these types of experiments as it leaves the top surface open and available for access with tubing, pipetting and/or electrodes etc.
1.4.5 Image processing

Images taken by a CCD camera are raw images. To extract experimental data and information from bright field or fluorescent images, the acquired images must be processed using a computer code. In this thesis two types of information needed to be extracted from captured images: 1) precise location of a two phase interface with time within a microchannel and 2) Concentration of a dispersed fluorescent dye inside a microchannel.

To extract concentration data from the fluorescent microscopy images, they must be normalized with respect to a bright-field and a dark-field image. Normalizing images also eliminates unwanted background features or defects from the raw images. Such defects may include noise, non-uniformity in excitation light, nonuniformity in background light, shadows, curved interfaces which appear in multiphase flows inside microchannels or marks or flaws made on the surface of the microchannel during the fabrication process. A bright-field image is an image captured when the microchannel is filled with the fluorescent dye only. A dark-field image is obtained when the microfluidic chip contains no fluorescent dye. A concentration of unity is
assigned to the bright-field image (image with highest pixel intensity) and zero to the dark-field image (image with lowest pixel intensity). Any given raw image of the microchannel can then be normalized with respect to the maximum and minimum intensities of the brightfield and darkfield images with a calculated concentration given by the following relationship:

\[ C = I_{\text{normalized}} = \frac{I_{\text{raw}} - I_{\text{dark}}}{I_{\text{bright}} - I_{\text{dark}}} \]  

(17)

where \( I \) represents the grayscale intensity of each pixel on the image and subscripts \( \text{raw} \), \( \text{bright} \), and \( \text{dark} \) correspond to the raw image, the bright-field image, and dark-field image, respectively. To normalize raw images according to Eqn. 19 and extract normalized concentration data for all pixels in an image, a computer program must be developed in MATLAB for instance.  

Interface tracking is done by imaging the interface with time and extracting the intensity data using a computer program. The images are first converted to black and white and location of the interface is captured by finding the location of a sharp change in the intensity.  

1.5 Numerical methods  
Computational fluid dynamics or CFD as it is often referred to is an important tool in studying fluid dynamics and has been employed across many different disciplines. CFD is advantageous in studying complex flow structures where experimental measurements would be too difficult or costly to perform or where supporting data is required to complement or validate experimental data. Generally speaking in computational fluid dynamics governing equations of fluid motion namely continuity and Navier-Stokes equations along with the other transport equations presented are solved computationally over a discretized solution domain. One advantage of computational fluid mechanics compared with experimental fluid dynamics is the ability to switch off certain equations or make additional assumptions in solving a fluid flow problem.

CFD has been adopted and used extensively in microfluidics particularly in microfluidic applications involving free interfaces or mixing of various fluid streams. For free interface
problems involving interfacial effects the so called volume of fluid (VOF) technique and the level set techniques have been established and employed\cite{111, 112}. A tremendous body of work has been devoted to computational modeling of multiphase flow in microchannels and microreactors\cite{87, 88, 113-118}. Numerical solutions have focused on various aspects of multiphase flow in microchannels such as hydrodynamics, bubble formation and interface shapes\cite{88, 115-117} and heat and mass transfer\cite{5, 113, 114, 118}.

Assumptions and simplifications are a common practice in numerical solution of fluid dynamics problems. Simplifications often are done to reduce the numerical cost and complexity of the computational model. As such simplifications have been done in modeling two phase flow in microchannels. For instance, a common assumption in modeling bubble train in microchannels is to neglect the gas phase due to its small viscosity and density compared with the liquid phase. This assumption is appropriate when the flow field in the liquid phase is of interest and the bubble shape and size is unchanging (i.e., steady). Assumption of a fixed interface shape is reasonable for very low capillary number flows. The gas-liquid interface in this case is modeled as a slip wall boundary for a single-phase liquid domain\cite{5, 100, 113}.

1.6 Overview of This Thesis

The contributions of this thesis are outlined below.

In chapter 1 an introduction to the topics covered in the thesis is given. Fundamental concepts behind microscale fluid flows and governing equations are briefly discussed. And the experimental and numerical tools and materials employed throughout this thesis are introduced and reviewed.

In chapter 2 a flow control strategy based on precisely controlled gas bubbles is introduced, characterized and applied. The flow control technique which we call a bubble gate takes advantage of a gas bubble to stop or allow liquid flow in microchannels. It offers significant advantages over state of art as it only requires a single fabrication layer and can be fabricated in both hard and elastic substrate materials to accommodate various lab-on-a-chip applications.

In chapter 3, a novel liquid routing technique for microfluidic and lab-on-chip devices is introduced that is based on self-synchronized motion of two gas bubbles. The liquid routing
technique which we call a bubble pump does not include any mechanical or moving parts and relies entirely on precisely controlled motion of gas bubbles. Similar to the bubble gate it is advantageous over state of art in terms of substrate material independence and single layer fabrication. We employ experimentation, computation and fluid mechanics theory to characterize and explain mechanism of action for the devised liquid routing technique.

In chapter 4, a microfluidic tensiometer is introduced that takes advantage of precise control over capillary pleasure across an interface to determine the interfacial tension between an immiscible pair of fluids. The devised tensiometer is simple with a small footprint and can be integrate into more complex lab-on-a-chip devices. Computation, theoretical analysis and experimentation were used to characterize the devised microfluidic tensiometer.

In chapter 5 an air-liquid culture platform for modeling the alveolar epithelium in vitro is introduced. The devised platform takes advantage of controlled motion of interfaces to manipulate epithelial cell layer and recapitulate alveolar function in vitro. Selective surface modification is employed to ensure desirable interface behavior for modeling of alveolar dynamics. Pulmonary cell culture and immunohistochemistry are conducted to characterize and validate the air-liquid cell culture model.

In chapter 6 a brief overview of potential future applications and extension of the topics covered in current thesis is presented.
1.7 References


98. D. Sinton and D. Li, Microscale flow visualization, 2002.


Chapter 2
Bubble gate for in-plane flow control

Summary

We introduce a miniature gate valve as a readily implementable strategy for active control of fluid flow on-chip, within a footprint of less than one square millimeter. The bubble gate provides for simple, consistent and scalable control of liquid flow in microchannel networks, is compatible with different bulk microfabrication processes and substrate materials and requires neither moving parts nor electrical connections. The bubble gate consists of two microchannels that meet at a T-junction. Liquid enters the first channel (i.e. liquid channel) from one side and flows past the T-junction towards the outlet. Gas is supplied to the second channel (i.e. gas channel) via the gas inlet and is maintained at one of two different pressure levels. The lower pressure level corresponds to the “open” state while the higher level corresponds to the “closed” state of the bubble gate. A gas bubble penetrates into the liquid-filled T-junction, confined on both sides with an array of equidistantly spaced micropillars, until the liquid flow is completely obstructed. Based on only a single masking layer we fabricated bubble gates using soft lithographic and bulk silicon micromachining procedures and evaluated their performance with a combination of theory and experimentation. We assessed the dynamic behaviour and consistency of the bubble gate operation, by determining the bubble’s dynamic response to an applied input pressure signal and evaluated the probability distributions for the bubble movement in over three hundred open-close cycles. Moreover, we report the operating pressure envelope for the bubble gates of varying geometry for different working liquids: de-ionized and filtered water, ethanol and a biological buffer. When plotted in terms of dimensionless gas and liquid input pressures, we obtained very good agreement between the experimentally determined bubble gate operational envelope and a theoretical prediction based on static wetting behaviour. We report case studies that serve to illustrate the utility of bubble gates in readily achieving fluid control in a Y-channel liquid sampling device and across three vertically bonded layers of a multilayer microfluidic device. Scalability of the bubble gate strategy is demonstrated by design and testing
of a device for digitized printing of the Canadian flag by entrapment of 59 colored liquid segments using 124 bubble gates.

2.1 Introduction

The unique characteristics of microfluidic and lab-on-a-chip systems have shown promise in potentially transforming a wide range of areas including analytical and clinical diagnostic devices\textsuperscript{1-5}, chemical discovery and early stage biopharmaceutical drug development\textsuperscript{6, 7}, the preparation of advanced colloidal and particulate materials\textsuperscript{8-12} and portable power devices\textsuperscript{13-15}. A common requirement for many lab-on-a-chip devices is their capacity to controllably manipulate, process, and analyze minute reagent volumes. To precisely deliver sample, precursor, or reagent solutions, readily implementable, consistent and effective flow control strategies are needed that comply with common fabrication protocols. The need for on-chip flow control increases the smaller the sample volumes become and the larger the number of different solutions involved in a given protocol.

A number of passive flow control strategies have been introduced based on different stop valve designs\textsuperscript{16-20} that can be actuated by either rising inlet pressures or centrifugal forces\textsuperscript{21, 22}. Many active flow control strategies have been previously devised a number of which are summarized in Table A1 (see appendices). These strategies employ actuation mechanisms that are based on optical (i.e. laser actuation), electrokinetic, pneumatic and piezoelectric effects. Alternatively some have utilized phase change, stimulus-responsive swelling, or two-phase flow\textsuperscript{23-26}.

A large number of the previously reported microvalves require multiple masks and multilayer fabrication, electrical or thermal contact, electrochemical reactions or other intrusive phenomena that limit the range of their application. Only a small subset of the many suggested valve designs has been adopted and routinely employed by the microfluidics community. In these designs, liquid flow inside a microchannel is interrupted by the deflection of microfabricated or sandwiched membrane that is actuated piezoelectrically, electrostatically or magnetically\textsuperscript{23-26}. Amongst those, pneumatically actuated elastomeric valves introduced by the Quake\textsuperscript{27, 28}, the Mathies\textsuperscript{29} and the Toner groups\textsuperscript{30} have become the most popular solutions for active flow control within microfluidic research community, due in part to their compliance with soft lithographic device fabrication and their scalability\textsuperscript{27-34}. 
Despite the many advantages of multilayer soft lithography in terms of prototyping and the impressive scalability of membrane-based valves, a number of device requirements are incompliant with PDMS as a substrate material. Examples are chemically resistant valves required for microreactors and for flow chemistry applications (acknowledging the fact that Quake valves have been implemented in devices that are solvent resistant and gas impermeable). In addition, a number of non-elastomeric substrate materials, e.g., poly(methyl-methacrylate) (PMMA), cyclic olefin copolymers (COCs), emerge as excellent candidates for commercial mass production of microfluidic devices by hot embossing and injection molding.

Characteristics of an “ideal” strategy for active flow control that is required in many microfluidic applications are as follows. Such a strategy: requires only one masking layer, has a small footprint, can be fabricated in different substrate materials (e.g. PDMS, poly(methyl-methacrylate) (PMMA), cyclic olefin copolymers, silicon and glass), can be fabricated using standard processes, does not require surface modification or electrical connections and is scalable.

Here, we introduce a flow control strategy, referred to as “bubble gate” that complies with the majority of the above requirements. The bubble gate requires only a single feature layer, is compliant with different substrate materials, several standard microfabrication processes and occupies only a small footprint.

Interfacial phenomena in general and gas bubbles in particular have been previously employed as active elements in volume-displacing microfluidic actuators. Bubble based flow control is of interest because it does not require complex fabrication procedures and gas-liquid interfaces have the ability of conforming to different channel geometries. In previous works on bubble-based flow control the bubbles were commonly generated and controlled either by phase change (i.e. boiling or explosive evaporation), by electrolysis or by thermo-electrically manipulating a previously trapped bubble. In our proposed strategy we employ a computer controlled gas stream that does not involve any of the aforementioned phenomena that may be intrusive for many applications.
The bubble gate strategy presented in this work is most suitable for well-based integrated microfluidics systems where the liquid samples are delivered to the microchannels via applied head pressure and are collected at the outlet. Schematic for such a target system is shown in Fig. 1.

Fig. 1 (a) Schematic illustration of different flow control strategies reported in the literature in terms of working liquids, substrate materials and number of masking layers required for device fabrication: (1, green colour) corresponds to electrical techniques, (2, blue colour) summarizes techniques based on the deflection of an elastomeric membrane, (3, yellow colour) summarizes conditions for flow control based on stimulus responsive materials and (4, light blue) corresponds to techniques employing flow obstruction via bubbles (see Appendix Table A1 for detailed references). Red region highlights “ideal” flow control strategy for microfluidic devices that is compliant with different working liquids and substrate materials and only requires a single masking layer. (b) Schematic illustration of “ideal” flow control strategy integrated in a device.
2.2 Experimental

2.2.1 Microfluidic device design

Figure 2a shows a schematic illustration of the bubble gate design that is composed of a stop valve and a bubble entrapment area where the gas and liquid channels meet. The stop valve holds the bubble outside of the T-junction allowing the liquid to pass through. However, it’s not necessary for the bubble to be locked right behind the stop valve for the liquid to flow through the main channel. Regardless of its location in the gas line, the bubble will cause a very high

Fig. 2 (a) Schematic illustration of bubble gate design and position of gas-liquid interface in “open” and “closed” states. (b) Flow resistance circuit diagram for bubble gate. (c)
Schematic illustration of gas-liquid interface The Young Laplace equation was used to analytically determine the operation envelope of the bubble gate at its margins when the equilibrium breaks down: when the bubble breaks into the stop valve gap of \( d = d_{\text{Stopvalve}} \) and when the bubble breaks into the liquid channel through the micropillars gap of \( d = d_{\text{Pillar}} \) (d) Schematic illustration of experimental setup. Scale bar in (a) is 250 μm.

Resistance in the gas line as compared with the main channel therefore the liquid will flow in the liquid channel. When the gas pressure is elevated sufficiently it forces the bubble to break into the T-junction (i.e. the bubble entrapment chamber) and block the flow in the liquid channel as seen in Fig. 2a. Two rows of micropillars are placed at the two sides of the T-junction inside the main channel to limit the bubble movement to one degree of freedom (i.e. inside the gas line only). Micropillars have been previously used to limit interface movement in microfluidic channels\(^{49}\). The distance between micro pillars (i.e. \( d_{\text{Pillar}} \)) is of significant importance since it determines the critical surface tension pressure required for the gas liquid interface to break up as will be discussed in the following paragraphs.

The bubble gate’s operation can be divided to three key states: (1) when the bubble is moving into the liquid channel closing the gate (see Fig. 2a) (2) the fully closed position where the bubble is occupying the entire chamber made by the liquid channel walls and the micropillars (see Fig. 2a) (3) when the bubble is withdrawing from the chamber hence opening the gate. These three states define the bubble gate’s operation envelope.

For the bubble gate to break into the stop valve opening, the pressure difference across the stop valve must be greater than the surface tension pressure (see Fig. 2c). In other words,

\[
P_G - P_{L,Gate} \geq \gamma \left( \frac{2}{d_{\text{Stopvalve}}} + \frac{2}{H} \right),
\]

where \( \gamma \) is the surface tension coefficient and \( H \) is the depth of the microchannel. Substituting for \( P_G \), the liquid pressure at the gate (see Fig. 2b), in terms of the applied liquid pressure head, \( P_L \), the gate closing constraint (state 1) is obtained as

\[
P_G - \frac{R_2 P_L}{R_1 + R_2} \geq \frac{2}{d_{\text{Stopvalve}}} + \frac{2}{H}
\]

(1)
where $R_1$ and $R_2$ are the liquid flow resistances from the inlet to the bubble gate and from the bubble gate to the outlet that were estimated by assuming Poiseuille flow and inserted in Eq. (1). To prevent bubbles to break into the downstream liquid channel while the bubble gate remains in its “closed” state, the gas pressure must remain below the surface tension pressure across the micropillar array (see Fig. 2c):

$$P_G \leq \Delta P_{\text{Pillar}} = \gamma \left( \frac{2}{d_{\text{Stopvalve}}} + \frac{2}{H} \right)$$

(2)

The two aforementioned constraints define the theoretical boundaries for effective bubble gate operation and will be used for comparison with experimental bubble gate operation data. Note that all the pressures in above relationships are gauge pressures (i.e. pressure differences with respect to atmospheric pressure). We non-dimensionalize pressures in Eqs. (1) and (2) in order to render them independent of the pillar distance and the working fluids. To obtain dimensionless form of the equations we divide both sides by $\Delta P_{\text{Pillar}} = \gamma \left( \frac{2}{d_{\text{Pillar}}} + \frac{2}{H} \right)$ and denote the dimensionless pressures as $P^* = P/\Delta P_{\text{Pillar}}$. The dimensionless form of the operation envelope margins is presented below.

$$\begin{cases} P^*_L - \eta P^*_G + \eta \lambda \leq 0 \\ P^*_G - 1 \leq 0 \end{cases}$$

(3)

where

$$\lambda = \left( \frac{2}{d_{\text{Stopvalve}}} + \frac{2}{H} \right) / \left( \frac{2}{d_{\text{Pillar}}} + \frac{2}{H} \right), \quad \eta = \frac{R_1 + R_2}{R_2}.$$ 

The first constraint in Eq. (3) is a linear boundary with the slope of $\eta$ which is a function of the resistances before and after the bubble gate (i.e. $R_1$ and $R_2$). The y-intercept for the line is $\eta \lambda$, which is a function of the channel height, pillar gap size, and the stop valve gap size. The second constraint is also linear with a slope of infinity. The constraints in Eq. (2) are useful as design guidelines for the bubble gate technique and will be used later in this work to validate the experimental data for the operation envelope of the bubble gate. A third constraint may also be defined and that is the minimum gas pressure required to overcome the capillary pressure across the bubble cap inside the gas channel. This minimum pressure is needed to push the gas-liquid interface inside the gas channel before it arrives at the bubble gate location.
Fig. 3 (a) Gas-liquid interface and contact angle evolution between the pillars of a bubble gate in closed position with increasing pressure. (b) Brightfield micrographs of gas-liquid interface entering the gap between the pillars as pressure rises from 45% to 93% of $\Delta P_{\text{Pillar}}$, the critical interface breakup pressure. Scale bar in (b) is 30µm.

The importance of surface tension forces in two-phase flow inside capillaries and microchannels is well established with a great amount of analytical and experimental work focused on solving
problems such as pressure drop, interface shape, pinning and contact angle hysteresis in two phase flows\textsuperscript{50}. Similarly, Surface tension effects are present in all three operation states of the bubble gate described earlier: 1) At the closing state when the gas-liquid interface moves towards the gate in the gas line a minimum applied pressure is needed to overcome the capillary pressure of the bubble cap and move the interface inside the channel. 2) At the closed position when the bubble is surrounded and pinned by the micropillars, the surface tension forces hold the bubble in place by overcoming the pressure forces across the micro pillars. As the gas pressure is increased, the contact angle of the gas liquid interface and the micropillars decreases as shown in Fig. 3a and b. This is in agreement with the theoretical relationship between pressure and contact angle for a slow moving bubble in a capillary, $\Delta P = (2\gamma/R)\cos(\theta)$. As the pressure rises, the contact angle approaches zero (i.e. $\cos(\theta)=1$). And 3) as the pressure is lowered the contact angle rises and the bubble moves out of the liquid channel. Dynamics effects such as pinning and contact angle hysteresis are present whenever the bubble is moving due to the presence of sharp channel geometries, defects on the surface or chemical contaminations\textsuperscript{50, 51}. Interface pinning and contact angle hysteresis are more pronounced when the surface tension and unperturbed contact angle between the gas and liquid are higher. This is caused by higher spring constant and rigidity of the interface in cases with higher surface tension and contact angles\textsuperscript{50, 51}. The more rigid interface will result in a stronger pining and a more unpredictable interface movement. This effect was observed in testing of the bubble gate technique with liquids of varying surface tension.

\subsection*{2.2.2 Device fabrication}

Microfluidic devices were fabricated in poly (dimethylsiloxane) PDMS (Sylgard 184, Dow Corning, Midland, MI, USA) using the soft-lithography technique and in silicon using deep reactive ion etching (DRIE) technique. To fabricate PDMS devices, a replica was first made on a silicon wafer using the well-known photolithography technique. The negative photoresist SU8-2050 (MicroChem Corp. Newton, MA) was spun on a silicon wafer (Silicon Quest International, Santa Clara, CA, USA) before it was baked on a hotplate at 65°C first and then at 95°C. The substrate was then exposed to the UV light through a photomask that contained the design for the devices. Following UV exposure, the substrate was baked again at 65°C and then 95°C to allow the exposed photoresist to polymerize. Lastly, the unexposed photoresist was removed from the
substrate using photoresist developer solution (MicroChem, Newton, MA, USA). Next, PDMS and curing agent were mixed and degassed in a vacuum chamber (model 280A, Fisher Scientific, ON, Canada) and applied to the substrate. The moulded PDMS was cured in an oven at 80°C for 30 minutes. The cured PDMS layer was removed from the substrate followed by, isolating individual devices and punching inlet and outlet holes. The holes were nearly 1/16 inch in outer diameter. The microfluidic device was then cleaned and placed in a Plasma Cleaner (model PDC, Harrick Plasma, Ithaca, NY, USA) with a glass slide. Following exposure to oxygen plasma for 30 seconds, the treated surfaces were brought into contact to form an irreversible seal.

The silicon devices were designed and fabricated using bulk silicon microfabrication techniques. The process flow started with RCA wafer cleaning of a 6” double-side polished silicon wafer (700 μm thick) and wet oxidation (1 μm). A thick resist (AZ9245) was spin coated and lithographically patterned for the microchannels (300 μm deep) using a high resolution chrome on glass mask. The oxide layer was then etched using buffered oxide etchant (BOE). At this point of the process, there were two masking layers on top of each other; patterned resist and oxide layers. After this step, the processed silicon wafer was placed in the deep reactive ion etching (DRIE) chamber (Tegal SDE1100), and the deep features were etched. The thick resist layer was removed during the DRIE process. The oxide layer was removed and a fresh 1 μm oxide was grown on both sides of the patterned silicon wafer. A thick resist was spin coated on the backside of the wafer and a thin resist layer was used to protect the etched front side of the wafer during BOE. The inlet and outlet holes were lithographically patterned on the backside. Using DRIE, the backside of the silicon wafer was etched (400 μm), and inlet and outlet holes were connected to the front side.

The oxide layer was removed using BOE, and a fresh 500 nm oxide layer was grown on the finalized processed silicon wafer. The wafers were then anodically bonded to a 6” Borofloat 33 wafer on wafer level. The silicon-based microfluidic devices were cut using a die saw machine.

2.2.3 Chemicals

To ensure the compatibility of the devised strategy with different chemical and biological applications, two different working liquids, one organic and one aqueous were adopted and used. Ethanol was used as a representative for organic solvents as its surface tension properties are
similar to most commonly used organic solvents. A hundred times diluted solution of micro particles (Flow Sphere, Invitrogen Inc., ON, Canada) in ethanol was used in micro particle image velocimetry (μPIV) experiments. Additionally, an aqueous biological buffer containing 3-(N-morpholino) propanesulfonic acid (MOPS) was prepared and used to represent aqueous solutions most commonly used in biological applications of lab-on-a-chip devices. The surface tension coefficient for the buffer solution was measured and found to be identical to that of water. All solutions were prepared and applied at room temperature (~25°C). Air was used as the working gas in all of the experiments.

A 4mM solution of fluorescein sodium dye (Sigma Aldrich, Canada) in ethanol was prepared and used for fluorescence microscopy in liquid sampling experiments.

2.2.4 Flow delivery and control

As seen in the schematics in Fig. 2d computer controlled gas pressure was employed to drive both liquid and gas streams. Pressure regulation was enabled by three servo pressure controllers (Marsh Bellofram, Newell, WV, USA) combined with electromagnetic valves (The Lee Company, Westbrook, CT, USA) that allowed precise gas pressure control via LabView software (National Instruments, Austin, TX, USA). The devices used in this work were all well-based devices to eliminate the need for bulky syringe pumps and facilitate compatibility and integrability. Therefore, liquid was delivered to the microchip by applying gas pressure head to liquid reservoirs that were positioned at the same elevation as the microchannels, as shown in Fig. 2d.

Two pressure controllers were connected to the electromagnetic valves to allow switching the gas pressures between $P_{\text{open}}$ and $P_{\text{closed}}$ and generate arbitrary control signals with square wave shape (see Fig. 4b) to control the bubble gates. The output of the electromagnetic valves was connected to the gas line on the microfluidic device using two sets of tubing: 0.5m of Tygon tubing with a 1/8” outer diameter and a 1/16” inner diameter followed by 0.8m of Teflon tubing (Upchurch Scientific, Oak Harbor, WA, USA) with a 1/16” outer diameter and a 1mm inner diameter. Tygon tubing of 0.7m length with a 1/8” outer diameter and a 1/16” inner diameter was used to connect a gas pressure head to the liquid reservoirs supplying the working liquid to the microfluidic devices.
2.2.5 Imaging and image processing

Brightfield microscopy was used to visualize the flow in the microchips. An inverted microscope (model Eclipse TE-2000-S, Nikon, Japan) was used to image the liquid and gas flow inside the microchannels. Images and videos were captured using a CCD camera (model QICam Fast 1394, QImaging, Surrey, BC, Canada) installed on the microscope.

To study the dynamics of gas-liquid interface, high speed imaging followed by image processing was employed. High speed imaging was performed using the aforementioned CCD camera with 8×8 binning and a frame rate of 40 frames per second, corresponding to a time interval of 25 ms between successive images. The captured images were processed in MATLAB R2010a (Mathworks, MA, USA). The MATLAB code detected the gas-liquid interface in every image and traced the bubble evolution over time by reading a sequence of images. Following the image processing, the interface’s dynamic behaviour (i.e. interface displacement vs. time) was normalized with respect to the channel width and the period of oscillations to facilitate comparison of the data.

Micro particle image velocimetry (µPIV) measurements were employed to quantify the velocity field in the microchannels and to evaluate the performance of the bubble gate. Fluorescent particles of 1µm diameter were added to the ethanol reservoirs as the tracer for µPIV measurements. Images were captured using a CCD camera (Sensicam model Doubleshot QE, PCO, Kelheim, Germany) via Davis (LaVision, Goettingen, Germany), a specialized PIV software. The images were then processed and the results were imported in Tecplot (Tecplot, Inc., Bellevue, WA, USA) in which the velocity fields were plotted.

Concentration measurements were done using fluorescent microscopy and imaging using a CCD camera (QImaging model QICam Fast 1394, Surrey, BC, Canada) followed by normalizing the images in MATLAB using bright field and dark field images. The concentration (i.e. intensity) distribution with time was extracted from a time series of normalized images.
2.3 Results

2.3.1 Characterization

Fig. 4a shows the brightfield images for the bubble gate in operation. Microscale particle imaging velocimetry (µPIV) measurements were employed to verify quantitatively whether the liquid flow is effectively obstructed by the gas bubble. Figure 4a at the top shows the liquid velocity field at a point immediately downstream of the valve when the bubble valve is open.
Fig. 4 (a) Local velocities obtained from μPIV measurements downstream of the location of bubble gate in the open (top) and closed (bottom) states. Ethanol and air were used as
working fluids. (b) Schematics of the control signal for bubble gate actuation. (c) Normalized measured gas pressure input versus normalized time. (d) Normalized bubble displacement versus normalized time for $P_L=0.02\text{psi}$ and $f=0.5\text{Hz}$ (blue line with diamond markers), $P_L=0.23\text{psi}$ and $f=0.5\text{Hz}$ (red line with triangular markers), $P_L=0.02\text{psi}$ and $f=1\text{Hz}$ (black line with square markers). (e) Histograms for normalized half cycle $t_1$ for 300 consecutive cycles. (f) Histograms for normalized full cycle $t_2$ for 300 consecutive cycles. Bin colours for histograms represent same experimental conditions as the colours in part (d). Scale bars are 250 $\mu\text{m}$ (a) and is 500 $\mu\text{m}$ (b).

As seen in the velocity fields, liquid is flowing with a maximum $x$-velocity of 15 mm/s near the center line of the channel. Fig. 4a at the bottom shows the velocity vectors at the same location when the bubble gate is closed. As demonstrated in the figure, in closed position the entire velocity field has a magnitude of zero. When inspected closely the velocity field in the closed position did not show any recognizable flow direction or pattern. With measured velocity magnitude of zero for the closed position, it is reasonable to conclude that the bubble gate strategy is effective in blocking liquid flow in microchannels. To investigate the dynamic behaviour of the bubble gate and its response to an applied pressure input, we used ethanol as the working liquid and varied the pressures at the liquid and gas inlets while the liquid outlet was connected to atmospheric pressure. A square wave pressure input shown in Fig. 4b with half cycle time of $t_1$ and full cycle time of $t_2$ (i.e. a frequency of $f = t_2^{-1}$) was applied at the gas inlet. The magnitude of the pressure input alternated between $P_{\text{closed}}$ and $P_{\text{open}}$ and the response was recorded using a CCD camera and high speed imaging (see section 3.5 for more details).

The pressure input was measured using a pressure transducer (Honeywell International, Morristown, NJ) and the normalized pressure was plotted against normalized time as seen in Fig. 4c. Pressure was normalized with the difference between the maximum ($P_{\text{closed}}$) and minimum ($P_{\text{open}}$) of the pressure input and time was normalized with the period of the applied control signal (i.e. $t_2$).

Fig. 4d demonstrates the bubble gate’s normalized displacement in response to a square wave input plotted against normalized time (normalized with the period of the input signal, $t_2$). The three different plots represent three different liquid pressure heads and/or input frequencies.
$P_{\text{closed}}$ and $P_{\text{open}}$ were constant for all three plots and were 0.26 psi and 0.11 psi respectively. The blue plot (i.e. diamond marker) represents $P_{\text{liquid}} = 0.02$ psi and $f = 0.5$ Hz. The red plot (i.e. triangular marker) represents $P_{\text{liquid}} = 0.2$ psi and $f = 0.5$ Hz. The black plot (i.e. square marker) represents the displacement response for $P_{\text{liquid}} = 0.02$ psi and $f = 1$ Hz. Figures 4e and 4f show histograms for the times $t_1$ and $t_2$ extracted from the bubble displacement plots in Fig. 4d for over 300 consecutive cycles. The bin colours for the histograms match those in the bubble displacement plot in Fig. 4d.

As seen from the displacement response plots and the histograms the bubble follows the square wave input with minor deviation from the control signal in the blue (diamond marker) and black (square marker) plots. The red plots (triangular marker) show more of a deviation as compared with the other flow conditions. This deviation can be attributed to the relatively high liquid pressure in this data set which approaches $P_{\text{closed}}$ hence causing a “pressure competition” between the liquid and gas streams to occupy the liquid channel at the T-junction. This competition results in instability in the gas-liquid interface movement as observed during the experiments and as a result caused the bubble movement to deviate from the control signal more than the other two conditions.

In order for the bubble gate strategy to be applied in various applications in a repeatable manner it is critical that the operating pressure envelope is known. The operating pressure envelopes were obtained by connecting the bubble gate to computer controlled gas and liquid pressure inputs and monitoring the bubble gate behaviour.

To find the operation envelope experimentally, gas and liquid pressures were varied in two different ways: In the first experimental approach the bubble gate was closed and the gas pressure was kept constant while the liquid pressure was increased from zero to a critical pressure at which the bubble interface failed either by retreating from the T-junction or by breaking through the micropillars. In the second approach, the liquid pressure was kept constant and the gas pressure was increased until it entered the liquid channel and blocked the liquid flow and eventually broke into the liquid channel through the micropillars. The gas and liquid pressures for each condition where the bubble gate operates successfully, were recorded and
normalized with respect to the critical capillary pressure, $\Delta P_{\text{Pillar}}$, as explained in detail in section 3.1.

Fig. 5 Normalized operating pressure envelope for PDMS made devices: (a) With $d_{\text{Pillar}}=20\mu m$, $d_{\text{stopvalve}}=75\mu m$, channel width of 500$\mu m$ and ethanol as working liquid. (b) With $d_{\text{Pillar}}=20\mu m$, $d_{\text{stopvalve}}=75\mu m$, channel width of 500$\mu m$ and a MOPS based buffer as working liquid. (c) With elliptical pillars and $d_{\text{Pillar}}=35\mu m$, $d_{\text{stopvalve}}=150\mu m$, channel width of 500$\mu m$ and ethanol as working liquid. (d) With $d_{\text{Pillar}}=35\mu m$, $d_{\text{stopvalve}}=150\mu m$, channel width 250 $\mu m$ and working liquid ethanol. Dashed lines represent the theoretical limit for
the gas to enter the microfluidic device. Solid lines indicate theoretical constraint for bubble gate’s “closed” state. Dash-dotted lines represent interface breakup margin.

Fig. 5a to d show the operating pressure envelope for bubble gate devices with varying geometry and working liquids. The lines in the figure represent the theoretical operation constraints discussed earlier. As seen from the data there is a very good agreement between the theoretical and experimental data with experimental data falling within the theoretical operation envelope in all cases except in Fig. 5b where a slight deviation exists. In Fig. 5b where the bubble gate is tested with an aqueous buffer, the experimental liquid pressures exceed the theoretical boundary which may be explained by the presence of significant interface pinning that caused the gas-liquid interface to withstand higher liquid pressures than predicted by theory. As seen in the bright field images of the bubble gate in the figure, in high liquid pressures the gas bubble is separated from the leading row of the micropillars and pushed against the downstream row of micropillars and is therefore marginally effective. As a result the experimental data in Fig. 5 do not cover the entire theoretical envelope at high liquid and gas pressures (i.e. near the point where the two lines representing the theoretical constraints intercept). This is due to the fact that the experimental data were collected for conditions that provide effective to marginal bubble gate closure only.

2.3.2 Case studies

To illustrate the bubble gate strategy as an effective and readily implementable flow control strategy two sampling experiments were performed the results of which are presented in Fig. 6. Figures 6a and 6b show a liquid sampling device that consists of two liquid reservoirs connected to two micro channels that meet at a Y-junction and merge into one channel that guides the flow towards the outlet.

The bubble gates were implemented downstream from the liquid reservoirs and upstream from the Y-junction. Gas pressure head of 0.23 psi was applied on the liquid reservoirs to drive the liquid. This pressure head corresponds to a flow rate of approximately 25 μL/min assuming Poiseuille flow in the channels. The open and closed pressure levels for the bubble gates were 0.22 psi and 0.32 psi respectively. Each bubble gate was subjected to a square wave signal with a period of six seconds (i.e. three seconds open and three seconds closed) with one bubble gate
half a cycle (three seconds) ahead of the other bubble gate. This control signal resulted in one channel being open for three seconds while the other was closed and vice versa.
Fig. 6 Liquid sampling with a square wave control signal (3 s open-3 s closed) (a) Experimental setup (b) The fluorescent images of the Y-channel at both open and closed positions (c) plot of concentration (or intensity) versus time (d) Histograms for the length of time each valve is open or closed (e) Schematics for a three layer liquid sampling microfluidic device and fluorescent images showing bubble gates blocking the flow of a fluorescently labelled liquid on each layer.

The concentration (i.e. intensity) distribution with time downstream Y-junction was measured by fluorescent imaging and image processing. The plot for the concentration distribution with time is shown in Fig. 6c. To investigate the sampling repeatability the open and closed times $\Delta t_{\text{Open}}$, $\Delta t_{\text{Closed}}$ were extracted from the plot in Fig. 6c for 130 consecutive cycles. The histograms for the open and closed times are brought in Fig. 6d. As seen in the histograms, the open and closed times followed the control signal precisely and resulted in a narrow histogram with the average and standard deviation of 3.03 s and 0.23 s for $\Delta t_{\text{Open}}$ and 2.93 s and 0.095 s for $\Delta t_{\text{Closed}}$.

The bubble gate strategy was also used for liquid sampling in a three layer microfluidic device as shown in Fig. 6e. The device consisted of three microchannels each on a separate layer with a common inlet and outlet for all channels and individual bubble gates controlling the liquid flow in each layer. Each bubble gate had a separate gas inlet and was controlled independently. Similar to the sampling experiment in Fig. 6a, pressure head of 0.23 psi was applied to drive the liquid into the channels (resulting in a flow rate of approximately 21μL/min). $P_{\text{Closed}}$ was varied between 0.3 and 0.35 psi and $P_{\text{Open}}$ was varied between 0.15 and 0.2 psi during the experiment. The fluorescent images in Fig. 6e show the bubble gate in each layer blocking the flow of the fluorescently labelled liquid in that layer while it flows in the other layers.

2.3.3 Scalability

To illustrate scalability of the bubble gate technology, a “Canada flag” device was designed and tested. The device was designed to print a digitized image of the Canadian flag by means of selective trapping of a dye in a network of microchannels. Entrapment of the dye was achieved using bubble gates operating in pairs which trapped a liquid segment in the middle of the gates. Figure 7a shows the rendered design of the device. Liquid segment entrapment using a pair of bubble gates is presented in figure 7b. The red liquid segment in figure 7b is trapped between
two bubble gates which are shown in blue. As seen in figure 7a, there are 124 bubble gates incorporated in the device, with a total of 19 liquid channels and 10 gas channels delivering the gas to the bubble gates. There is one liquid inlet and one gas inlet on the device.

The Canadian flag was printed in three main steps: 1) The entire device was filled with a solution of red dye in ethanol delivered to the device through the liquid inlet, 2) The bubble gates were closed by introducing gas via the gas inlet, trapping the red solution in designated locations on the liquid channels, and 3) Keeping the bubble gates closed, clear ethanol was introduced to the device via the liquid inlet flowing through bypass channels.
Fig. 7 Microfluidic printing of the Canadian flag using 62 pairs of bubble gates (a) Rendered schematic of the device with the bubble gates and bypass channels patterned to represent the Canadian flag. The liquid inlet is on the right and the gas inlet and liquid outlets are on the left. All liquid channels have the same resistance (b) Close up schematic of a pair of bubble gates trapping a red liquid sample (c) Digitized image of the Canadian flag produced by liquid dye entrapped using the bubble gates. the scale bar is 5mm long.
This flushed the red solution out of the parts of the liquid channels that represent the colour white on the Canadian flag. Following the third step, a digitized microfluidic image of the Canadian flag was produced which is shown in figure 7c. Throughout the entire experiment gas pressure was varied between 0.2 psi and 0.3 psi and the liquid was delivered to the device through pressurized reservoirs with pressures ranging from 0.2 psi to 0.4 psi.

2.4 Conclusions

A readily implementable on-chip flow control strategy was developed, characterized and illustrated. The devised strategy did not include any mechanical or moving parts. It consisted of a controllable gas stream that intercepts a stream of liquid at a T-junction and is confined within micropillars that prevent it from flowing upstream or downstream. Presence of the gas bubble in the liquid channel seals the channel downstream the T-junction from the upstream liquid flow. In order for the bubble gate to be applied reliably its dynamic behaviour and controllability was investigated. It was observed that the bubble gate follows the control signal with minor deviation. The operating pressure envelope within which the bubble gate is effective for flow control was determined for devices with different bubble gate structures and using different working liquids (i.e. ethanol and an aqueous buffer).

The bubble gates were employed to control and sample liquid flow from different reservoirs in a single layer as well as a multilayer microfluidic device. It was shown that the bubble gate strategy can be used to control the flow of different liquid samples within microchannels by allowing or blocking flow of different liquids in a network of microchannels within an integrated well-based microfluidic device.
2.5 References


Chapter 3: Bubble pump: Strategy for in-plane liquid routing

Summary

We present the “bubble pump”, an on-chip liquid routing technique targeted for application in scaled up well-based microfluidic systems requiring long-term pumping at low to medium flowrates. The bubble pump requires only a single layer fabrication, a single pneumatic control line and does not contain any flexible membranes and mechanical or moving parts making it compatible with both elastomeric and rigid substrate materials. Our liquid routing technique relies on an in-series configuration of two gas-bubble enabled micro valves (i.e. bubble gates) acting in a liquid channel, one upstream and one downstream. Each bubble gate consists of a gas bubble with a precisely controlled pressure, which can move controllably in and out of a liquid channel at a T-shaped junction, blocking or allowing liquid flow. The gas bubble movement inside the liquid channel is confined by two arrays of tightly packed micro-pillars flanking the T-junction. The upstream bubble gate is active while the downstream gate is passive and merely responds to pressure fluctuations induced by the active gate. In order to achieve directional flow the active bubble gate is set in a reciprocating motion by alternating the gas bubble pressure between a higher (actuation) and a lower (withdrawal) pressure level while the passive bubble gate is subjected to a constant gas pressure lower than the actuation pressure for the active gate. In its unexcited state, the passive gate is in “closed” position and will only open briefly, allowing liquid flow downstream, when the pressure behind it rises in response to the actuation pressure of the active gate. The bubble pump was tested and characterised in this work and shown to be capable of long-term pumping (>72 hours) with a variety of working liquids (organic solvents, oils and aqueous biological solutions). The bubble pump consistently delivered flow rates ranging from 0 to 5.5μl/min depending on the pump dimensions, working fluids and pumping frequency chosen. We achieved flowrate enhancements of up to 100% by applying a parallel configuration of two bubble pumps operating within a single microchannel without any need for additional control lines or setup modifications. Achievable flowrates are comparable to or exceeding the flow rates of available on-chip pumps without the limitations or complexities pertaining to fabrication, biocompatibility, operation and control or substrate material elasticity.
Scalability of the bubble pump was demonstrated by its successful implementation onto a well-based device with twelve independent liquid lines. All twelve bubble pumps were operated simultaneously using the same controls as a single pump yielding uniform flowrates with only seven percent variation across twelve microchannels.

3.1 Introduction

In the past decades microfluidic and lab-on-a-chip technologies have shown promise in a wide range of applications such as: analytical and diagnostic devices, platforms for chemical and material synthesis and microphysiological systems. Flow control and sample delivery remains a critical function in microfluidic devices. External pumps have routinely been used to direct flow in microfluidic channels. External pumps (e.g. syringe pumps) have traditionally been used in research laboratories and are suitable for continuous flow microfluidic applications involving fluid volumes of, for example, 1-50 ml. However, a number of disadvantages render external pumps ultimately not practical for integrated lab-on-a-chip applications and chemical or biological screening tasks that require only minute sample volumes but large numbers of samples. Such disadvantages include: the large size of external pumps (e.g. the weight of a typical syringe pump exceeds one hundred times the weight of a typical microfluidic device), limited scalability, high cost, limited response time for applications that require rapid changes in flow rate, the large dead volume of fluids contained in external reservoirs and tubing compared with the volume of the microfluidic channels. It is therefore desirable, particularly for portable well-based analytical devices, to provide a relatively low-cost, easy-to-fabricate, and scalable solution for flow control.

A large number of active on-chip pumping strategies have been suggested over the past decade. These techniques have harnessed a wide range of physical and chemical phenomena to induce flow in microchannels including: pneumatically induced deflection of membranes, piezoelectricity, electrostatic effects, electrochemical reactions, thermo-pneumatic and acoustic effects. To date, the most widely used of these techniques are the pneumatic membrane pumps such as the peristaltic pump developed by Quake’s group. As described by the schematic illustration in Fig. 1a, many of the flow control strategies developed to date include features that limit their utility in a range of applications. For instance most biological
applications involving living cells or microorganisms are incompatible with high electrical
voltages$^{54}$, high temperatures and electrochemical reactions. On the other hand membrane based
strategies rely on the use of elastic membrane materials that are susceptible to degradation when
exposed to reactive fluids. For instance polydimethylsiloxane (PDMS) based micro-pumps are
not compatible with organic solvents$^{55, 56}$. Many of the previously discussed techniques require
complex multilayer fabrication and precise alignment that increase the fabrication cost.
Therefore, consistent, programmable, scalable and substrate-independent delivery of small fluid
volumes remains a critical requirement for well-based portable microfluidic devices (Fig. 1b).

We present an on-chip flow routing strategy that overcomes or improves upon the
aforementioned limitations through operation of a pair of “bubble gates”$^{57}$ in series. In our liquid
routing technique the upstream bubble gate is active and serves as an actuator displacing a
volume of the working liquid while the downstream bubble gate is passive and serves as a flow
rectifier ensuring liquid flow in the downstream direction. Our devised technique requires only a
single layer fabrication and does not require elastic substrate materials or moving parts and is
therefore implementable in hard materials such as silicon$^{57}$. It is also readily scalable without a
need for additional controls or setup modifications. The schematic illustration in Fig. 1b
describes the target microfluidic device for our devised strategy. Details of the design and
operation principle of our technique will be discussed in detail in the following sections.
Fig. 1 (a) An overview of different types of on-chip micropumps weighed against a set of parameters affecting utility of a microfluidic flow control technique. Sizes of bars represent a qualitative score for each type of micropump. Each pumping technique is represented by a distinct color (b) A single layer well-based analytical device with on-chip sample routing strategy that is ready for use following a single layer fabrication and requires a single pneumatic control line. The schematic illustration shows a well-based integrated device with on-chip pumps that draw various samples from the inlet wells and drive them through the microchannels towards the outlet.
3.2 Experimental

3.2.1 Device design and experimental set up

The bubble pump consists of an in-series configuration of two bubble gates we previously developed and characterized. Each bubble gate consists of a gas bubble that intercepts a liquid channel at a T-shaped junction and can allow or stop the liquid flow by precise control of the bubble movement inside the liquid channel. The bubble movement inside the liquid channel is confined by means of two rows of closely packed micro-pillars that flank the T-junction. In our pump design we employ an active bubble gate (i.e. upstream gate) for liquid actuation and a passive bubble gate (i.e. downstream gate) that serves as a flow rectifier allowing flow towards downstream only.

The design for the bubble pump is shown in Fig. 2a with the active bubble gate on the upstream (i.e. left) side and the passive bubble gate on the downstream (i.e. right) side. To operate the pump the active gate is set in a reciprocating motion by alternating the gas pressure between a higher (actuation pressure, $P_{act}$) and a lower (withdrawal pressure, $P_{wd}$) level as described in Fig. 2b. As shown in Fig. 2c the passive bubble gate is maintained at a constant pressure and is “closed” (i.e. occupying the full width of the liquid channel) in its unperturbed state. During the actuation step the active gate protrudes the liquid channel displacing a volume of liquid and increasing the liquid pressure behind the passive gate and consequently opening the passive gate. The brief opening of the passive gate allows a fraction of the displaced liquid volume to flow downstream. The passive gate rapidly bounces back to its resting closed state when the active gate withdraws from the liquid channel, hence, preventing back flow and ensuring a directed liquid flow downstream. As shown in Fig. 2c, the passive gate is designed with a tapered micro-pillar arrangement, a configuration that minimizes the contact area of the gas bubble with the liquid channel’s top wall. The tapered configuration facilitates detachment of the interface from the upper wall during actuation. Other taper angles and contact areas were explored and will be discussed in section 2.2. Figure 2d schematically illustrates the experimental setup used in our experiments. Two gas lines with computer controlled gas pressures were involved in pumping of a working liquid from the inlet reservoir to the outlet. The working liquid was pipetted in the inlet well and was collected at the outlet. Only one control line generating a square wave pressure input with adjustable upper ($P_{act}$) and lower ($P_{wd}$) pressures and frequency was needed.
to pump the liquid from the inlet to the outlet. Details of flow control and delivery will be discussed in more detail in section 2.6.

Fig. 2 (a) Schematic illustration of the bubble pump design consisting of an active (upstream) and a passive (downstream) bubble gate in series forming a flow routing unit. The bright field micrograph depicts the bubble pump in operation. Scale bar 500µm. (b) The active bubble gate in “withdrawal” and “actuation” states along with the magnified design of the active gate. Two arrays of micro-pillars limit the bubble motion inside the
liquid channel to one degree of freedom perpendicular to the liquid channel (c) The passive gate in its “closed” and “open” states associated with the withdrawal and actuation states of the active gate respectively. (c) The experimental setup for experiments with the bubble pump. Liquid was displaced from the inlet reservoir to the outlet using the bubble pump. Both the inlet reservoir and the outlet were at atmospheric pressure. The inlet reservoir could be refilled by pipetting fresh liquid in the reservoir. All gas pressure levels were computer controlled and the bubble pump was activated by applying a square wave pressure input at the inlet of the active gate. Scale bar 500µm.

Schematic illustrations and bright field micrographs in Fig. 3a show the bubble pump completing its three-step pumping cycle. The gas pressure for the passive gate is maintained constant at \( P = P_{ps} \) in all steps of the cycle while the gas pressure for the active gate is alternated between a higher actuation pressure level of \( P = P_{act} \) and a lower withdrawal pressure of \( P = P_{wd} \). As shown in the pressure profiles in Fig. 3a, for successful operation of the bubble pump the actuation pressure must exceed the passive gate pressure and the withdrawal pressure must remain lower than the gas pressure for the passive gate (\( P_{wd} \leq P_{ps} \leq P_{act} \)).

3.2.2 Numerical simulation

Numerical simulations were performed using the numerical modeling software, ANSYS (V14.5, Canonsburg, PA, USA), to investigate, compare and optimize different design configurations and to ensure that the adopted design will generate the maximum output in the desired direction when subjected to the operation cycle shown in Fig. 3a. We employed a volume of fluid (VOF) numerical model \(^{58,59}\) to examine the pumping ability and achievable flowrates.

We adopted a pressure-based, double precision, two-dimensional, transient VOF solver with pressure boundary conditions at the gas and liquid inlets and the liquid outlet. No-slip boundary condition was applied at the channel walls. The pressure inlet boundary condition for the active gate was a time dependent boundary condition and was programmed as a user defined function (UDF) in C++ and was implemented in FLUENT to model the square-wave pressure input for the active gate. The numerically obtained volume fraction contours across the solution domain for the bubble pump in withdrawal and actuation steps are shown in Fig. 3d. As shown in Fig. 64
3d, during actuation the passive gate opens partially allowing for the liquid to move downstream. Figure 3e shows the time variation of the liquid flowrate at the inlet (red line) and outlet (black line) of the bubble pump in our solution domain. A negative flowrate corresponds to a flow exiting the domain and a positive flowrate is a flow that enters the domain (i.e., the bubble pump). As seen in Fig. 3e, the inlet flow rate periodically switches from positive to negative and vice versa as the bubble pump operates according to the cycle in Fig. 3a meaning that a fraction of the volume displaced by active bubble gate flows upstream while the rest travels downstream towards the outlet of the bubble pump. The purely negative flow rate at the outlet of the domain demonstrates that the flow at the outlet is an outward flow with no back flow at all times (i.e. net flow downstream). The plot in Fig. 3f shows the computed net liquid volume routed by the bubble pump towards the outlet with time. The net volume was achieved by integration of the instantaneous flowrate at the outlet of the solution domain. The bubble pump flow rate can be determined from Fig. 3f to be approximately 3.6µl/min which agrees favorably with our experimental data for the same pumping frequency (i.e.10Hz) and working fluids (i.e., ethanol and air).
Fig. 3 (a) Active and passive bubble gate pressures at each step of the pumping cycle. (i) Represents the active gate and (ii) represents the passive gate. (b) Schematic illustrations of the bubble pump in its three-step pumping cycle (c) Bright field micrographs showing the bubble pump in each step of its pumping cycle. (d) Numerically modeled liquid (ethyl alcohol) volume fraction contours across the solution domain at actuation and withdrawal
steps (e) Numerically modeled instantaneous flowrate with time at the inlet (red) and outlet (black) of the bubble pump solution domain for $f=10\text{Hz}$, $P_{act}=700\text{Pa}$, $P_{wd}=0$, $P_{ps}=600\text{Pa}$ (d) the net liquid volume pumped downstream obtained by integration of instantaneous flow rate data. Scale bar is 250µm.

Different bubble pump configurations were investigated numerically. Figure 4 demonstrates a numerical study of three different passive gate geometries that were considered for our bubble pump.

Fig. 4 Volume fraction contours for three bubble pump designs with different passive gate geometries: (a) $\alpha=90^\circ$ and $l=500\mu\text{m}$ (b) $\alpha=110^\circ$ and $l=330\mu\text{m}$ (c) $\alpha=120^\circ$ and $l=220\mu\text{m}$ (d) Flowrate with time at the outlet of the pump for the three different geometries studied:
\( \alpha=90^\circ \) and \( l=500\mu m \) (dashed line), \( \alpha=110^\circ \) and \( l=330\mu m \) (dash-dotted line) and \( \alpha=120^\circ \) and \( l=220\mu m \) (solid line). The design in (c) has the highest outlet flowrate for the same pumping conditions \( (P_{act}=0.7KPa, P_{ps}=0.6KPa, f=10Hz) \). (e) Net volume displaced with time for designs in (a), (b) and (c) confirming that (c) displaces the largest volume followed by (b) configuration while (a) produces no net flow. Scale bar 250\mu m.

Figure 4a shows the volume fraction contour predicted numerically for the bubble pump with a passive gate geometry that is identical to the active gate with a pillar array taper angle (the angle between the micro-pillar array and the liquid channel) of \( \alpha=90^\circ \) and wall contact length of \( l=500\mu m \). As shown in the contour, during actuation the gas-liquid interface in the passive gate is pushed against the downstream pillar array rather than withdrawing from the liquid channel to allow a downstream flow. In volume fraction contours of Figures 4b and 4c the pillar arrays are tapered at angles of \( \alpha=110^\circ \) and \( \alpha=120^\circ \) and wall contact lengths of \( l=330\mu m \) and \( l=220\mu m \) respectively. Figure 4d shows the outlet flowrate data with time superimposed for the three different pump configurations studied. As demonstrated by the instantaneous flowrate data the greater the taper angle and the smaller the wall contact area, the higher the downstream flow rate will be for the same operating pressures and frequency. Figure 4e quantifies the net volume displaced by the bubble pump over time by integration of the instantaneous flowrate data and confirms that the passive gate design in Fig. 4c delivers the highest displaced fluid volume while the design in Fig. 4a allows no net flow downstream. The higher flowrate for the design in Fig. 4c can be explained by more effective opening of the passive gate due to smaller wall contact area that results in a smaller disjoining force\(^{60-62} \) \( F_{dj}=P_{dj}A_{c} \) required to open the passive gate. As a result of its superior output, we adopted the design in Fig. 4c in our experiments throughout this work.

3.2.3 Operational envelope

To explain the pumping mechanism of the bubble pump and to determine the operational pressure envelope within which it can be applied successfully we have employed the flow circuit representation of the bubble pump shown in Fig. 5a. The bubble pump is connected to an inlet reservoir with a pressure of \( P_{well} \). Pressure at the outlet of the bubble pump is denoted by \( P_{out} \). In our experimental setup inlet and outlet pressures were nearly atmospheric (i.e., zero gauge
pressure). As discussed earlier the pressure of passive gate is maintained steady at $P_{ps}$ while the active gate pressure oscillates between $P_{act}$ and $P_{wd}$. $P_{wd}$ was atmospheric (zero gauge) in the majority of our experiments and therefore, the active gate pressure oscillated between zero and $P_{act}$. The flow resistance before and after the active gate are denoted by $R_1$ and $R_2$.

Fig. 5 (a) Flow circuit for the bubble pump. The liquid inlet is connected to a reservoir at $P = P_{well}$ while the liquid exists the device at $P = P_{out}$. The flow resistance upstream the active bubble gate is denoted by $R_1$ and the resistance downstream of the active bubble gate is denoted by $R_2$. (b) Numerically obtained intermediate pressure, $P_m$, with time for one
pumping cycle. \( P_m \) has been normalized with respect to \( P_{ps} \) (\( P_{*m}=P_m/P_{ps} \)) and flow time has been normalized with respect to the period of oscillations (\( t^\#=t/T_{total} \)). (c) Operating pressure envelope for the bubble pump is shown by the hatched triangular area. All pressures are normalized with respect to the maximum capillary pressure at which the interface breakup occurs through micro-pillars (\( \Delta P_{Pillar} \)). Both active and passive pressures must remain below the maximum pressure (dash-dotted line) and above the minimum pressure required to enter the channel (dashed line). Active pressure must be greater than passive pressure (below the solid line). (d) Flowrate plotted against the outlet pressure head for the bubble pump. Flow rate decreases with increasing outlet pressure and the bubble pump stops operating at an outlet pressure of \( P_{ps} \) when the passive gate is no longer preventing backflow.

During the actuation step, the active bubble gate acts as a plunger driving the liquid towards the liquid channel where the displaced fluid volume is split in two flow streams one flowing upstream (\( Q_u \)), and the other flowing downstream (\( Q_d \)). \( Q_u \) is a backflow towards the inlet reservoir and \( Q_d \) is the desirable bubble pump output that flows past the passive gate and is collected at the outlet. The liquid pressure behind the passive gate inside the channel connecting the active and passive gates is denoted by \( P_m \). The intermediate liquid pressure, \( P_m \), is initially zero when the pump starts operating and as the active bubble gate begins oscillating and driving the liquid towards the liquid channel, \( P_m \) rises until it surpasses the passive gate pressure momentarily (\( P_{ps}<P_m<P_{act} \)). The elevated liquid pressure behind the passive gate opens the gate allowing a fraction of the displaced liquid (\( Q_d \)) to flow downstream. Figure 5b shows the intermediate pressure, \( P_m \), with time obtained from numerical simulations to determine the evolution of the intermediate pressure during one pumping cycle. The pressures are normalized with respect to \( P_{ps} \) for a case where \( P_{act}=1.6 P_{ps} \). It is noteworthy that to ensure successful bubble pump operation, \( P_m \) must surpass the passive gate pressure (\( P_{ps} \)) and therefore, the active gate pressure must exceed the passive gate pressure at all times (\( P_{ps}<P_{act} \)).

In order to understand and explain the flowrate delivered by the bubble pump, we assume a pressure driven flow along the flow passage depicted by the red dashed line in Fig. 5a. As shown in Fig. 5a the pressure along the flow passage decreases from \( P_m \) to \( P_{out} \) at the outlet. Therefore, the higher the intermediate pressure, \( P_m \), the higher the bubble pump output is. The magnitude of
\(P_m\) however is dependent on the actuation pressure \((P_{act})\) and the pressure of the passive gate \((P_{ps})\) and cannot be increased arbitrarily. The pressure in both the active and passive bubble gates is limited by \(\Delta P_{Pillar}\), the maximum capillary pressure the gas-liquid interface can withstand before it penetrates the liquid channel through the narrow gap between the micro-pillars. Therefore, the pressure difference across the interface must remain lower than \(\Delta P_{Pillar}\) to ensure successful pumping without interface breakup. On the other hand, there is a lower limit for the gas pressure in the bubble gates imposed by the minimum capillary pressure required for the gas to enter the gas channels leading to the bubble-pump. We denote this pressure with \(\Delta P_{min}\). The minimum and maximum capillary pressures are dependent on the bubble pump geometry and the working fluids:

\[
\begin{align*}
\Delta P_{Pillar} &= 2\gamma \left( \frac{1}{\delta} + \frac{1}{H} \right) \\
\Delta P_{min} &= 2\gamma \left( \frac{1}{w} + \frac{1}{H} \right)
\end{align*}
\]

\(\rightarrow \frac{\Delta P_{min}}{\Delta P_{Pillar}} = \frac{\left( \frac{1}{w} + \frac{1}{H} \right)}{\left( \frac{1}{\delta} + \frac{1}{H} \right)} \tag{1}
\]

where \(\gamma\) is the interfacial tension coefficient, \(H\) is the channel height and is constant throughout, \(\delta\) is the gap size between the micro-pillars and \(w\) is the width of the gas channel. The ratio of the minimum capillary pressure to the maximum capillary pressure is purely geometrical as shown in Eqn.1 and is nearly 0.2 in all bubble pump devices tested in this work except for the design we employed for pumping of culture medium which we will be discuss in detail in section 3.1.

Figure 5c shows the operation envelope for the bubble pump as a general guideline for interfacial pressure difference across the active and passive bubble gates. All pressures were normalized with respect to the maximum capillary pressure, \(\Delta P_{Pillar}\). As discussed earlier, both active and passive gate pressures must remain between the minimum and maximum pressures represented by dashed and dash-dotted lines in Fig. 5b respectively. Moreover, the pressure of the passive gate must remain lower than that of the active gate to ensure effective pumping. The hatched area in Fig. 5b represents the operation envelope for the bubble pump.

As discussed earlier during the actuation step the intermediate pressure, \(P_m\), behind the passive bubble gate rises as the active bubble gate protrudes the liquid channel and this elevated pressure results in opening of the passive gate and displacement of the working liquid towards the outlet.
Assuming pressure driven flow through the flow passage starting behind the passive gate and ending at the outlet (the red dashed conduit in Fig. 5a) the resulting flow rate is obtained as:

\[ Q_d = \frac{P_m - P_{out}}{R_2} \rightarrow P_m - P_{out} = R_2 Q_d \]  

(2)

Following a similar process the upstream flow rate (i.e., the back flow) can be obtained as:

\[ Q_u = \frac{P_m - P_{well}}{R_1} \]  

(3)

From Equations 2 and 3 the ratio of downstream to upstream flow rate will be:

\[ \frac{Q_d}{Q_u} = \frac{(P_m - P_{out})R_1}{(P_m - P_{well})R_2} \]  

(4)

According to Eqn. 4, for a device with similar flow resistances before and after the active bubble gate and for similar pressures at the inlet and outlet the upstream and downstream flowrates are equal. This was confirmed by our numerical studies also.

Figure 5d shows the plot for Eqn. 2 and indicating that the bubble pump output decreases with increasing outlet pressure. Mathematically, it is expected from Eqn. 2 that the bubble pump stops pumping when the outlet pressure is equal to \( P_m \). However this does not hold true in practice as the passive bubble gate will fail to perform its flow rectifying role when the outlet pressure approaches \( P_{ps} \) (red line in Fig. 5d). As a result the pressure head at the outlet of bubble pump must remain well below the passive gate pressure for effective pumping.

3.2.4 Device fabrication

Devices were fabricated in PDMS (SYLGARD 184, Dow Corning Corporation, Midland, MI) using the soft-lithography technique. As the first step to fabricate PDMS devices, a replica was made on a microscope glass slide as the substrate using photolithography technique. In order to fabricate the replica, a negative photoresist (SU8-2050, MicroChem, Newton, MA, USA) was spun on the glass slide and baked on a hotplate first at 65°C and then at 95°C. The baked photoresist was then covered with a photomask containing the design features and exposed to UV light. Following exposure to the UV light through a photomask, the substrate was baked again on a hotplate at 65 and 95 degrees. Following the post-exposure bake, the substrate was
allowed to cool down and the unexposed photoresist was removed by immersing the substrate in photoresist developer solution (MicroChem, Newton, MA, USA) for ten minutes while stirring the solution to enhance SU8 dissolution. The final replica fabrication step was to heat up the wafer at 150 degrees for five minutes to remove any liquid residues. PDMS resin and curing agent were mixed next and applied to the replica in a petri dish and degassed in a vacuum chamber (Model 280A, Fisher Scientific, ON, Canada).

The molded PDMS was then heated in an oven at 80°C for 30 minutes. The bubble pump devices were then cut and removed from the replica and the inlet and outlet holes were punched. The whole for the inlet well was nearly 1/8” in diameter and the outlet holes were nearly 1/16 inch in diameter. The microchip was then cleaned and placed in a Plasma Cleaner (Harrick, Ithaca, NY, USA) with a glass slide. Following exposure to oxygen plasma for 30 seconds, the treated surfaces were brought into contact to form an irreversible seal. A 3ml syringe (BD-Canada, Mississauga, ON, Canada) is cut to form a 1ml liquid reservoir and is then placed at the device inlet.

To ensure hydrophilic PDMS surface for measurements done with cell culture medium the devices were plasma bonded and were tested immediately after plasma treatment to ensure a wetting channel surface and formation of a lubricated interface.

### 3.2.5 Chemicals

Ethanol (100%, Commercial Alcohols, ON, Canada) was used as a representative for organic solvents and was used in characterization experiments. Mineral oil light grade (Bioshop Canada, Brulington, ON, Canada) was tested as a representative for oils and the resulting flow rate data were collected and are reported. RPMI 1640 culture medium (Life Technologies Inc., Burlington, ON, Canada) supplemented with fetal bovine serum (10%) and penicillin was used to verify utility of the bubble pump for mammalian cell culture applications. Air was used as the working gas in all experiments. All working fluids were prepared and applied at room temperature (~20 to 25°C).
3.2.6 Flow delivery and control

Computer-controlled digital servo pressure controllers (Type 3110, Marsh Bellofram, Newell, WV, USA) that allowed precise pressure control ($\varepsilon_p=0.005\text{psi}$) via LabView (V12.0.1, National Instruments, Austin TX, USA) were employed to control the gas pressure levels for the active and passive bubble gates. The passive gate was maintained at one constant pressure level while there were two pressure levels for the active gate for each of activation and withdrawal states. The pressure of the active gate was alternated between the two fixed pressure levels using an electromagnetic valve (The Lee Company USA, Westbrook, CT, USA) controlled using a LabView software. The withdrawal pressure level was atmospheric in all experiments except for pumping experiments with culture medium. In case of culture medium pumping a slightly higher pressure than atmospheric was used to prevent abrupt withdrawal of the interface and foam formation. As a result, two pressure controllers were employed for culture medium pumping while all other experiments required only one pressure controller. All working liquids were delivered to the device by pipetting the liquid inside the inlet reservoir.

3.2.7 Imaging and image processing

Bright field microscopy was used to visualize the two phase flow inside the microfluidic devices. An inverted microscope (Eclipse TE-2000-S, Nikon, Japan) was used for visualization and imaging of the experiments. Images and videos were captured using a CCD camera (QImaging QICam Fast 1394, Surrey, BC, Canada) installed on the microscope.

3.3 Results and Discussion

3.3.1 Flowrate characterization

The bubble pump is similar to a macroscale plunger pump with the difference that it only includes one passive valve and the stroke or the volume of the displaced fluid is variable unlike the mechanical plunger pump. Therefore, the flowrate delivered by the bubble pump is dependent on the stroke length ($\Delta y$) of the active bubble gate that acts as the piston in a plunger pump. In our bubble pump assuming a negligible film thickness, the displaced fluid volume, $\Delta V$, can be written as:

$$\Delta V = \Delta y A_{ch} = Ut_{wd}A_{ch}$$

(5)
where $U$ is the withdrawal velocity of the interface and $t_{wd}$ is the amount of time the active gate is withdrawing away from the liquid channel drawing a liquid volume back into the active gate channel and $A_{ch}$ is the cross-sectional area of the channel. Converting the displaced volume to the total flowrate produced by the active gate we can write:

$$\begin{cases}
Q_{total} = Q_u + Q_d \to \frac{\Delta V}{T} = Q_u + Q_d \\
T = t_{wd} + t_{act} = 1/f
\end{cases} \quad (6)$$

where $Q_{total}$ is the total flow rate and $T$ is the time period of the reciprocating motion of the active bubble gate including the time it is withdrawing and the time it is actuating. Substituting for parameters in Eqn. 6 from Equations 2 to 5 we obtain:

$$\begin{cases}
U t_{wd} A_{ch} f = Q_d + x Q_d \\
x = \frac{(P_m - P_{well}) R_2}{(P_m - P_{out}) R_1}
\end{cases} \quad (7)$$

Rearranging Eqn. 7, the net downstream flow rate of the bubble gate can be written as:

$$\begin{cases}
Q_d = \frac{U t_{wd} A_{ch}}{(1+x)(t_{wd}+t_{act})} = \frac{U r A_{ch}}{(1+x)(r+1)} = \frac{U t_{wd} A_{ch} f}{(1+x)} \\
r = \frac{t_{wd}}{t_{act}}
\end{cases} \quad (8)$$

where $r$ is the ratio of the time the active bubble withdraws and the time it actuates.

To ensure utility of the bubble pump with different working fluids and to characterize the pumping performance we tested the bubble pump with different liquids and measured the flowrate produced. Flowrates were measured by measuring the volume of the liquid output over time. The schematic in Fig. 6a illustrates the experimental setup employed for flowrate measurement experiments. As shown in the schematic illustration, straight clear tubing with inner diameter of 1mm was fixed onto a ruler and connected to the outlet to allow for the continuous measurement of the liquid volume exiting the pump. The bubble pump was allowed to operate at different combinations of $t_{wd}$ and $t_{act}$ (i.e., different frequency, $f$, and stroke size, $\Delta y$). Figures 6b to 6f present the experimental data for flowrate characterization of the bubble pump with air and ethanol as working fluids.
In Fig. 6b the flowrate data is plotted versus frequency for a case where the withdrawal time is kept constant at 300ms (i.e., constant stroke length, $Δy$) while the actuation time and as a result the operation frequency is varied. As shown in Fig. 6b the flow rate increases linearly with increased frequency. However there is a limit as to how low the actuation time can be decreased (i.e. the frequency increased) without compromising flowrate due to incomplete or partially-complete pumping cycles. The effect of partial or incomplete cycles can be seen towards the highest frequencies in the plot where the slope of the flowrate line begins to diminish. The initial linear behavior however is predictable according to Eqn. 8 when written in the format below with $t_{wd}$ as a constant:

$$Q_d = \frac{Ut_{wd}Achf}{(1+x)}$$  \hspace{1cm} \text{(9)}$$

Equation 9 represents the red dashed trend line in Fig. 6b with a constant slope for the frequencies where the bubble pump operates with complete pumping cycles.

Figure 6c shows the flowrate data plotted against frequency for varying operation frequencies while withdrawal and actuation times are equal ($r=1$). Substituting for $r$ in Eqn. 8 we obtain the outlet flowrate as:

$$Q_d = \frac{UA_{ch}}{2(1+x)}$$  \hspace{1cm} \text{(10)}$$
Fig. 6 (a) Experimental setup used to measure the flowrate for the bubble pump. A capillary of known inner diameter (1mm) was connected to the outlet to measure the volume of displaced liquid over time. Ethanol and oil were used as working fluids for characterization experiments. (b) Flow rate plotted against actuation frequency with withdrawal time kept constant at 300ms. (c) Flowrate plotted against actuation frequency for pumping with a balanced cycle when actuation and withdrawal times were equal ($t_{wd} = t_{act}$). (d) Superimposed flowrate-frequency data from (b) and (c). The agreement between pumping with a balanced cycle ($t_{wd} = t_{act}$) and pumping with a constant withdrawal time indicates that for low frequencies flowrate is independent of the withdrawal time. (e) Flowrate plotted versus actuation time while keeping the withdrawal time constant at 300ms. (f) Flowrate plotted against $r$, the ratio of withdrawal time ($t_{ret}$) and actuation time ($t_{act}$). The ratio $r$ was varied while maintaining a constant frequency of 1Hz.

Equation 10 indicates that in the case where withdrawal and actuation times are equal flowrate is not a function of frequency. As shown in Fig. 6c, the independence of flowrate from pumping frequency holds true for frequencies ranging over nearly an order of magnitude from frequency of two to ten. For frequencies higher than ten the active bubble gate fails to actuate as the time interval between actuation and withdrawal of the interface is too brief for the gas-liquid interface to respond to the pressure input and as a result the attainable flowrate is zero. On the other hand, at frequencies lower than two the flowrate begins to decrease as the actuation and withdrawal times increase. At low frequencies (i.e., long withdrawal times) the gas-liquid interface has sufficient time to withdraw all of the available gas channel length on the device regardless of the frequency and as a result is similar to the case in Fig. 6b where the withdrawal time is constant and the flow rate decreases linearly with increasing actuation time (i.e., decreasing frequency). Figure 6d shows the flowrate data from Fig 6b and 6c superimposed. As seen in the superimposed plot the two data sets agree for low pumping frequencies ($f<2$Hz) as discussed above.

In Fig. 6e flow rate is plotted versus actuation time ($t_{act}$) while maintaining the withdrawal time (i.e., pumping stroke length) constant at 300ms. As demonstrated by the data the flowrate decreases with increased actuation time as it reduces the pumping frequency while maintaining
the displaced liquid volume in a single cycle ($\Delta V$) constant. The red dashed trend line for the experimental data shown in Fig. 6d is predicted analytically by Eqn. 8 when written in terms of withdrawal and actuation times as below:

$$Q_d = \frac{Ut_{wd}A_{ch}}{(1+x)(t_{wd}+t_{act})}$$

(11)

The flowrate data presented in Fig. 6f point out the effect of varying values of withdrawal time to actuation time ratio, $r=t_{wd}/t_{act}$. As shown by the experimental data with increased $r$ the flowrate initially increases sharply but the slope gradually declines until the output flowrate reaches a plateau. The effect of varying $r$ on flowrate can also be explained analytically by Eqn. 8 when written in terms of $r$:

$$Q_d = \frac{UrA_{ch}}{(1+x)(r+1)}$$

(12)

The red dashed trend line for the experimental data in Fig. 6f is predicted analytically by Eqn. 12 when plotted against $r$. At high values of $r$ corresponding to extremely low actuation times the flowrate begins to drop as a result of partially-complete pumping cycles caused by the interface’s inability to actuate long enough to travel the full length of the pumping stroke.

Microfluidic systems have been extensively used for cell culture and cell biology studies. In order to demonstrate utility of the bubble pump for cell culture applications we conducted pumping experiments with a common mammalian cell culture solution consisting of RPMI 1640 culture medium supplemented with 10% FBS and penicillin. Our initial experiments with the original device dimensions (i.e., design of Fig. 4c) were hindered by occasional foaming of the culture medium due to the mechanical stresses caused by abrupt motion of the active bubble gate and high concentration of surfactants present in the culture medium. To enhance reliability of our pumping strategy with culture mediums and solutions with high surfactant concentration we redesigned the bubble pump and reduced the gas channel width to one fifth of the original size and the liquid channel width to one half. The reduction in channel dimensions increased the interfacial tension and rigidity of the gas bubble and reduced the likelihood of interface breakup and foaming during bubble pump operation. To further reduce the likelihood of foam generation we limited the withdrawal distance of the active bubble gate by raising the withdrawal pressure.
(\(P_{wd}\)) slightly to a non-zero pressure unlike all previous experiments with other working fluids. These modifications resolved the foaming problem and reliable pumping was achieved for more than 72 hours of uninterrupted operation. The achievable flowrates, however, were reduced by the design and operational modifications as they decreased the displaced liquid volume. A flow rate of nearly 0.043µl/min (or 0.72nl/s) was the maximum flow rate achievable after modifications. For long term cell culture applications, however, high flow rates are not required as the flow rate is limited by the shear stress threshold that living cells can undergo\(^{65,66}\).

Perfusion flow rates of below 0.1µl/min have commonly been used for culture medium delivery in microfluidic cell culture applications\(^{67-69}\). Figure 7b shows the flow rate data obtained for pumping with the culture medium. As seen in Fig.7b flow rate increases linearly with pumping frequency as a result of our intentional maintenance of a constant stroke distance. Similar to the flowrate data in Fig. 6b the linear behavior for the flowrates in Fig. 7b can be explained by Eqn. 9.

In order to demonstrate utility of our bubble pump for different applications with various working liquids of different viscosities and interfacial tensions with air we tested the bubble pump with light mineral oil and a common mammalian cell culture medium supplemented with all the necessary ingredients for cell culture. Figure 7c shows the bright field image of the bubble pump operating with mineral oil and air as working fluids. The experimental flowrate data for pumping with mineral oil is presented in Fig. 7d. The flowrate data in Fig. 7d were acquired for pumping with a balanced cycle where the withdrawal and actuation times were equal (i.e. \(r=1\)). As seen in the plot the flowrate increases rapidly with increased frequency and peaks at a maximum flowrate of nearly 0.26µl/min before starting to decline. The sharp decline in the flowrate for pumping with mineral oil occurs due to the high viscosity of the mineral oil slowing down the bubble movement during actuation which in turn results in partial actuation and a decreased flow output. It is noteworthy that the average flowrate produced with mineral oil (\(\mu=23cP\), \(Q_{avg}=3.27\mu l/min\)) as the working liquid was 19.2 times smaller than the average flowrate obtained with ethanol (\(\mu=1.1cP\), \(Q_{avg}=0.17\mu l/min\)), a ratio that is comparable with the ratio of viscosities (i.e., \(Q_{Ethanol}/Q_{oil} \approx \mu_{oil}/\mu_{Ethanol}\)). This observation further confirms our assumption of pressure driven flow in Eqn. 2 indicating that the output flowrate decreases with
increasing flow resistance ($R_2$) as was the case in our experiments with mineral oil (i.e. increased viscosity results in increased flow resistance).

Fig. 7 (a) Micrograph showing the bubble pump in operation with working fluids of RPMI 1640 culture medium+10% fetal bovine serum (FBS) and air. The dimensions were reduced to ensure reliable long-term pumping for cell culture applications. Scale bar 250µm. (b) Flowrate versus actuation frequency for pumping with a balanced cycle ($t_{wd}=t_{act}$) and working fluids of culture medium and air. (c) Bright field micrograph for the bubble pump operating with working fluids of mineral oil and air. Scale bar 500µm. (d) Flowrate versus actuation frequency data for pumping with a balanced cycle ($t_{wd}=t_{act}$) and working fluids of mineral oil and air.
3.3.2 Parallel configuration and scalability

To enhance the flow output of the bubble pump we devised and investigated a configuration that consisted of two bubble pumps operating in parallel in the same liquid channel displacing the working liquid from the inlet well towards the. Figure 8a shows the schematic illustration for the parallel pump design and a bright field micrograph showing the pump in operation. Active and passive gates for both pumps were connected to common gas inlets and as a result the controls required to operate the parallel pump were identical to a single bubble pump. The total flowrate output of the pump is the sum of flowrates for each individual pump \( Q_{\text{total}} = Q_1 + Q_2 \). Figure 8b shows the flowrate data for the parallel pump operating with working fluids of mineral oil and air (black line) superimposed with the flowrate data for a single bubble pump operating under the same conditions (red line). As shown in the plot the flowrate of the parallel pump is twice as high as that of a single bubble pump for frequencies higher than two. The flow rates of the parallel pump and the single pump converge as the pumping frequency decreases. At higher frequencies the stroke size of the bubble pump is small and the interface remains in the section of the gas channel that is identical to a single pump design as a result the volume displaced (i.e., flowrate produced) by the parallel pump is exactly two times that of a single pump. At low frequencies (i.e., long withdrawal times) however, the interface withdraws over a longer period of time traveling past the 90 degree bend in Fig. 8a that was designed differently from the single pump to allow for a simultaneous control of both pumps. The difference in gas channel geometry for the parallel pump hinders the interface movement compared with the single pump and as a result the total volume displaced is less than twice that of a single gate. The flowrates for the parallel pump were however higher than that of a single gate for all pumping frequencies with both maximum flow rates and the average flow rate for the parallel pump being 30 percent higher than that of a single pump.

In order to demonstrate utility of the bubble pump technique in applications where a large number of concurrent processes take place such as in biological culture or drug screening we developed and tested a well-based scaled up pumping device. Figure 8c shows the schematic illustration of the scaled up device. As shown in Fig. 8d the device consists of twelve independent microchannels each connected to an independent inlet well, a bubble pump and an independent outlet. Each of the twelve bubble pumps consists of an active and a passive gate.
Similar to the parallel pump, all active and passive gates were connected to a common active and passive inlet respectively and therefore the controls required to operate all twelve pumps were identical to a single bubble pump. The twelve inlet wells were filled with the working liquid (mineral oil) and the bubble pumps were started by setting the active gates in a reciprocating motion. We successfully controlled all twelve pumps with a single control line with minimal variation. The active gates demonstrated a consistent stroke length and oscillation timing across all twelve channels. The histogram in Fig. 8f shows the flowrate distribution for the twelve microchannels. The data show a mean flowrate of 0.13µl/min and a standard deviation of 0.01µl/min across the twelve microchannels, a seven percent deviation from the mean flowrate. The variation in flow rates is due to the slight variations in the stroke size caused by nonlinearities in the interface movement.
Fig. 8 (a) The Schematic illustration showing a parallel bubble pump configuration with two bubble pumps working in parallel in the same liquid line pumping the liquid from the inlet well towards the outlet. The bright field micrograph shows the parallel pump in operation. Scale bar 500µm. (b) Measured flowrate data versus frequency for the parallel pump configuration (◆) superimposed with those from a single bubble pump (■) with
working fluids of mineral oil and air. (c) Schematic illustration of the scaled-up pumping device with twelve independent liquid channels. The working liquid is fed into twelve inlet wells each connected to an independent microchannel with a bubble pump displacing the inlet liquid towards an outlet. All twelve active gates are controlled by a single pneumatic control line while all passive gates are connected to a common gas input. (d) The schematic illustration showing the close-up for structure of the scaled-up pumping device. (e) Photograph of the scaled-up pumping device with channels filled with red dye (f) Flowrate distribution for the twelve microchannels on a scaled-up pumping device operated with mineral oil and air at \( f = 0.5 \text{Hz} \). Scale bar 1cm long.

3.4 Conclusion

We presented an on-chip “bubble-pump” liquid routing strategy that tackles some of the unresolved problems associated with state of art microfluidic flow control techniques. Designed to deliver long term, low to medium flowrates in well-based integrated microfluidic devices, our technique is single layer, independent of substrate material elasticity, biocompatible and scalable with no additional controls required. The bubble pump was designed based on the bubble gate technique, a valving method that exploits computer controlled movement of a gas bubble to alter or block liquid flow in a microchannel. The bubble pump takes advantage of two bubble gates for liquid routing, one active and one passive. The active bubble gate drives the working liquid and the passive gate spontaneously rectifies the flow in the direction of interest. We employed numerical modeling, analytical modeling and experimentation to verify, optimize and characterize the bubble pump. We tested the bubble pump with three representative liquids for three groups of liquids commonly used in microfluidic applications: ethanol (organic solvents), mineral oil (oils) and culture medium (aqueous and biological solutions). The bubble pump was found effective for long term (i.e., more than 72 hours) uninterrupted pumping with aforementioned liquids with maximum flow rates of 5.5\( \mu \text{l/min} \), 0.26 \( \mu \text{l/min} \) and 0.043 \( \mu \text{l/min} \) for working liquids of ethanol, mineral oil and culture medium respectively. Enhanced flowrates were achieved by employing a parallel pumping configuration consisting of two bubble pumps operating within one liquid channel thereby producing flowrates twice as high as that of a single pump at high frequencies and a 30 percent higher average flowrate across all frequencies. The bubble pump was proven to be readily scalable through successful development and operation of
a twelvefold scaled up device. All twelve bubble pumps of the scaled up device were operated by the same controls as a single pump producing uniform flowrates with only a seven percent variation in the flowrates measured across all 12 microchannels.

3.5 References


Chapter 4: Microfluidic technique for in-situ interfacial tensiometry

Summary

We present a microfluidic strategy for real time in-situ measurement of interfacial tension between pairs of immiscible fluids within integrated microfluidic reactors. Our tensiometer consists of two microchannels that meet at a T-shaped junction and form a small chamber (volume of 0.025 µl) at the intersection of two channels. The intersection chamber is isolated from the horizontal channel except for two narrow micro-slits, one on each side of the T-junction. To perform an interfacial tension measurement for an immiscible pair of fluids, the horizontal channel is fed with the wetting liquid while the less wetting fluid (i.e. the disperse phase) is introduced via the vertical channel and brought in contact with the wetting liquid to form a two-phase interface at the intersection chamber. Interfacial tension is determined from precise measurement of the capillary pressure required to drive the two-phase interface out of the T-junction through the micro-slit. The interfacial pressure is automatically recorded and the interfacial tension is determined from the Young-Laplace equation. Using our tensiometer, each measurement took less than a minute once the samples were loaded in the feed reservoirs with sample volumes required for a measurement on the order of microliters. Samples containment within the microfluidic device prevented contamination or external disturbances from skewing the measurement results. We validated our tensiometer both experimentally and numerically. To validate our technique experimentally, we compared the measurement results from our tensiometer with those obtained using a commercial pendant drop system with a discrepancy of less than three percent. We demonstrated utility and versatility of our tensiometer by measuring interfacial tension values ranging from 6mN/m to 72mN/M for a variety of commonly encountered fluid-fluid interfaces (i.e. organic solvent-air, aqueous-air, oil-air, oil-water and oil plus surfactant-water). Capability of our technique in performing real-time in-situ measurements was demonstrated by a case study involving real-time periodic measurement of interfacial tension downstream of a sampling device. Two liquids with different interfacial tensions with
air were periodically sampled and allowed to flow downstream where the interfacial tension was measured using our tensiometer in real-time without disruption of the upstream sampling.

4.1 Introduction

The interfacial tension of fluids has tremendous impact on life. Examples are irrigation and nutrients uptake in plants and pulmonary function\textsuperscript{1,2}. Real life applications such as coatings, painting, welding, electroplating, production processes, ink jet printing, lubrication, detergents, cosmetics and oil recovery all depend on interfacial phenomena\textsuperscript{3-5}.

In sub-millimeter length scales encountered in lab-on-a-chip and microfluidic devices for chemical\textsuperscript{6-10} and biological analysis\textsuperscript{11-15}, interfacial phenomena are critical in two ways: Firstly by directly affecting the flow structure and behavior in such devices as surface tension forces dominate fluid flow in microchannels\textsuperscript{8,16,17}. For instance, formation and size distribution of bubbles and droplets\textsuperscript{18,19} or pressure drop\textsuperscript{20} in multiphase flow microfluidics. Secondly, when the knowledge of interfacial properties of the fluids being analyzed or produced within the micro-device is of interest.

Interfacial tension may vary locally in a microchannel as a result of a progressing chemical reaction or biochemical process. In such cases the removal of the product and subsequent measurement in a separate tensiometry setup is impractical and necessitates local on-chip interfacial tension measurements within the microfluidic system. A particularly important group of microfluidic devices that benefit from local real-time interfacial tension monitoring are multiphase flow droplet reactors\textsuperscript{21,22} with applications in chemistry\textsuperscript{23-25} and biology\textsuperscript{26-28}. Reactor performance and quality of products in these reactors are directly dependent on the size distribution of droplets which in turn is a function of interfacial tension.

Common interfacial tension measurement methods are based on either direct or indirect measurements\textsuperscript{29}. Wilhelmy plate technique\textsuperscript{30,31} and Du Noüy\textsuperscript{29,32} ring method are common direct measurement methods that measure the interfacial force by means of a microbalance. Indirect methods on the other hand calculate the interfacial tension value from other measured variables such as pressure, shape of interface, volume and weight. The most common indirect methods are pendent drop method\textsuperscript{33-35}, drop weight method\textsuperscript{4,36,37} and sessile drop method\textsuperscript{34,38-41}.
that rely on the force balance between gravitational and surface tension forces. While these measurement methods are well established for most common applications they are not transferable to in-situ measurements within confined microchannels relevant to microfluidic and lab-on-a-chip systems.

There have been a limited number of microfluidic-based tensiometers developed to date. Previous microfluidic efforts have determined interfacial tension from measurement of the size and shape of droplets formed in microchannels. Quasi-static tensiometry techniques for immiscible liquids have also been achieved by controlling the position of a liquid-liquid interface within a tapered microchannel with known geometry. Nguyen et al. performed gas-liquid interfacial tensiometry using dynamic generation of a segmented flow and correlation of the bubble generation frequency and interfacial tension. The microfluidic tensiometry techniques developed to date were predominantly designed for standalone tensiometry of two phase interfaces. They are particularly not suitable as a modular technique that can be integrated in an existing microfluidic reactor and perform real time in-situ measurements of the reagent or products.

For a microfluidic tensiometry technique to be applicable as an in-situ measurement unit it must require: a small footprint, minute volume of samples and minimal user intervention. Additionally the tensiometer module must not disturb the normal operation of the upstream microchannels to be applicable within an integrated microfluidic device. Here, we present a readily implementable automated technique with above characteristics for determining both gas-liquid and the liquid-liquid interfacial tension. Our method is based on the precise measurement of the maximum pressure required to force a two-phase interface through a micro-slit. It requires a footprint of less than 1mm$^2$, a sample volume of the order of 1µl and minimal user intervention. Our microfluidic tensiometer performs all measurements inside a micro-device sealed from the outside which prevents outside disturbances to affect the measurement results. Furthermore, our technique allows precise control over the pressures in both fluids forming a fluid-fluid interface which enables us to perform interfacial tension measurements under high pressures.

We start with the presentation of the device design and experimental setup. We then perform a sensitivity analysis based on theory followed by experimental and numerical validations of our
tensiometer. In the end we perform a case study to illustrate utility of our technique as a modular tool for real-time in-situ tensiometry.

4.2 Experimental

4.2.1 Device design and experimental setup

Our microfluidic tensiometer relies on the precise measurement of maximum pressure required to push a fluid-fluid interface out of a micro-slit of known geometry. As shown in Fig. 1, our strategy consists of a T-junction connecting two perpendicular channels, with each channel delivering one fluid to the T-junction where the two immiscible fluids meet and form a two-phase interface. The less wetting fluid (Fluid A) is engulfed by the more wetting fluid (Fluid B) forming a lubricated interface\textsuperscript{48}. The two phase interface is confined within the T-junction by narrow slits that share similarities with narrow features or micro-pillar arrays used previously to stabilize fluid-fluid interfaces\textsuperscript{49-51}. The T intersection and the two micro-slits form a measurement chamber. The micro-slit of the width, $d_{us}$, between the upstream side-channel and the measurement chamber is connected to a pressurized liquid reservoir (see Fig. 1) and delivers the more wetting liquid (Fluid B) to the measurement intersection. Downstream side of the T-intersection is connected to the atmospheric pressure via a micro-slit of the width $d_{out}$. The maximum pressure measurements are performed at the downstream micro-slit where the interface is pushed out towards the tensiometer outlet. For in-situ measurements in integrated micro-devices, the upstream slit ($d_{us}$) must be narrower than the outlet slit ($d_{out}$) size ($d_{us} < d_{out}$) in order to prevent the maximum pressure technique to disturb the upstream flow.

Once the two-phase interface was established inside the vertical channel, the pressure of fluid A (i.e. $P_A$) was gradually increased until fluid A filled the entire intersection and the fluid-fluid interface was held behind the outlet micro-slit with the pressure difference across the interface being the difference between the atmospheric pressure (i.e. $P_{atm}$) and the pressure of fluid A ($\Delta P$)
The precise computer control over the pressure across the interface made it possible to increase the pressure of fluid A in small increments and capture the critical pressure ($P^*$) at which interface breakup occurs. In our measurements $P_A$ was increased in small increments of 0.005 psi until the interface breakup was captured by a MATLAB generated software. At the point of interface breakup through the outlet micro-slit, $P_A$ was recorded as the maximum pressure ($P^*$). With the knowledge of $P^*$ and the geometry of the slit (i.e. $d_{out}$ and the channel height, $H$) the interfacial tension was calculated from the Young-Laplace equation as:

$$P^* = 2\gamma \left(\frac{1}{\delta} + \frac{1}{H}\right) \rightarrow \gamma = \frac{P^*}{2} \left(\frac{1}{\frac{1}{\delta} + \frac{1}{H}}\right)$$

(1)
Fig. 1 Schematic of the experimental setup and the micrograph for the microfluidic device employed in our measurements. All pressures were computer controlled. Liquid pressures were adjusted using a computer controlled gas pressure head where applicable. Scale bar is 500 micrometers.

4.2.2 Sensitivity analysis:

As seen in Eqn.1 the parameters affecting the accuracy of our measurements are the pressure $P$, and the geometrical dimensions of the outlet slit, $\delta$ and $H$, which are shown in the schematic illustration of Fig. 2a. Pressure affects accuracy of our tensiometry technique via resolution of pressure measurements (i.e. resolution of the employed pressure controller), $\varepsilon_P$ as follows.

$$\frac{\partial \gamma}{\partial P^*} = \frac{1}{2} \left( \frac{1}{\delta^* H} \right) \rightarrow d\gamma = \frac{\varepsilon_P}{2} \left( \frac{1}{\delta^* H} \right)$$    (2)
As seen in Eqn. 2, the interfacial tension measurement has a linear relationship with the resolution of pressure measurements, with a slope that is defined by the geometry of the outlet micro-slit. Substituting for $\delta$ and $H$ in Eqn. 2 with the dimensions of the micro-slit used in our measurements ($\delta = 20\mu m$ and $H = 75\mu m$) we can plot the error in interfacial tension measurement as a function of the pressure resolution as shown in Figure 2b. As predicted by Eqn. 2, the larger the resolution of pressure measurements the bigger the measurement error will be. Our pressure controllers had a resolution of 0.005 psi (~34 Pa) that corresponds to an error of nearly 0.2 mN/m. In order to assess sensitivity of the interfacial tension measurements to the geometrical parameters we need to determine derivatives of $\gamma$ with respect to $\delta$ and $H$ starting with the Young-Laplace equation:

$$\gamma = P \frac{H\delta}{2(H+\delta)}$$

(3)

and finding partial derivatives of interfacial tension with respect to the micro-slit width ($\delta$) and channel height ($H$) we obtain:

$$\begin{align*}
\frac{\partial \gamma}{\partial \delta} &= \frac{P}{2 \left(1 + \frac{\delta}{H}\right)^2} \rightarrow \partial \gamma = \frac{P \delta}{2 \left(1 + \frac{\delta}{H}\right)^2} \\
\frac{\partial \gamma}{\partial H} &= \frac{P}{2 \left(1 + \frac{H}{\delta}\right)^2} \rightarrow \partial \gamma = \frac{P \delta}{2 \left(1 + \frac{H}{\delta}\right)^2}
\end{align*}$$

(4)

Dividing Eqn. 4 by Eqn. 3 we will have:

$$\begin{align*}
\frac{\partial \gamma}{\gamma} &= \frac{\partial \delta}{\delta (H+\delta)} = \frac{\delta (1 + \frac{1}{r})}{\delta (1 + \frac{1}{r})}, r = \frac{\delta}{H} \\
\frac{\partial \gamma}{\gamma} &= \frac{\partial H}{H (H+\delta)} = \frac{\delta (1 + \frac{1}{r})}{\frac{H}{\delta} (1 + \frac{1}{r})} \\
\frac{\partial \gamma}{\gamma} &= \frac{\partial H}{H (H+\delta)} = \frac{\delta (1 + \frac{1}{r})}{\frac{H}{\delta} (1 + \frac{1}{r})}
\end{align*}$$

(5)

Equation 5 indicates the mathematical relationship between relative change in the micro-slit dimensions $\delta$ and $H$ and the corresponding relative change in the measured interfacial tension $\gamma$. In Fig. 2c and d Eqn. 5 is plotted for a relative change from zero to one in $\delta$ and $H$ and the corresponding change in $\gamma$ for micro-slits of different aspect ratio ($r = \delta/H$). As shown in the plots the slit width $\delta$ has the highest relative impact on the measurement when it is significantly smaller than the channel height, $H$ (i.e. at low $r$) and conversely, the channel height has the highest impact on the measurement of $\gamma$ when the slit is much shallower than it is wide (i.e. at
high \( r \). Plots in Fig. 2 are a useful reference for the design of our devised tensiometer and for prediction of the accuracy of measurements that can be achieved with different designs. Devices used for the measurements presented in this work had a \( r \) value of 0.26 indicating that the slit width was the major determining parameter in the measurements.

![a) Schematic illustration for the micro-slit geometry with the width of \( \delta \) and height of \( H \) (b) Sensitivity of the interfacial tension measurement \( (d\gamma) \) with respect to the resolution of pressure measurements \( (\varepsilon_p) \) (c) Relative error in the measurement of \( \gamma \) as a function of a relative change in the slit width \( (\delta) \) for various outlet slit geometries (i.e. values of \( r=\delta/H \)) (d) Relative error in the measurement of \( \gamma \) as a function of a relative change in channel height \( (H) \) for various micro-slit geometries (i.e. values of \( r=\delta/H \)).

Fig. 2 (a) Schematic illustration for the micro-slit geometry with the width of \( \delta \) and height of \( H \) (b) Sensitivity of the interfacial tension measurement \( (d\gamma) \) with respect to the resolution of pressure measurements \( (\varepsilon_p) \) (c) Relative error in the measurement of \( \gamma \) as a function of a relative change in the slit width \( (\delta) \) for various outlet slit geometries (i.e. values of \( r=\delta/H \)) (d) Relative error in the measurement of \( \gamma \) as a function of a relative change in channel height \( (H) \) for various micro-slit geometries (i.e. values of \( r=\delta/H \)).
4.2.3 Numerical validation

In order to verify validity of the theoretical concept behind our devised technique we developed a volume of fluid (VOF) numerical model \textsuperscript{52,53} using a commercial code (ANSYS Fluent V14.5, Canonsburg, PA, USA) and compared the maximum bubble pressures predicted by young-Laplace equation with those obtained from transient numerical models and close agreement was noted with a discrepancy of less than two percent.

We employed a pressure-based, double precision, two dimensional, transient VOF solver with constant pressure boundary condition applied at the fluid inlet and outlets and no-slip boundary condition applied at channel walls. The two dimensional model represented a design in which the slit width, $\delta$, is significantly smaller than the height, $H$ (i.e. $r=0$). As a result, in comparing the numerical data with static Young-Laplace equation only the slit width was taken into account.

Additionally, it was noted in our numerical models that in order for the tensiometer to follow the Young-Laplace equation the variations in pressure must occur gradually and in a quasi-static fashion. Figure 3a shows the evolution of the gas-liquid interface (Phase A: air and Phase B: ethanol) in our numerical model. In our transient simulation, the pressure of phase A ($P_A$) was gradually increased at the bottom inlet while the pressure of the phase B ($P_B$) was maintained at atmospheric levels for two side channels (the outlets). As a result, the interface began moving from the bottom inlet towards the measurement intersection (i.e. i and ii) and occupied the entire intersection (i.e. iii) and eventually underwent breakup through the micro-slit when the pressure in Phase A reached the maximum pressure (i.e. iv).
Fig. 3 (a) Transient contours for volume fraction of phase A at the T-junction. With increased pressure the interface occupies the entire measurement chamber and eventually is pushed out of the micro-slit when the maximum pressure is reached (b) Plots demonstrating the evolution of pressure profile across the T-junction along the section line of LL’. PA is increased from 28% of the maximum pressure to the maximum pressure where breakup occurs as indicated by the sharp declines in the pressure profile in black.

Figure 3b shows the pressure evolution across the measurement intersection encompassing both input and output slits. The sharp rise in the pressure profiles indicates the locations of the gas-
liquid interface in inlet and outlet slits. As seen in Fig. 3b with increased pressure the interface moves in further towards the micro-slit until it reaches the maximum pressure (i.e. $P^*$) where the interface breakup through the micro-slit occurs. The interface breakup can be seen in Figure 3b in the pressure profile associated with $P = P^*$ where sudden decline in the pressure profile occurs at the location of the two micro-slits ($x=0.5$ and $1.5\text{mm}$). The post-breakup pressure decline occurs as a flow is established inside the micro-slit towards the outlet. Micrographs in Figure 4 demonstrate the interface evolution with increased pressure from 30 percent of the maximum pressure to 99 percent of the maximum pressure. As seen in the figure the lubricated interface proceeds towards the outlet slit until breakup occurs at the maximum pressure and at an interface angle of zero.

![Micrographs showing interface evolution](image)

**Fig. 4** Bright field micrographs showing the evolution of the two-phase interface as the pressure is gradually increased from $P_A=0.3P^*$ to just below the maximum pressure $P_A=P^*$ where the interface is pushed through the micro-slit. The micrographs, in agreement with
figure 3b, show the interface traveling towards the point of breakup. Scale bar is 300 µm. The working fluids are mineral oil and air.

4.2.4 Device fabrication

Devices were fabricated in poly (dimethylsiloxane) PDMS (SYLGARD 184, Dow Corning Corporation, Midland, MI, USA) using the soft-lithography technique. To fabricate the PDMS devices, a replica was made on a microscope glass slide as the substrate using photolithography technique. To do so, a negative photoresist (SU8-2050, MicroChem, Newton, MA, USA) was spin-coated on the substrate and baked on a hotplate sequentially at 65C and 95C. Following exposure to the UV light through a photomask, the substrate was baked again on a hotplate at 65 and 95 degrees and the unexposed photoresist was removed using photoresist developer solution (MicroChem, Newton, MA, USA). The final step in replica fabrication was to heat up the replica at 150C for five min to remove any liquid residues. PDMS resin and curing agent were mixed next and applied to the replica and degassed in a vacuum chamber (Model 280A, Fisher Scientific, Ottawa, ON, Canada).

The replica was then heated in an oven at 80C for 30 minutes until the PDMS was cured and hardened. The microchips were then cut and removed from the replica and the inlet and outlet holes were punched. The holes were nearly 1/16 inch in outer diameter. The microchip was then cleaned and placed in a Plasma Cleaner (Harrick, Ithaca, NY, USA) with a glass slide (coated with PDMS for liquid-liquid interfacial tension measurement experiments). Following exposure to oxygen plasma for 30 seconds, the treated surfaces were brought into contact to form an irreversible seal.

To ensure hydrophilic PDMS surface for measurements done with water the devices were plasma bonded and were used immediately after plasma treatment to ensure a wetting channel surface and formation of a lubricated interface. Conversely for liquid-liquid experiments with oil and water the PDMS surface was restored to the native hydrophobic state by overnight heating at 80C following plasma sealing of the devices.
4.2.5 Chemicals

Several pairs of fluids were used in interfacial tension measurements. Ethanol (100%, Commercial Alcohols, ON, Canada) was used as a representative for organic solvents. Mineral oil light grade (Bioshop Canada, Brulington, ON, Canada) was used to conduct mineral oil-air and mineral oil-water interfacial measurements. Span®80 surfactant (Sigma Aldrich Canada, Oakville, ON, Canada) was added to mineral oil to measure the reduced interfacial tension between oil and water in presence of Span®80. Deionized water was used wherever water was needed in the measurements.

4.2.6 Flow delivery and control

Computer-controlled digital servo pressure controllers (Type 3110, Marsh Bellofram, Newell, WV, USA) capable of precise pressure control ($\epsilon_p=0.005$psi) via LabView (V12.0.1, National Instruments, Austin TX, USA) were employed to drive the non-wetting phase (fluid A) via applying pressure head while the wetting phase (fluid B) was simply delivered to a well at the device inlet and spontaneously filled the channels before fluid A was introduced to the device to form an interface. The devices used in this work were all well-based devices which eliminated the need for bulky syringe pumps.

4.2.7 Imaging and Image processing

Bright field microscopy was used to visualize the two phase interface in the microchips. An inverted microscope (Eclipse TE-2000-S, Nikon, Japan) was used for visualization and imaging of the experiments. Images and videos were captured using a CCD camera (QICam Fast 1394, QImaging, Surrey, BC, Canada) installed on the microscope.

In order to capture the moment when breakup of the bubble through the micro-slit occurs, a real-time image processing code (MATLAB, Mathworks, MA, USA) was developed to monitor the movement of the gas-liquid interface and detect the moment the two-phase interface moves out of the micro-slit. Utilizing the developed automated strategy allowed us to determine the onset of interface break up through the micro-slit and capture the value of the corresponding pressure (in LabVIEW) as the critical pressure for determining the interfacial tension.
4.3 Results and discussion

In order to verify accuracy of our devised interfacial measurement technique we validated our technique against one of the most common and commercially available interfacial measurement techniques, the pending drop method. To validate the measurement technique for a wide range of interfacial tension values (from 22 to 72 mN/m) we prepared solutions of ethanol in water with different concentrations of ethanol ($\varphi_e$) and measured the interfacial tension of the solutions with air using our devised in-situ technique as well as a commercial hanging drop measurement system (DSA 100, Kruss, Hamburg, Germany).

Each measurement taken by our tensiometer took on average a minute or less until the interface was pushed through the micro-slit and the resulting bubble or drop was subsequently flushed towards the outlet by the incoming flow from the upstream channel. This often entailed a reduction in the pressure of the non-wetting phase (or Phase A) to allow for the incoming liquid stream (or Phase B) clear the T-junction of disperse drops or bubbles and prepare the tensiometer for the next measurement (i.e. completely filled with the wetting phase).

Measurements using the hanging drop technique however required a longer time for preparation and acquisition of acceptable measurements. Using the commercial hanging drop system, the liquid (or one of the liquids) involved in the two-phase interface of interest needed to be loaded in a the syringe was then secured inside a moving mechanical part which lowered the syringe to the appropriate position where a needle entered a reservoir filled with the second phase (air or a second immiscible liquid). Once the needle was inside the second phase droplets were formed at the tip of the needle until break up occurred while the system recorded images of the drop shape just before the breakup. The captured images were then analyzed by the proprietary software and an interfacial tension measurement was returned by the software. The entire process to take a measurement was found to require nearly 8 to 10 minutes for each data point once the system is setup which underlines the time advantage of using the microfluidic technique.

Figure 5 shows the interfacial tension measurements acquired using our microfluidic device (filled dots) plotted along with the measurements using the commercial hanging drop tensiometer (squares). As shown in the figure there was only a slight discrepancy of below three percent between the measurements taken by our tensiometer and those taken using the hanging drop
system. As shown in Fig. 5a the measured interfacial tension of pure deionized (DI) water and ethanol with air agree well with the room temperature values reported in the literature 54.

In order to further determine utility of our tensiometer and the range of different fluid-fluid interfaces it is capable of measuring we performed measurements for different sets of fluid-fluid interfaces as shown in Fig. 5b. The highest interfacial tension measured using our tensiometer was for the deionized (DI) water and air interface at 72mN/m and the lowest measurement was 6.2mN/m for the interface between DI water and mineral oil with span80 at a concentration above critical micelle concentration (CMC).
Fig. 5 (a) Interfacial tension measurements for solutions of varying concentration of ethyl alcohol in water ($\varphi_e$) with air measured using our tensiometer (dot) and a commercial hanging drop measurement system (square). Scale bar is 250µm (b) Interfacial tension values for five common pairs of immiscible fluids measured using our technique. Each pair represents a common two-phase system encountered in microfluidic applications (i.e. aqueous-gas, organic solvent-gas, oil-gas, oil-aqueous, oil+surfactant-water) demonstrating the utility and versatility of the microfluidic tensiometer. All measurements were performed at room temperature.
As a case study to demonstrate the utility of our technique in the context of integrated microfluidic systems we implemented our tensiometry technique downstream of a sampling device where two streams of ethanol and water-ethanol solution (φ_e=10%) enter the device from two different inlets using a pair of on-off on-chip valves\textsuperscript{49} and the interfacial tension of each of these liquids with air was then measured in real time downstream of the device as the liquids were leaving the microfluidic device. Our inline measurement module was fitted with an independent outlet to ensure atmospheric pressure on the outlet side of the micro-slit and to provide a rapid exist for the bubbles resulting from the measurement to avoid interference with the sampling experiment taking place upstream. Figure 6a shows the rendered device design and bright field micrographs for the tensiometer. Figure 6b shows the periodic measurements conducted in-situ using the tensiometer. After each measurement the solutions were altered and the device was flushed with the next solution before a measurement was performed again to ensure the device was free of any residuals.
Fig. 6 In-situ real-time measurement of interfacial tension downstream of a sampling device where the working liquids were switched periodically between ethanol, a solution of water and 10 percent ethanol ($\phi_e=10\%$). (a) Device design and a bright field micrograph showing the tensiometer in operation (b) Values of interfacial tension with air for the alternating working solutions with time. Each new measurement was performed following one minute of flushing to clear device of any residues. Scale bar is 500 micrometers. All measurements were performed at room temperature.
4.4 Conclusion

We presented a novel technique for in-situ interfacial tensiometry in integrated microfluidic systems. Our technique relies on computer controlled measurement of the breakup pressure for a two-phase interface through a micro-slit. We investigated the technique theoretically and determined the sensitivity of interfacial measurements to the parameters involved: measured breakup pressure, micro-slit geometry (height and width). The resolution of pressure measurements were 0.005psi (~34pa) which resulted in a measurement error of 0.2 mN/m. The measurement sensitivity with regard to the micro-slit dimensions was found to be dependent on the aspect ratio of the micro-slit. At high aspect ratios where either the height or the width was much greater than the other, tensiometry measurements were most sensitive to variations in the smaller dimension. We validated the conceptual basis for our technique numerically using a volume of fluid (VOF) ANSYS model. Experimental validation was performed by comparing the interfacial tension measurements done using our tensiometer and a commercial hanging drop system. The measurement results showed strong agreements with discrepancies below three percent for a wide range of interfacial tensions measured from 23mN/m to 72mN/m. We demonstrated that our tensiometer can be successfully employed to measure interfacial tension for the most common interfaces encountered in microfluidic applications: water-air, organic solvent-air, oil-air, oil-water and oil plus surfactant-water. We showcased the utility of our technique for real-time in-situ measurements in integrated microfluidic systems by performing measurements downstream of a sampling device as the working liquid was varied periodically with time every two minutes.
4.5 References


Chapter 5: In-Vitro Model of the Alveolar Epithelium Barrier

Summary

We developed a microfluidic platform that allows routine culture and systematic investigation of lung epithelial cells in proximity of a gas-liquid interface. Our approach enables achieving and maintenance of physiological conditions including gas composition, periodic applied strain on the cultured cells with physiological magnitudes and frequencies. Additionally, our proposed platform is scalable and is made using standard microfabrication procedures. Pulmonary epithelial cells are cultured on top of an array of microscale holes with hole diameters on the order of human alveolar diameter (i.e. 50-100 micrometers). We have developed the platform and the biological methods needed to investigate formation of an intact cell layer supported by a gas-liquid interface. We expect that a confluent cell layer will be formed in proximity of the gas-liquid interface that can be mechanically manipulated by changing the pressure difference across the gas-liquid interface. If successful in forming a confluent epithelial cell layer, the future work will involve culture of a layer of endothelial cells on top of the epithelial cells and perfusion of culture medium at flow rates corresponding to physiological shear levels in order to recapitulate physiological features of the alveolus-capillary barrier.

5.1 Introduction

Drug development and screening is hindered by the poor prediction ability of preclinical animal models that often are not accurate enough surrogates to predict the effect of drug compounds on humans and as a result lead to failure of drugs in late stages of their development when human clinical trials are conducted\textsuperscript{1-6}. Considering the high cost and lengthy timeline of developing new drugs\textsuperscript{7-8}, there is a need for new technologies and models that can efficiently and accurately predict drug safety and efficacy in humans during preclinical studies\textsuperscript{2-5}. In addition to drug development, identification of new environmental toxins has also been hampered by the inefficiency of existing animal models\textsuperscript{4} and further points out a need for new solutions. *In vitro* cell culture models have been employed as successful surrogates to replace animal studies and provide reliable predictions for clinical trials. Biological barriers such as the intestine for
instance, have been modeled by well characterized in vitro surrogates such as Caco-2 intestine cell line which has been used as a standard model for investigating oral bioavailability of new drug compounds\textsuperscript{9,10}.

Not all biological barriers, however, have such well characterized in vitro surrogates. The distal lung in particular is challenging in regards to developing cell culture models\textsuperscript{10}. The lack of a well characterized in vitro model of human alveolar epithelium presents a serious limitation in epithelial transport studies and inhalation biopharmaceutics as systemic drug absorption predominantly occurs by trans-epithelial transport in the alveoli\textsuperscript{9,10}. One reason the development of well characterized epithelial barrier models of the distal lung has been hampered is the lack of reliable, cell cultures that form functional tight junctions\textsuperscript{9-12}. The NCI-H441 cell line has been shown to form tight junctions and been presented as a viable model for alveolar epithelial barrier\textsuperscript{9}. H441 cells were originally isolated from the pericardial fluid of a patient with papillary adenocarcinoma of the lung\textsuperscript{10} and has been shown to form electrically tight, polarized cell monolayers and express a number of important membrane transporters very similarly to primary culture of human alveolar epithelial cells\textsuperscript{9,13}.

An additional challenge and a crucial requirement for modeling alveolar epithelium in vitro is the ability to take into account the dynamic mechanical forces involved in physiological function of the alveoli. The consideration of mechanical forces is critical as in the past two decades it has become evident that most cells throughout the body sense their mechanical environment and respond to mechanical changes or stimuli\textsuperscript{14-17}. Mechanical forces play an important role in the development, homeostasis and repair of tissues. This is predominantly due to the mechanosensitivity of many biological remodeling processes at the cellular level such as proliferation, migration, differentiation, apoptosis and extracellular matrix synthesis\textsuperscript{18,19}. The lung in particular is a mechanically dynamic organ, and pulmonary cells are subjected to several complex physical forces including breathing forces, forces resulting from pulmonary blood flow, and surface tension forces in the pulmonary epithelium\textsuperscript{20-23}. Interfacial tension at the air-liquid interface affects compliance of the lung and resistance of small airways\textsuperscript{21,22,24}. Mechanical forces play an important role in regulating the structure, function, and metabolism of the lung and abnormal mechanical forces can lead to many pathological situations and physical force-
related disorders such as: pulmonary hypoplasia, barotrauma, pulmonary hypertension, asthma, and chronic obstructive pulmonary diseases\textsuperscript{21, 25}.

Although progress has been made in the development of alveolar epithelial cell culture models\textsuperscript{4, 10, 26-28}, cultured monolayers often fail to differentiate and express lung-specific functions. Improved tissue organization have been achieved by growing cells in three-dimensional (3D) cultures\textsuperscript{29-33}, however, these methods still fail to reproduce physical, structural and mechanical features of the alveolar epithelium barrier.

The disadvantages of existing \textit{in vitro} models has led new efforts to adopt engineering tools and techniques including microfabrication techniques originally developed in microelectronics industry to create platforms and engineered microenvironments that combined with appropriate cell culture models offer superior physiological accuracy as \textit{in vitro} models\textsuperscript{2-6}. For instance in recent years, a significant body of work has been devoted to cell culture in microfluidic devices\textsuperscript{34-48}. These microfluidic studies have demonstrated that cell viability can be achieved successfully in microfluidic culture platforms, and with appropriate protein coatings, culture medium and flow conditions cultured human cells have been shown to express tissue-specific differentiated functions in these platforms\textsuperscript{49-51}.

In recent years, microfluidic technologies have been exploited to develop microenvironments for pulmonary cell culture and to recapitulate lung function, including the alveolar epithelial barrier, \textit{in-vitro}\textsuperscript{3-5, 52-54}. These efforts have focused on studying various respiratory disorders\textsuperscript{54} or effects of environmental toxins\textsuperscript{3, 4} by implementing various pulmonary cells in a microfluidic platform. A more detailed review of some of the prominent works is brought in the appendix Table A2. Huh et al\textsuperscript{4} introduced a unique membrane based microfluidic platform to mimic the alveolar epithelium barrier. The microfluidic platform consisted of a porous membrane sandwiched between two vacuum chambers on the sides and two liquid channels on the top and the bottom. Epithelial and endothelial cells were cultured on two sides of the membrane with culture medium fed through top and bottom channels while the vacuum channels on the side applied periodic tension in the synthetic membrane and in turn dynamically exposed the cells to uniaxial strain. Additionally, the microfluidic model involved an epithelial cell layer that was submerged in
culture medium. This is while epithelial cells *in vivo* are in direct contact with a gas-liquid interface and are subjected to radial expansion in a spherical alveolar structure.

In this work we describe cell culture platform that allows culture of H441 epithelial cells in direct contact of an air-liquid interface and also enables precisely controlled manipulation of the cell layer through expansion of the gas-liquid interface. We expect the combination of our adopted cell culture model and our developed ability to accurately manipulate air-liquid interfaces to overcome some of the existing shortcomings of current microfluidic models for alveolar epithelium.

5.1.1 Technological Hypothesis

Our controlled gas-liquid interface culture platform is capable of recapitulating, in an accurate and dynamic manner, the alveolar epithelial barrier, including periodic applied stress and strain and gas composition. The proposed platform consists of a supporting structure containing micro-holes (e.g. a capillary plate) on the order of alveoli diameter that allow H441 pulmonary epithelial cell line to grow on a side that is immersed in culture medium while gas is maintained on the opposite side forming a gas liquid interface in proximity of the cell layer (see Fig. 1). The gas-liquid interface exposes the cell layer to physiological levels of mechanical stress and strain and as a result, creates a microenvironment that is a better representative of the physiological conditions compared with state of the art laboratory procedures for pulmonary cell culture that rely on stretched polymer membranes for modeling the alveolar epithelial barrier.

5.1.2 Biological Hypothesis

NCI-H441 epithelial cells attach to the solid surface surrounding the microfabricated array of holes and proliferate until they form a tight cell layer on top of the micro-holes verified by ZO-1 immunostaining. The gas liquid interface interacts with the cells and causes the cells to respond to the mechanical stimuli in a measurable manner.

5.2 Materials and Cells

H441 cell line, human lung adenocarcinoma epithelial cell line, were used in our experiments as the cell lines has been shown to be the only cell line capable of forming tight junctions needed
in our alveolar epithelial barrier model. Culture medium (RPMI 1640, ATCC, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Sigma Aldrich, Oakville, ON, Canada) and 1% Penicillin-Streptomycin (Life Technologies, Burlington, ON, Canada) was used to culture H441 cells. Deionized water was used wherever water was needed in experiments. Air was used as the gas phase in all experiments. Phosphate-Buffered Saline (PBS) (7.4 (1X), Life Technologies, Burlington, ON, Canada) was used to rinse the cells and to dilute solutions where needed. Trypsin-EDTA (0.25% Trypsin with EDTA 4Na, Life Technologies, Burlington, ON, Canada) was used in passaging H441 cells. Fibronectin Bovine Protein (Life Technologies, Burlington, ON, Canada) was used to cover glass surfaces prior to culture.

Alexa fluor conjugated ZO-1 antibody (ZO1-1A12, Life Technologies, Burlington, ON, Canada) was used to evaluate tight junction formation in H441 cell layers. A 4% solution of formaldehyde (36.5-38% in H2O, Sigma Aldrich, Oakville, ON, Canada) in PBS was used to fix the cells for immunostaining. Solution of 0.1% Triton X-100 in PBS was used for membrane permeabilization. A 5% solution of Skim milk in PBS was used as the blocking solution for immunostaining. Octadecyltrichlorosilane (Sigma Aldrich, Oakville, ON, Canada) was vapor-deposited on glass capillary plates to render them hydrophobic for air-liquid cell culture experiments.

5.3 Air-Liquid Interface Cell Culture

The concept of developing a platform with precisely controlled air-liquid interface as a cell culture model for alveoli epithelial layer stemmed from our work on controlled interaction of gas-liquid interfaces with micro-pillars. Figure 1a shows evolution of a gas liquid interface inside a micro-slit sandwiched by micro-pillars. As shown in the figure with increased capillary pressure the interface advances in the slit. The initial objective was to confirm whether a confluent cell layer can be formed and supported on top of a gas-liquid interface between micro-pillars with micro-sized gaps as shown in Fig. 1b. The initial assumption was that the epithelial form a confluent layer above the gaps according to the illustration depicted in Fig. 1b.
Fig. 1 (a) Bright field images of the gas liquid interface evolution with increased capillary pressure. $\Delta P_{\text{Pillar}}$ is a characteristic pressure at which the gas-liquid interface breaks and gas enters the liquid channel. (b) Schematic illustration of the cell culture at the gas liquid interface showing two different scenarios that may occur if lung epithelial cells form a confluent layer on top of the micropillars or micro-holes.

5.4 Air-Liquid Culture Platform Designs

Two sets of designs, pillar-based and capillary plate-based were developed for the air-liquid culture platform. These designs are presented in the following sections in detail.

5.4.1 Pillar-based Design

Based on initial assumptions illustrated in Fig. 1, an initial design was made that was based on an array of equidistant micropillars with gaps of 20-50µm as shown in Fig. 2. Gas is introduced at the gas inlet while the culture medium is delivered to the device at the liquid inlet. It was assumed that a gas-liquid interface will be formed at the location of micropillars and cells will be able to rest on the interface, proliferate, and form a confluent layer similar to the schematic illustration in Fig. 1b.
However the design was found unsuitable for supporting and manipulating a cell layer as the interface in its static position was not aligned with the top surface of the micro-pillars and would not provide the required support for the cells when they are seeded as it is desirable and expected that the cell layer will rest on top of the interface without breaking through the interface which can cause serious damage to the cells\textsuperscript{56-58}. It is also important that the gas liquid interface is capable of stimulating the cell layer mechanically by applying physiological levels of stress and strain on the cells. The pillar-based design in Fig. 2 does not meet this requirement as the interface is confined by the surrounding micro-pillars and cannot exert the required levels of strain on the cells.

An additional problem faced with this design was the device orientation and issues related to optical access and imaging. To seed the cells on the micro-pillar array and form a tight layer the soft lithography fabricated device would needed to be rotated 90 degrees to allow the cells to settle on the pillar array plane. This configuration however would not allow optical access and imaging with conventional microscopy techniques.
Fig. 2 Design for a pillar-based device for cell culture at the gas-liquid interface. (a) A gas-liquid interface will be formed at the location of micropillars while the liquid can continue to flow in the liquid channel and exit the device at the outlet (b) Gas and liquid are introduced through gas and liquid inlets respectively. The scale bar is 1mm.

5.4.2 Capillary Plate Design

To tackle the problems associated with the initial pillar based design a capillary plate or disc format was adopted. In this design the supporting structure for the cell layer is a plate (or disc) with hundreds of through-holes (or capillaries) that span from the top to the bottom surface of the plate as shown in Fig. 3a. The plates used in this study were commercially available (Collimated Holes, Campbell, CA) glass plates with a 50 percent open area (i.e. half of the plate’s surface
area was covered with capillaries) and various capillary diameters from 5µm to 50µm. Figure 3b shows the photograph for one such capillary plate with a thickness of 500µm and capillaries of 50µm in diameter. The capillaries act as the pillar gaps in the pillar-based design meaning the cell layer is expected to form on the flat surface of the capillary plate with culture medium on top surface of the plate and gas on the other side.

Fig. 3 (a) Rendered 3D illustration of a round capillary plate 25mm in diameter with 50µm capillaries spanning over the plate thickness of 1mm from the top surface to bottom surface. (2) Photograph of a square glass capillary plate, 25mm wide, with a thickness of 0.5mm covered with capillaries of 50µm diameter.
In order to inspect the surface quality and capillary shape of the procured capillary plates we performed scanning electron microscopy (SEM) and confocal microscopy of the capillary plates. Figure 4a and b show the SEM images of a capillary plate with 25µm capillaries. As shown by the SEM images the capillaries are approximately circular in cross section and the unetched areas surrounding the capillaries possess a smooth surface with minor imperfections. These observations were also confirmed by performing confocal microscopy of the plate. A solution of rhodamine in ethanol was applied to the surface before confocal images were captured including a z-scan as shown in Figures 4c and d. The smooth surface area engulfing capillaries shown in Fig. 4 will be the anchoring point for the cells to hold on to as they proliferate and cover the surface of the plate including the gas filled capillaries.

Fig. 4 (a) and (b) SEM images of the surface of the capillary plate showing the smooth surface of the plate where the pulmonary cells are expected to adhere. (c) and (d) Confocal
microscopy images for the same capillary plate captured with rhodamine applied to the surface for imaging.

As the capillary plates used in this study were glass made they were hydrophilic and any aqueous solution applied at the surface of the plate would instantly wet the surface of the plate and fill the capillaries with liquid. We devised two solutions to this problem to ensure capillaries will not be flooded with liquid and that a gas-liquid interface will be present: 1) by applying a precisely controlled gas pressure to keep the liquid out of the capillary and maintain the interface at a steady location and 2) by selectively modifying the surface properties of the capillary plate so that the liquid is stopped at a predefined location where a gas-liquid interface is formed.

Figure 5 shows three different capillary surface properties: wetting, partially wetting and non-wetting. The schematic illustration in Fig. 5a corresponds to culture medium on a wetting glass capillary with a contact angle of less than 20 degrees. As shown in the figure the liquid wets the capillary as a result to maintain a gas-liquid interface in this case the bottom surface of the plate must be pressurized with precisely controlled air pressure.
Fig. 5 Schematic illustrations showing the cross section of the capillary plate with culture medium on top for three different capillary surface conditions (a) A wetting capillary surface with a contact angle below 20 degrees. This condition applies to clean native glass capillaries. (b) Partial wetting capillary surface with a contact angle ranging between 60 and 90 degrees. This condition may apply to contaminated glass capillaries or mild surface modifications with a hydrophobic coating. (c) Non-wetting or hydrophobic capillary surface with a contact angle of higher than 90 degrees that ensures the interface is pinned at the top of the capillary.

Same applies to the partial wetting capillary shown in Figure 5b. A partial wetting capillary surface would be wetted by the liquid although at a larger contact angle. As a result to maintain an interface in Fig. 5b active pressure control would be required.
To develop an air-liquid culture platform the static position of the interface must be well defined and uniform across the different capillaries. However, even with active pressure control, maintenance of a uniform static position for the gas-liquid interface across the capillary plate is challenging when the plate is wetting or partially wetting.

However, if all surfaces of the capillary are rendered hydrophobic while maintaining the top surface (where cells are cultured) hydrophilic, the gas-liquid interface will be formed and supported by surface tension forces without the need for pressurization at the bottom surface. The schematic illustration in Fig. 5c shows a gas-liquid interface pinned on top of one such hydrophobic capillary with a contact angle higher than 90 degrees. Selective surface modification will ensure a uniform location of the gas liquid interface (i.e. at the top of the capillary) across entire surface area of the plate. For these reasons we adopted selective surface modification of the capillary plate for our air-liquid culture platform. The experimental process for selective surface modification is discussed in the next section.

5.5 Experimental

5.5.1 Surface modification and ECM coating

To modify surface of the glass capillary plates we adopted surface treatment using organosilanes a group of chemicals widely used in fabrication of microfluidic devices and microelectromechanical systems (MEMS)\(^{59,60}\). We chose Octadecyltrichlorosilane (OTS) which has been extensively used to produce hydrophobic surfaces via liquid or vapor deposition\(^{59-64}\). Figure 6 describes the method used and the results obtain by selectively depositing vapor-phase OTS on capillary plates.
Fig. 6 Selective surface modification of capillary plate (a) Schematic illustration showing experimental setup for selective vapor deposition of Octadecyltrichlorosilane (OTS) on the surface of a capillary plate cross section of the capillary plate. Culture surface of the plate was protected from deposition of OTS by sealing capillary plate with parafilm and a microscope glass slide. Surface to be treated was held 5mm away from a drop of OTS in presence of ~ -30 inHg vacuum overnight. (b) Photograph of a drop of DI water on the protected surface of the capillary plate showing a contact angle of 37° following surface modification. (c) Photograph showing a contact angle of 123° for a drop of water on the treated surface of the capillary plate following surface modification with OTS.

To treat the capillary surface with OTS vapor deposition at room temperature was adopted. The treatment process used is as follows:
1) Capillary plate was exposed to oxygen plasma to oxidize the glass surface and create hydroxyl groups on the surface that silane molecules can react with and attach to the surface resulting in surface silanization.

2) One surface of the plate was sealed to protect it from silane and remain hydrophilic for cell culture. Surface protection was achieved by attaching the surface to a glass microscope slide and sealing the edges using parafilm and adhesive tape.

3) The capillary plate was held at a distance of 5mm from a second capillary plate where a drop of OTS was placed to evaporate as shown in Fig. 4a. Evaporation was done under a vacuum of nearly -30 inHg inside a vacuum chamber. The capillary plate was left in vacuum overnight (8-12 hours).

Figure 6b and 6c show a capillary plate after surface modification. As shown by the photographs the protected surface remained hydrophilic with a contact angle of nearly 37° while the unprotected surface demonstrated a contact angle of more than 120°. As shown in the figure surface treatment resulted in a drop of water remaining on the surface of the capillary plate without penetrating the capillaries while prior to surface modification a drop of water entered the capillary plate completely and no sessile drop was formed on the surface.

Following surface treatment, the hydrophilic surface of the capillary plate was coated with fluorescently labeled Fibronectin (HiLyte Fluor™ 488 labeled, Cytoskeleton, Denver, CO, USA) at 5µg/cm² by covering the surface with the solution and allowing it to dry at room temperature. The coated surface of capillary plate is shown in Fig. 7. The boundary between coated and uncoated regions can be seen in the fluorescent images confirming that the green fluorescent hue is from the Fibronectin coating and that a uniform layer of Fibronectin is present on the surface.
Fig. 7 Confocal microscope images of Fibronectin on a surface modified capillary plate (a) and (b) Micrographs captured at 20× and 40× magnification showing a uniform layer of fibronectin on top of the capillary plate. (c) and (d) Micrographs taken at 20× and 40× magnification showing the boundary of the coated and uncoated areas of the plate confirming that the green fluorescent color originated from the fibronectin coating. Capillary diameter is 25µm.
Following Fibronectin coating of the capillary plates we tested the treated and coated capillary plate to ensure the modification and coating processes did not alter functionality of the capillaries in terms of forming an air-liquid interface at the top of capillaries. To do this labeled culture medium was dispensed on the Fibronectin-coated surface of the capillary plate and the interface was imaged using an upright confocal microscope. The confocal micrographs of the air-liquid interface are shown in Fig. 8. The 3D reconstruction in Fig. 8a demonstrates presence of bubble caps at the surface of capillary plate as shown by the dark dome-shaped structures present at the top surface of the capillary plate.

Fig. 8 Confocal microscope images of gas liquid interface formed on on the capillary plate modified and coated with Fibronectin. (a) Z-scan 3D reconstruction of the capillary plate close to the surface indicating gas bubbles inside the capillaries. (b) Brightfield image shows the optical fringe of bubble caps confirming presence of a gas-liquid interface at the upper surface of the capillary plate.
Presence of a gas liquid interface at the upper surface of the capillary plate is further shown by the bright field micrographs in Fig. 8b where optical fringes of the bubble caps prove their presence at the top surface where imaging was conducted. Observations in Fig. 8 demonstrated functionality of the treated and coated capillary plate in forming a gas liquid interface at the top surface of capillary plate where H441 cells can hypothetically form an intact layer.

5.5.2 Integrated capillary plate device designs

Following successful treatment and testing of the capillary plates it was necessary to develop platforms that can host the capillary plates and form a standalone air-liquid culture platform complete with a controlled gas inlet and a liquid well for culture medium. The capillary plate design does not require a classical microfluidic device in the sense that it does not involve a complex network of channels or on-chip valves and pumps. Figure 9 shows a device assembly design we developed for the capillary plate integrated device. The integrate culture device will be loaded with culture media and cells using a pipette similar to commonly used cell and tissue culture well plates. However, as discussed before for the cell culture platform to be capable of cell layer manipulation by means of a controlled gas-liquid interface a sealed supporting platform with a gas inlet is required to host the capillary plate. As a result, we developed a simple multilayer platform with a gas inlet delivering controlled gas pressure to a gas cavity below the capillary plate by means of a straight microchannel. The capillary plate is mounted on top of the gas cavity with the bottom face of the capillary plate exposed to the computer-controlled gas pressure inside. Figure 9 shows the detailed 3D design of assembled culture platform including the multilayer supporting device with the capillary plate mounted and sealed onto it. The reservoir (i.e. well) engulfing the top face of the capillary plate is open and culture media and cells can be supplied to the reservoir through this opening.
Fig. 9 Three-dimensional rendered images of single-well multilayer air-liquid culture platform consisting of supporting platform and capillary plate assembled together. (a) Computer controlled gas pressure with a pressure resolution of 0.005 psi is applied at the device inlet while the culture medium and the cells will be introduced to the device via the culture well (b) The capillary plate is mounted on top of a gas cavity with the bottom surface of the capillary plate exposed to the computer-controlled gas pressure inside the cavity to allow mechanical manipulation of the cells once a confluent layer of cells is formed.

As an alternative design it is also possible to modify the single-well design so that it can accommodate multiple independent culture wells which will allow multiple parallel experiments with isolated cell populations to be performed on the same device and as a result, scaling up the
device utility. 3D rendered images of one such modified design are shown in Fig. 10. For the purpose of this work however we only focus on the single-well design shown in Fig. 9. The supporting platforms shown in Figures 9 and 10 can be made in PDMS using the common photolithography and soft lithography techniques.

Fig. 10 (a) 3D rendered image for multi-well air-liquid culture platform showing the supporting platform and the capillary plate assembled to form an integrated device. (b) Modified multi-well culture platform allows multiple parallel experiments with isolated cell populations to be performed on the same device.
However PDMS is not an ideal candidate for some biological applications due to its high gas permeability, especially if it is desired that the cells are exposed to a specific gas composition. To tackle the permeability problem the devices can be made in thermoplastics such as Poly(methyl methacrylate) (PMMA) using fabrication techniques such as hot embossing or reactive ion etching (RIE). Thermoplastics are an attractive option for biomedical devices as they are biocompatible and disposable due to their low price. As a substrate material for microdevices, thermoplastics are desirable due to their transparency, wide range of mechanical and chemical properties, impermeability to gases and their compatibility with replica-based micro-fabrication techniques. In this work, however, we used PDMS made supporting platform for our air-liquid culture device. Figure 11 shows a PDMS made air liquid culture platform with integrated capillary plate.
Fig. 11 (a) Photograph of a multilayer supporting platform made in PDMS for integrated capillary plate device (b) Photograph of a circular capillary plate with 25µm holes assembled and sealed on top of the PDMS platform. (c) Photograph of the integrated capillary plate culture platform with the reservoir filled with water. The top PDMS layer provides a reservoir on top of the capillary plate and will contain the culture medium. (d) Close up of the device filled with water. Scale bar in (a) 25 mm.

5.5.3 Cell culture protocol for capillary plate culture platform

As discussed before the advantage of employing a capillary plate with a hydrophobic bottom surface and capillary walls is the spontaneous formation and stabilization of the gas liquid interface upon contact with the culture medium without the need for extensive gas pressure control and visualization to adjust the gas-liquid interface. The hydrophobic walls of the micro-holes prevent the liquid culture medium to flow down the holes due to capillary forces even in the absence of gas pressure control. This spontaneous equilibrium of the interface simplifies cell
culture using the capillary plate platform to levels similar to a simple culture well plate. However gas pressure control is needed to mechanically manipulate the cell layer in experiments where mechanical stresses need to be taken into account.

The experimental protocol for cell culture using the capillary plate platform is described below.

1. The device is sterilized using by UV radiation in a biosafety cabinet.
2. The capillary plate is coated with an ECM protein such as Fibronectin as described before.
3. Cells will be introduced into the reservoir(s) on top of the capillary plate using a pipette or syringe.
4. The device will be maintained at static conditions for 3-4 hours to allow the cells to settle and adhere onto the plate and the interface.
5. Culture medium can be partially changed over time but replacing the entire liquid volume is not possible as it will disrupt the gas-liquid interface.
6. Visualization of the cells is possible using an upright fluorescent or bright field microscope.

5.5.4 Alveolar epithelium model validation

In order to validate our air-liquid culture platform as a viable model for alveolar epithelial barrier, strong evidence of formation of a tight cell layer on top of the capillary plate and in presence of the air-liquid interface was required. Trans epithelial electrical resistance (TEER) measurement and immunostaining for junctional proteins such as Occludin and ZO-1 have been previously used to investigate tightness of pulmonary epithelial cell layers. Therefore, we elected to use ZO-1 immunostaining as a measure for tightness of our cultured cell layers. To validate our model two sets of cultures would take place 1) NCI-H441 culture atop of glass coverslips 2) NCI-H441 culture on top of the capillary plate in the static condition with no interface movement using our devised air-liquid culture platform. Cell layer tightness will be
verified by comparing ZO-1 distribution for the cell layer cultured using our platform with the cell layer cultured on top of a coverslip.

NCI-H441 cells were seeded on top of cover slips and cultured in six well plates for a week before ZO-1 immunostaining was performed. Figure 12 shows the bright field micrographs for the cells cultured on coverslips at day one, three and seven. ZO-1 immunostaining was done at full confluence according to the following recipe:

1) Cells were fixed using a 4% solution of formaldehyde in PBS for 15 minutes
2) Cells were permeabлизed using a 0.1% solution of Triton X-100 in PBS for 5 to 10 minutes.
3) Cells were blocked using a 5% solution of nonfat milk in PBS for an hour
4) Cells were incubated with the ZO-1 antibody for an hour before being rinsed three times and imaged.

![Fig. 12 Bright field micrographs showing H441 cells in culture. (a) day 1 post culture (b) day 3 post culture and (c) at 100% confluence. NCI-H441 cells were cultured on glass microscope coverslips.](image)

Figures 13 (a) to (d) show ZO-1 distribution for NCI-H441 cells cultured on coverslips at day 7 of the culture. In Fig. 13, (a) and (b) belong to the same culture at different locations and (c) and (d) belong to a different culture at different locations within that culture. Figure 13e and 13f show H441 culture inside a regular six well plate. As shown in the figure, ZO-1 distributions
suggest presence of a tight epithelial cell layer. As seen in Fig. 13, there are sporadic areas with lower fluorescent intensity that appear darker. This effect is caused by the variable height of the cell layer meaning the dark spots are of a different height and as a result are slightly out of focus and do not appear as bright as the parts that are in focus.
Fig. 13 Fluorescent micrographs showing distribution of junctional protein ZO-1 following immunostaining NCI-H441 cells at day 7 in culture. (a)-(d) Cells were cultured on glass microscope coverslips (e) and (f) cells were cultured in 6 well plate.

We proceeded to cell culture atop capillary plates in presence of a gas-liquid interface, following our successful culture and immunostaining of H441 cells in control experiments. Very high seeding density was adopted to provide a sufficient number of cells for formation of a tight cell layer adjacent to the gas liquid interface. Confluent day-7 H441 cells in a standard T75 culture flask were detached and a 500µl solution containing one sixth of the total cell population was delivered to the top surface of the capillary plates. The surface area of the seeded portion of the capillary plates was nearly 1.75 cm² (i.e. a circular area with a diameter of 1.5cm). The seeded capillary plate and the PDMS-made supporting platform were fitted inside a well within a standard 6-well plate and were incubated for 3 to 4 hours to allow cell attachment before the well was filled with culture medium. Culture medium was changed daily during the course of the experiment. The air cavity beneath the capillary plate was optically accessibly allowing us to monitor the bottom surface of the capillary plate to ensure no liquid has penetrated through the capillaries and therefore, a gas-liquid interface is present at the top surface in proximity of the cells. Visual access to the top surface was not easily achievable as the capillary plate was opaque and an upright microscope was needed for visualization of the cells while in culture. Three experiments with three different capillary plates were performed. In one experiment the top surface of the capillary plate was left uncoated with cells coming in contact with the native glass surface while in the other two experiments the surface was coated with bovine fibronectin as described earlier.

Following a culture period of 4 to 7 days, immunostaining for ZO-1 protein in conjunction with a Hoechst DNA stain were conducted to visualize the cells and investigate cell growth and cell layer formation on the top surface of the capillary plates. Figure 14 shows the double stain results for a capillary plate with uncoated glass surface. As shown in the fluorescent micrographs the cells show a strong preference for deposition at the locations of capillaries. In order to further investigate and understand the strong preference of the cells for the gas-liquid interface as compared with the glass surface we repeated the experiment this time with the glass surface
coated with a later of bovine fibronectin. The immunostaining results are shown in figures 15 and 16.

Fig. 14 Fluorescent micrographs for N=1 experiment showing distribution of junctional protein ZO-1 and the cell nuclei following double stain of NCI-H441 cells at day 7 in culture atop an uncoated capillary plate surface. (a)-(d) cells in various locations within the seeded area on top of the capillary plate. Cells appear to have a preference for the
capillaries and avoid attachment to the flat glass surface in between the capillaries. Scale bar 50µm.

In micrographs in Figure 15a to 15d the strong preference for the capillaries is repeated this time with sporadic single cells observed on the glass surface. In Figure 15e and 15f cells appear to be attached to the glass surface more and not follow the general trend. It is noteworthy that such areas were rare within the capillary plate and the vast majority of the cells were deposited at capillary locations.

The third culture experiment the results of which are shown in Figures 16 and 17 further confirmed the previously observed trend. As shown in Figures 16a to 16d the majority of cells accumulate at the capillary location while there are sporadic areas within the capillary plate where cells are covering both the glass surface and the capillary entrance. An example of such areas can be seen in Figure 16a or in the extended view shown in Figure 17 where cells appear to be covering both the solid surface and the gas-liquid interface.

A gas-liquid interface offers a more physiologically relevant microenvironment for pulmonary epithelial cells and as a result, we attribute the trend observed in our air-liquid culture experiments to the natural preference of H441 pulmonary epithelial cells to be in the vicinity of a gas-liquid interface in comparison to a solid glass surface. However, further investigation and isolation of parameters involved are needed to pin-point the underlying reasons for such apparent preference of H441 cells for deposition at the gas-liquid interface.
Fig. 15 Fluorescent micrographs for N=2 experiment showing distribution of junctional protein ZO-1 and the cell nuclei following double stain of NCI-H441 cells at day 4 in culture atop a bovine fibronectin-coated capillary plate surface. (a)-(d) cells in various locations within the seeded area on top of the capillary plate. Similar to results in Fig. 14.
cells appear to have a preference for the capillaries and avoid attachment to the flat glass surface in between the capillaries. (e) and (f) In few locations on the capillary plate surface, cells could be seen attached to the glass surface rather than crowding at the capillaries. Scale bar 50µm.

Fig. 16 Fluorescent micrographs for N=3 experiment showing distribution of junctional protein ZO-1 and the cell nuclei following double stain of NCI-H441 cells at day 4 in culture atop a bovine fibronectin-coated capillary plate surface. (a)-(d) cells in various locations within the seeded area on top of the capillary plate. The trend observed in N=1,2
experiments is repeated as shown by selective distribution of cells at the capillaries while few cells attach to the glass surface in between capillaries.

Fig. 17 Fluorescent micrograph for N=3 experiment showing distribution of junctional protein ZO-1 and the cell nuclei over an extended area of the capillary plate surface following double stain of NCI-H441 cells at day 4 in culture atop a bovine fibronectin-
coated capillary plate surface. At the top the micrograph shows a cell layer extending over both glass surface and the capillaries.

5.6 Conclusion

We developed a novel platform for pulmonary epithelial cell culture as an in-vitro model for the alveolar epithelial barrier taking into account the dynamic mechanical stresses that occur during respiration. Our model is unique in the sense that epithelial cells are directly cultured and supported by a gas-liquid interface and mechanical stress in the cell layer is induced by varying the capillary pressure across the supporting gas-liquid interface. Our air-liquid culture platform consists of a capillary plate and a supporting microfluidic platform. The capillary plate is a small disc or plate (~2cm) with a large number of through-holes or capillaries extending from one surface of the disc to the other. By selectively rendering surface of the capillary plate hydrophobic we demonstrated the ability to achieve uniform formation of gas-liquid interfaces at the surface of the capillary plate when it is exposed to cell culture liquid. Pulmonary epithelial cells would be seeded in presence of the gas-liquid interface and form a tight layer above the interface mimicking the alveolar epithelium. The cell layer can be manipulated mechanically by altering the gas pressure in the capillaries which provides a more physiologically accurate model than the previously used stretched membranes. We designed and fabricated a supporting microfluidic platform that hosted and sealed the capillary plate and allowed for precisely controlled delivery of air pressure on the bottom surface of the capillary plate for mechanical manipulation of the cell layer. Formation of a tight layer of H441 pulmonary epithelial cells on top of the air-liquid interface was adopted as a validation for our cell culture model. ZO-1 junctional protein immunostain was employed to verify tightness of cell layers. H441 cells cultured on a glass microscope coverslip were used as a control for cell layer tightness. ZO-1 immunostains were performed and optimized for the control cell layers and showed formation of tight junctions between cells. ZO-1 and Hoechst immunostain for cells cultured on the air-liquid interface was performed for three different experiments with three different capillary plates and cell populations. In all three experiments cells demonstrated a strong preference for growth at the gas-liquid interface with few cells attaching to the glass surface in between the capillaries. Further investigation is needed to explain the underlying reason for this preference. Further investigations including TEER measurements are needed to verify tightness of the cell layer.
formed at the capillaries before the cultured cells can be used an in-vitro model for alveolar epithelium.

5.7 References


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Chapter 6: Future work

Abstract

In this chapter a number of ideas and designs are presented that can be pursued as an extension or continuation of some of the topics covered in this thesis. In the first two chapters of the thesis flow control and manipulation strategies were introduced and characterized that relied on controlled motion of gas bubbles by through precise pressure control and employment of arrays of closely packed micropillars. In the first section of this chapter an “on-chip incubator” concept is introduced that also takes advantage of micropillars in combination with gas liquid interfaces to create a dynamic and tunable microenvironment for cell culture applications. The on-chip incubator is an excellent platform for studying applications were dynamic real time change in the conditions of cell culture microenvironment is required. It can for instance, be applied for in vitro modeling of a lung transplant procedure to investigate the effect of varying temperatures and gas composition on the longevity of pulmonary cells. In this chapter we cover some preliminary designs and studies that indicate feasibility of such platform from the theoretical and conceptual standpoint. In the second part of this chapter a number of case studies and fabrication methods are presented and discussed that can be pursued in continuation of the devised alveolar epithelium model platform introduced in chapter 5. The proposed fabrication methods and substrate materials are aimed at design and fabrication of customized capillary plates in-house rather than using commercial plates as was done in chapter 5. The case studies presented are based on the assumption that a confluent monolayer of epithelial cells will be formed at the gas-liquid interface.

6.1 Lung Transplant-on-a-Chip

This section is devoted to development of automated incubation platforms for microfluidic culture based on the technologies developed in this thesis. These techniques are aimed at
modeling lung transplant procedure on chip and eventually help understand and tackle acute lung injury (ALI) in lung transplantation.

6.1.1 Introduction

Lung transplantation is the most effective strategy to treat patients with end-stage lung disease. It involves donor lung harvesting followed by a storage step before the donor lung is transplanted to the recipient patient (see Fig. 1). Over 2,400 lung transplants are performed globally on an annual basis using a variety of lung transplant procedures. Despite the large number of transplants and the variety of organ transport methods, there is no consensus regarding the most effective procurement strategy to maximize lung longevity with mild to severe lung injury occurring in 20–30% of recipients. Currently the safe storage times (i.e. $t_{\text{Storage}}$) for donor lung grafts are considered to be around 6 to 8 hours irrespective of the procurement technique used. After this time, ischemia-reperfusion (IR) related lung injury hinders successful lung transplant procedures, and may result in graft failure. The IR syndrome typically occurs within 72 hours after the transplantation and is characterized by alveolar damage, lung edema and hypoxemia. The major causes of IR-related injury are drastic reduction in temperature and oxygen concentration during donor lung transport. Fig. 1 illustrates the long transplantation process from harvesting donor lung to lung transplant to the recipient.
Fig. 1 Schematic illustration showing different steps of a lung transplant surgery from left to right: Harvesting the donor lung, donor lung storage under cold ischemia condition, and finally transplantation and post-transplantation steps.

Understanding the damaging cellular and molecular mechanisms during graft transport and developing procurement and preservation strategies that inhibit them can be an effective way of addressing IR-related donor lung injuries. Furthermore, the study of how the pulmonary cells change over time during the preservation period and subsequent reperfusion process is critical since the success of the transplantation depends on the duration of organ preservation. Additionally, the ability to study multiple treatment conditions on multiple pulmonary cell samples in a given experiment is needed to facilitate comparative studies to maximize graft preservation times.

While recent studies have pointed out certain drugs that have improved pulmonary grafts in rats such studies do not allow for precise regulation of the cellular environment. Additionally, most experimental methods do not allow for continuous real time study of the cellular changes and can
only collect data at certain points during each experiment. Traditional cell culture systems such as well plates or culture dishes do not allow dynamic and precise manipulation of the cellular microenvironments which have a significant impact on cell behaviour. These experimental challenges hinder acquisition of crucial data that is needed for better understanding of pulmonary cell behavior under transplant conditions.

Microfluidics is a proven technology in the field of cell biology that allows for precise regulation of cellular conditions during cell culture. As a result our collaborators at the Toronto General Hospital are interested in employing a microfluidic culture platform to conduct the pulmonary cell viability experiments they currently perform using traditional cell culture methods (i.e. flasks and well plates). An outline of the proposed experiment for studying the effect of varying gas composition and temperature on pulmonary cell viability is shown in Fig. 2.
Fig. 2 Flow chart for the experimental conditions involved in the study of ischemia-reperfusion in pulmonary cells. The cells are exposed to dissolved gas and temperature conditions over time and imaging GFP labeled cells will determine cell viability in predefined points during the experiments as shown in the diagram.

6.1.2 Objective

To develop a scalable microfluidic cell culture model capable of accurately recapitulating physical parameters encountered in a standard lung transplant procedure including: dissolved gas composition, temperature, wall shear stress and substrate strain. Micropillar-based technology for on-chip gas control developed in this thesis that will be implemented to achieve an integrated microfluidic cell culture device. The on-chip strategy will allow us to deliver the gasses at exact location on the culture channel where they are needed without the need for membranes or permeable substrate materials. Temperature control is achieved through a combination of a PID controlled thermoelectric element and a thermistor which will be discussed in detail later in this document along with the on-chip gas dissolution techniques.

6.1.3 Control Parameters

- Temperature: accurately controlled between 4 and 37C with a resolution of 0.2C
- Gas pressure: maintained below the capillary pressure between microfabricated pillar arrays (~1 psi). The capillary pressure between the pillars was estimated based on surface tension values at 4C and 37C which are nearly 75 mN/m and 70 mN/m respectively\(^6\).
- Gas mixture composition: different mixtures of air, CO2 and O2 are needed for different experiments (e.g. 95% air +5%CO2)
- Shear rate: shear stress applied on ECs will closely match the physiological shear levels (1-20 dyne/cm\(^2\))
6.1.4 Device Design Considerations

From the technological point of view the devised microdevice must allow dynamic control of the control parameters listed above including temperature, gas pressure, gas composition and shear rate. The parameter space for all the above parameters versus time are shown in Fig. 3. From the engineering point of view it is important that the devised microfluidic platform is capable of meeting the parameter space requirements in Fig. 3 in a dynamic and repeatable manner that allows reliable and repeatable culture and screening of pulmonary cells.

![Parameter space diagram](image)

Fig. 3 Parameter space showing the dynamic control required over time for (a) culture temperature (b) Gas pressure applied at the gas liquid interface (c) dissolved gas
composition under which the cells are cultured (d) the wall shear rate exerted on the adherent cells\textsuperscript{5} (e) The strain rate applied to lung cells.

6.1.5 Preliminary Designs and Studies

Here a selection of preliminary strategies and studies that have been performed for dissolved gas composition control, temperature control and shear stress control are presented along with computational models developed to date to design and assess different aspects of the lung transplant-on-chip model.

![Diagram of culture channel](image)

**Fig. 4** Device design showing the culture channel sandwiched between two gas delivery channels as the preliminary gas delivery strategy for pulmonary cell culture.

6.1.6 Control of Dissolved Gas Composition

Gas mixtures will be made by adjusting partial pressures of each component. For instance to achieve a 95% air +5%CO2 mixture we will need to premix two separate streams of air and CO2 at a pressure ratio of 19 to 1 respectively. The gas mixture can then be delivered to the culture channel location.
• A series of micro pillars can be employed to lock gas liquid interface outside of the culture channel that contains cells adhered to the top and/or bottom walls (as shown in Fig. 4)

• Time required for the gas to diffuse completely into the liquid channel is in the order of \((L^2/4D)\) or a few minutes. As an example, for \(L=1\,\text{mm}\) the oxygen diffusion time is around 229 seconds at 4°C (i.e. under 4 minutes) and 99 seconds at 37°C (i.e. 1.5 minutes)

• The devices should be sealed for gas permeation or non-permeable materials be used

• The pressure of the gas mixture will be maintained using the pressure controllers

• Purely diffusive gas dissolution in the media is possible only if there is no liquid flow (i.e. shear stress) in the micro channel (see Fig. 5). Presence of fluid flow will result in boundary layers that can hamper mixing and therefore, forced advection will be required to achieve effective mixing.

• Moving gas bubbles can be used to create convection in the liquid stream and enhance mixing of the gas in the liquid significantly. Designs based on bubble movement are presented in Fig. 6 and Fig. 7. Both these designs are based on the bubble gate and bubble pump designs presented in chapters 2 and 3 in this thesis⑦.
Fig. 5 Finite element model for oxygen diffusion (c=0.2) using the pillar based gas exchanger in absence of any fluid flow. Diffusion of oxygen was modeled according to Fick’s law. As shown in the figure oxygen will diffuse into the liquid filled channel and eventually saturates the liquid in absence of fluid flow.
Fig. 6 The proposed gas delivery strategy based on the bubble gate design. A series of bubble gates are opened and closed in an oscillatory manner to promote convective dissolution of the gas mixture in the culture media.

Fig. 7 The proposed comb exchanger gas delivery strategy. A series of quasi-bubble gates in a comb configuration are opened and closed in an oscillatory manner to promote convective dissolution of the working gas in the culture media.
6.1.7 Control of Wall Shear Stress

- Wall shear stress which is the shear stress felt by the cells attached to the channel walls will be controlled by adjusting the flow rate of the culture media entering the culture channel.
- We initially control liquid flow by adjusting the applied pressure head over a reservoir filled with culture media.
- Wall shear stress can be calculated based on Poiseuille flow assumption inside the microchannel.

6.1.8 Temperature Control

Temperature control is of critical importance for the lung transplant-on-chip project as the preservation needs to be done under hypothermic conditions and local on-chip cooling can be challenging. There are two aspects to temperature control for the devised microdevice, the on-chip temperature control for the culture channel and the temperature control of the culture media reservoir. The two aspects will be considered in combination, to ensure the cells in the culture channel are constantly exposed to desired temperatures. The acceptable temperature accuracy is below one degree Celsius and is ideally 0.2°C or below which is the temperature resolution offered by commercial incubators.
- Based on calculated heat diffusion lengths, it was found that the heat diffusion length is under 5mm for a wide range of flow rates of the culture medium in the culture channel. This means that no off-chip heating is necessary for the culture medium reservoir as long as a 5mm temperature equilibrium zone is allocated on the designed microchannels so that the culture medium can equilibrate with the culture channel temperature before it reaches the cells.
- Thermoelectric temperature controllers will be used to precisely control and maintain the temperature at the culture channel location.
- The thermoelectric element requires a heat sink that will keep the hot side of the thermoelectric element at room temperature as a result a cooling jacket with a channel for cooling water to pass through has been devised and developed.
- A thermistor detects the temperature adjacent to the culture channel and the control system will automatically adjust the heating or cooling to maintain the temperature at the desired level. The temperature control system is shown in Fig. 7.

Fig. 8 Lung transplant-on-chip device and the temperature control unit assembled. Temperature control is achieved using thermoelectric elements attached to a sapphire disc that will sit on the microdevice. The sapphire disc with a relatively high conduction coefficient helps transfer heat to and from the TE element for cooling and heating respectively. An aluminum made cooling jacket will be attached to the top surface of the
TE element as a heat sink. The cooling jacket has four tubular channels inside. Water at room temperature will be flown inside the cooling jacket using a syringe pump. (c) the cooling jacket designed to be placed on top of a thermoelectric element as a heat sink. The thermoelectric element will heat and cool the culture channel accurately and in case of cooling the absorbed heat by the TE element will be removed by the proposed cooling jacket heat sink. The maximum power of a TE element is 4.4W applied to the bottom surface in the model.

6.1.9 Potential Experiments:

Model validation:
- Culture A549 cells in the microfluidic chip.
- 12 ([n=3]X 4) independent cell populations (4 cell culture channels repeated 3 times)
- Expose cells to 24 h of simulated ischemia at 4°C in 100% O2 in D10\(^{1}\) (DMEM plus 10% FBS), DMEM, or low potassium dextran glucose (LPDG)\(^{1}\) or ViaSpan\(^{1}\) followed by 2 h of reperfusion at 37°C.
- Double-stain with fluorescein diacetate-propidium iodide for viability
- Extract percentage of surviving cell data for all four preservation mediums

Case study 1: LT-On-a-Chip model case study 1; effect of preservation solution flow rate in ex-vivo lung perfusion (EVLP)
- Human pulmonary microvascular endothelial cells (HPMEC)
- 10 culture channels with varying length or width (i.e. varying flow resistances) resulting in 10 cell populations exposed to 10 different wall shear stress values
- Dynamic bright field imaging every 10 minutes
- study the dynamic effect of shear stress on cell longevity via cell morphological studies over the full span of an EVLP cycle
Case study 2: rearrangement of cytoskeletal structure in response to EVLP conditions

- Actin, Tubulin, Cytokeratin, Vimentin immunostains.
- Verify how cell cytoskeleton, cell-cell and cell-substrate adhesions are affected during the preservation and recovery using EVLP technique.

Case study 3: drug screening for prevention/treatment of ischemia-reperfusion induced lung injury

- Use GFP and Renilla labeling to study the effect of various drugs on the cells.
- Our scalable platform allows testing a large number of samples at once ideal for drug screening.

6.2 Future work on alveolar epithelium model platform

6.2.1 Custom fabrication of the capillary plate

The capillary plate can be made in silicon, glass or thermoplastics such as PMMA and COC using common microfabrication techniques. Commercial glass capillary plates with hole diameters ranging between 5µm to 50µm are also available for purchase (e.g. capillary plates offered by Collimates Holes Inc.). For the scope of this thesis the initial experiments were performed using commercially available plates. In-house fabrication of the plates in silicon or PMMA will provide more flexibility for tuning device design and function. PMMA-made plates can be made using hot embossing or reactive ion etching (RIE)\(^8\)\(^-\)\(^11\) (fabrication procedure shown in Fig. 8). PMMA-made capillary plates are the preferred option as unlike silicon they are transparent and allow optical access from either top or the bottom of the plate. Silicon-made capillary plates can be fabricated using either KOH wet etching (fabrication procedure shown in Fig. 9) or Reactive Ion Etching (RIE) (fabrication procedure shown in Fig. 10).
Fig. 9 Process flow for deep reactive ion etching of PMMA (or similar thermoplastics such as polycarbonate) (a) raw PMMA sheet (white) (b) a thin layer of titanium (yellow) sputtered on PMMA (c) A layer of photoresist (red) coated on top of the titanium layer (d) The photoresist layer is patterned by UV exposure and rinsing with photoresist solvent (e) Reactive ion etching (RIE) patterning of the titanium layer using CF4 (f) O₂ RIE of PMMA using the patterned titanium as a mask (g) stripping the patterned PMMA sheet of the titanium layer using 1% HF solution.
Fig. 10 Process flow for KOH wet-etching of a through-hole in silicon (a) clean silicon wafer (dark grey) (b) a thin layer of silicon nitride (light grey) is deposited on the wafer using chemical vapor deposition (LPCVD or PECVD). Alternatively, silicon wafers with silicon nitride may be purchased to save fabrication time\textsuperscript{12}. (c) A layer of positive photoresist (red) coated on top of the nitride layer (d) The photoresist layer is patterned by UV exposure and rinsing with photoresist solvent (e) Reactive ion etching (RIE) patterning of the silicon nitride layer using CF\textsubscript{4} or CHF\textsubscript{3}\textsuperscript{12} and photoresist is removed using acetone\textsuperscript{13}. (f) KOH wet-etching of the silicon until the etched hole from top and bottom sides of the silicon wafer meet. The hole is slightly tapered (i.e. the diameter of the hole decreases with distance from the surface) which is natural to wet-etching of silicon\textsuperscript{13}. 

Fig. 11 Process flow for DRIE etching of a through-hole in silicon (a) clean silicon wafer (dark grey) (b) a thin layer of silicon oxide (light grey) is deposited on the wafer using chemical vapor deposition (LPCVD). Alternatively, silicon wafers with silicon oxide layer may be purchased to save fabrication time. (c) A layer of positive photoresist (red) coated on top of the nitride layer (d) The photoresist layer is patterned by UV exposure and rinsing with photoresist solvent (e) Buffered oxide etch (BOE) patterning of the silicon oxide layer. (f) DRIE etching of the silicon until the etched hole from top and bottom sides of the silicon wafer meet. The hole has vertical walls using this fabrication technique. Photoresist layer can be removed using either piranha cleaning or using acetone. The oxide layer can be removed using BOE.
6.2.2 Future biological problems to be addressed

1. More realistic recapitulation of the alveolar inflation and deflation by varying the gas pressure that the cell layer is exposed to rather than mechanically stretched membranes as have been previously used\textsuperscript{14-16}.

2. Evaluating the pulmonary epithelial cell-to-cell binding strength by increasing the gas pressure in minute steps (currently our pressure control resolution is 0.005psi or 34.47 Pa) until the cell layer ruptures. With the current pressure resolution the smallest force that can be applied (i.e. measurement resolution of the force) assuming a spherical gas-liquid interface with a diameter of 25\(\mu\)m is 16920 pN. Intercellular binding strength has been previously evaluated using Atomic Force Microscopy (AFM) and has been shown to be in the order of hundreds of Pico Newtons (pN)\textsuperscript{17, 18}. It is unclear whether it is possible to evaluate the binding strength of individual epithelial cells by varying the applied gas pressure. It is especially important to decouple the gas pressure that is exerted on the gas-liquid interface versus the pressure that is applied on the cell layer. This is important as the cells will not be fixed to the gas-liquid interface and may simply float over the pressurized interface rather than being exposed to the stress caused by the applied gas pressure.

We speculate that past a certain pressure threshold the cells will inevitably be exposed to the stress from the gas liquid interface. We expect that with increased pressure the cells will undergo one of the following scenarios:

a) Cells are damaged as a result of exposure to the high stress of the gas-liquid interface and the cell layer ruptures at points of contact with the gas-liquid interface

b) The cell layer is detached from the supporting structure
c) The cells will secrete surfactants as they do in vivo to lower the mechanical stress applied onto to them and prevent cell injury\textsuperscript{19,20}.

A combination of scenarios (a) and (b) is possible. Whether the cell layer ruptures or detaches from the device surface depends on the comparative strengths of cell-cell and cell-surface bonds. The ideal scenario for the purpose of this project is the scenario in (c) where cells demonstrate their physiological functions.

3. If formation of a confluent epithelial cell layer on top of the micro-pillars or micro-holes is achieved successfully, it is possible to co-culture a layer of endothelial cells on top of the epithelial cell layer to obtain a more physiologically accurate cell culture. Such a culture system could potentially be used for recapitulating and studying certain pulmonary diseases and conditions such as: Pneumonia caused for example by bacteria or chronic obstructive pulmonary disease caused by smoking tobacco.
6.3 References


13. C. Liu, Electrical and computer Department University of Illionis at Urbana-Champaign Pearson Education International, 2006.


## Appendices

### Table A1. Examples of the flow control techniques developed to date with details on their fabrication and operation

<table>
<thead>
<tr>
<th>Authors</th>
<th>Type of flow control</th>
<th>Mechanism of Action</th>
<th>Substrate Material</th>
<th>Number of Layers</th>
<th>Working Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacobson et al.</td>
<td>valving/sampling</td>
<td>electrokinetic</td>
<td>glass</td>
<td>one</td>
<td>Sodium tetraborate buffer</td>
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<tr>
<td>Schasfoort et al.</td>
<td>sampling/pumping</td>
<td>electro-osmosis</td>
<td>silicon, glass</td>
<td>one</td>
<td>Phosphate buffer</td>
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<td>Kaigala et al.</td>
<td>valving</td>
<td>phase-change</td>
<td>glass, PDMS</td>
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<td>PCR mix</td>
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<td>Sundararajan et al.</td>
<td>sorting/pumping/mixing</td>
<td>pneumatically</td>
<td>glass, PDMS</td>
<td>one</td>
<td>water</td>
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<tr>
<td>Grover et al.</td>
<td>valving/pumping</td>
<td>pneumatically</td>
<td>glass, PDMS</td>
<td>three and four</td>
<td>water</td>
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<td>Irimia et al.</td>
<td>valving/sampling</td>
<td>pneumatically</td>
<td>glass, PDMS</td>
<td>three</td>
<td>blood</td>
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<tr>
<td>Unger et al.</td>
<td>valving/pumping</td>
<td>pneumatically</td>
<td>glass, PDMS</td>
<td>two</td>
<td>water</td>
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<td>Thorsen et al.</td>
<td>multiplexed valving</td>
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<td>glass, PDMS</td>
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<td>water</td>
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<td>Zhang et al.</td>
<td>valving/pumping</td>
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<td>PMMA, PDMS</td>
<td>three</td>
<td>water, blood</td>
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<tr>
<td>Authors</td>
<td>Function</td>
<td>Actuation Method</td>
<td>Materials</td>
<td>Quantity</td>
<td>Solution</td>
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<td>Kim et al.</td>
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<td>mouse immunoassay mix</td>
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<td>Weaver et al.</td>
<td>gain valve</td>
<td>pneumatically actuated</td>
<td>PDMS</td>
<td>four</td>
<td>water</td>
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<td>Jian et al.</td>
<td>pumping/flow</td>
<td>laser-induced thermal</td>
<td>glass, PDMS</td>
<td>one</td>
<td>water/ethanol</td>
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<td>Wijngaart et al.</td>
<td>valving</td>
<td>thermally controlled</td>
<td>glass, PDMS</td>
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<td>water</td>
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<td>Lee et al.</td>
<td>valving</td>
<td>membrane/electro-chemically actuated</td>
<td>PDMS, PMMA, SU-8, silicon</td>
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<td>NaCl solution</td>
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<td>Sánchez-Ferrer et al.</td>
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<td>Liquid-Crystalline Elastomer</td>
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<td>water</td>
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<td>Weibel et al.</td>
<td>valving/pumping</td>
<td>mechanical squeezing of</td>
<td>PDMS</td>
<td>one</td>
<td>human serum/water/dimethylsulfoxide</td>
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<td>Pemble et al</td>
<td>valving</td>
<td>mechanical squeezing by</td>
<td>Silicone Microbore tube</td>
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Table A2. Summary of previous investigations on microfluidic perfusion cell culture.

<table>
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<th>Author</th>
<th>Year</th>
<th>Culture platform type</th>
<th>Fabrication Technique</th>
<th>No. Of Layers</th>
<th>Cell type</th>
<th>Dynamic gas control</th>
<th>Gas sensing method</th>
<th>Dynamic temperatur e control</th>
<th>Dynamic applied stress control</th>
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<tr>
<td>P. C. Thomas et al</td>
<td>2011</td>
<td>PDMS channel</td>
<td>soft lithography</td>
<td>two</td>
<td>none</td>
<td>Oxygen control with gas-equilibrated water stream</td>
<td>oxygen-sensing, based on a layer of platinum tetra(pentafluor ophenyl)porphin e (PtTFPP)</td>
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<td>Jr-Lung Lin et al</td>
<td>2010</td>
<td>PDMS chamber</td>
<td>soft lithography</td>
<td>three</td>
<td>Chondrocytes</td>
<td>None</td>
<td>None</td>
<td>indium tin oxide (ITO) heater in closed loop temp control (0.2C resolution)</td>
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<tr>
<td>S. P. Forry et al</td>
<td>2011</td>
<td>PDMS device</td>
<td>soft lithography</td>
<td>two</td>
<td>mouse fibroblasts</td>
<td>CO2 control using CO2-equilibrated water stream</td>
<td>ratiometric pH sensitive dye (5-and-6)-carboxy SNARF-1</td>
<td>None</td>
<td>none (quake valve flow control)</td>
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<td>A. Takano et al</td>
<td>2012</td>
<td>PDMS/PM MA</td>
<td>soft lithography/assembly</td>
<td>multilayer (&gt;3)</td>
<td>African green monkey kidney cells (COS-7)</td>
<td>water jacket with NaHCO3/Na2CO3 solution CO2 regulation through PDMS membrane</td>
<td>PH measurement with a pH electrode</td>
<td>none (37C hotplate)</td>
<td>none (Brail cell flow control)</td>
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<td>Ivar Meyvantsson et al</td>
<td>2008</td>
<td>PDMS channels</td>
<td>soft lithography</td>
<td>one</td>
<td>NMuMG, GFP-NMuMG, Hs578Bst, HMT-3522 S1, MCF10A</td>
<td>none (incubator)</td>
<td>none</td>
<td>none (passive capillary pumping)</td>
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<td>Jianbo Shao et al</td>
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<td>PDMS channel</td>
<td>soft lithography</td>
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<td>Human umbilical vein endothelial cell line (HUVEC-2C)</td>
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<td>none (incubator)</td>
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<td>D. Huh et al</td>
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<td>Lichuan Zhang et al</td>
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<td>PDMS channel with membrane for ALI culture</td>
<td>soft lithography</td>
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<td>Human alveolar epithelial cells (A549, ATCC)</td>
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<td>Nicholas J. Douville et al</td>
<td>2011</td>
<td>PDMS chamber with membrane for ALI culture</td>
<td>soft lithography</td>
<td>three</td>
<td>Human alveolar basal epithelial (A549; CCL-185, ATCC) and primary, murine AECs</td>
<td>none (incubator)</td>
<td>none (incubator)</td>
<td>combination of solid mechanical and surface-tension stresses (cyclic propagation of air–liquid interface and wall (membrane) stretch</td>
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<td>Divya D. Nalayanda</td>
<td>2009</td>
<td>PDMS culture wells with PET membrane for ALI culture</td>
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<td>A549 cells, human alveolar basal epithelial cells</td>
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<tr>
<td>Dongeun Huh et al</td>
<td>2010</td>
<td>PDMS channel with porous PDMS membrane for ALI culture and vacuum chambers to stretch the membrane</td>
<td>soft lithography/chemical etching of PDMS</td>
<td>three</td>
<td>human alveolar epithelial cells and human pulmonary microvascular endothelial cells cultured on opposite sides of the membrane</td>
<td>none (not mentioned)</td>
<td>none (not mentioned)</td>
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<td>Dongeun Huh et al</td>
<td>2012</td>
<td>PDMS channel with porous PDMS membrane for ALI culture and vacuum chambers to stretch the membrane</td>
<td>soft lithography/chemical etching of PDMS</td>
<td>three</td>
<td>human alveolar epithelial cells and human pulmonary microvascular endothelial cells cultured on opposite sides of the membrane</td>
<td>none (incubator)</td>
<td>none (incubator)</td>
<td>10% cyclic strain at 0.2 Hz via stretching porous membrane hosting the cells and flow shear stress of 0.2 dyne/cm² but effect of shear was not studied</td>
<td></td>
</tr>
<tr>
<td>Henry W. Glindmeyer et al</td>
<td>2012</td>
<td>silicone tube coated with fibronectin</td>
<td>none</td>
<td>silicon tube</td>
<td>human lung airway epithelial cell line NCI H441</td>
<td>none (incubator)</td>
<td>none (incubator)</td>
<td>no dynamic stress control but the effect of surfactants and oscillatory flow on cell damage during airways reopening was investigated</td>
<td></td>
</tr>
</tbody>
</table>
Section A1. Solid mechanics considerations in bubble gate design

The most important solid mechanics consideration regarding the bubble gate design is the potential bending in micropillars that may result in the bubble gate failure if the displacement caused by bending is significant when compared with the pillar gap size, $d_{\text{Pillar}}$. To evaluate the effect of bending analytical solution based on the classical beam bending problem were obtained and the maximum micropillar displacement was calculated and compared with the pillar gap size. To determine the load applied to each micropillar a force balance analysis is performed as follows:

$$\sum F = 0 \rightarrow WH - (P_{\text{Gas}} - P_{\text{Liquid}})DH = 0 \rightarrow W = (P_{\text{Gas}} - P_{\text{Liquid}})D = \Delta PD$$

As shown above the only force affecting the micropillars is the pressure difference across the micro pillar as shown in Fig. A1. To calculate the maximum displacement, the micropillars were modeled as classical fixed end beams with an applied uniform load of $W$ with the governing equation and of $d^4y/dx^4 = W/EI$ where $y$ is the pillar bending displacement, $x$ is the coordinate along the pillar length, $W$ is the load, $E$ is the module of elasticity for the material and $I$ is the second area moment for the pillars’ circular cross section (i.e. $I_{\text{circle}} = \pi R^4/4$). Solving the beam bending differential equation for $y$ the displacement distribution along the pillar length is found as $y = (Wx^2/24EI)(H-x)^2$. With the maximum bending occurring at the half length of the pillars or $x=H/2$, the maximum displacement is given by $y_{\text{max}} = \Delta PH^4/48E\pi R^3$. The table below summarizes the calculated values of $y_{\text{max}}$ for silicon and PDMS devices and under two different pressure gradients.

<table>
<thead>
<tr>
<th>$\Delta P$</th>
<th>0.35 psi</th>
<th>1 psi</th>
<th>$d_{\text{Pillar}}$</th>
<th>$y_{\text{max}}/d_{\text{Pillar}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device</td>
<td>Displacement (nm)</td>
<td>Gap Distance (μm)</td>
<td>Ratio</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>PDMS device</td>
<td>62.789</td>
<td>179.397</td>
<td>20</td>
<td>0.009</td>
</tr>
<tr>
<td>Silicon device</td>
<td>0.2452</td>
<td>0.7</td>
<td>5.4</td>
<td>1.30E-07</td>
</tr>
</tbody>
</table>

As seen in the above table all calculated displacements are significantly smaller than the pillar gap distance, $d_{\text{Pillar}}$, with the largest displacement to gap size ratio being 0.009 in the worst case scenario. As a result, it can be concluded that the pillar bending due to the pressure difference is not significant and can be assumed negligible for the purpose of this work.

**Figure A1.** (a) micropillars under uniform load, $W$, due to the pressure gradient across the pillars (b) the classical fixed-fixed beam model for the micropillars with applied load, $W$, (top) and the diagram showing the bending moment and shear force and an arbitrary location $x$ along the beam.
Section A2. Investigation of transient pressure disturbances on bubble gates

As a starting point for investigation of the transient phenomena affecting the bubble gate’s dynamic behavior, a set of experiments were designed which aimed at measuring the bubble gate’s gas pressure right at the gas inlet when the bubble gate is in an oscillatory motion (i.e. half cycle closed and half cycle open). The bubble gate’s inlet pressure was measured for varying liquid pressure and oscillation frequency (i.e. half cycle times) as shown in Fig. A2. Slight pressure oscillations and pressure overshoot related to digital pressure controllers were observed.

To investigate whether increasing the dead volume of the gas in the system would dampen the oscillations and the overshoots a volume of nearly 60 cm$^3$ was added to the gas line and the pressure measurements in Fig. A2 were repeated to allow comparison of the pressure signal before and after the added volume. The pressure measurements for the bubble gate with additional volume are presented in Fig. A3. As seen in Fig. A3 the overshoot in the blue plot with the half cycle time of three seconds seems to have been eliminated following the addition of the extra volume, however, the red plot with a half cycle time of 300ms shows random fluctuations even after the volume addition.

Figures A4 and A5 present the pressure measurements similar to the experiments in Fig. A2 and A3 with the difference that in these experiments the liquid pressure is also oscillating between a lower and higher value. These experiments were designed to investigate the effect of liquid pressure perturbations on the gas pressure in a bubble gate. These artificial perturbations were meant to simulate the liquid pressure perturbations that may arise when a series of bubble gates operate in concert within a peristaltic pumping unit. As seen in Fig. A4 and A5, there are fluctuations in the pressure signal both before and after the addition of the extra volume and for different oscillation frequencies. This may indicate that external perturbations in fact cause slight changes in the gas pressure that cannot be compensated for by the current pressure controllers that we employ in our experiments.

It is also noteworthy that the pressure sensor used in the measurement had a resolution of 0.05 psi, which may invalidate some of the pressure fluctuations that are below the resolution. In order to find out whether the pressure sensor or the pressure controller are responsible for the small pressure fluctuations it is proposed that a very sensitive pressure sensor is used to repeat
the experiments presented here. Following the proposed experiments it should be clear whether
the pressure controllers are sufficiently fast and accurate to allow peristaltic pumping using the
bubble gate strategy.

Figure A2. Pressure measurements in the gas line of a bubble gate device (a) For half cycle times of
3000ms (blue) and 300ms (red) and $P_{\text{Liquid}}=0$psi (b) For half cycle times of 1000ms (blue) and 300ms
(red) and $P_{\text{Liquid}}=0.2$psi
Figure A3. Pressure measurements in the gas line of a bubble gate device with added gas control volume of 60cm³ (a) For $P_{\text{Liquid}}=0$ psi and half cycle times of 3000ms (blue) and 300ms (red) (b) For $P_{\text{Liquid}}=0.2$ psi and half cycle times of 3000ms (blue) and 300ms (red)
Figure A4. Pressure measurements in the gas line of the bubble gate when the gate is exposed to liquid pressure perturbations at the liquid inlet and outlet (a) $P_{\text{Gas}}=0.17\text{psi}-0.425\text{psi}$, $P_{\text{Liquid}}=0.06\text{psi}-0.17\text{psi}$ (b) $P_{\text{Gas}}=0.24\text{psi}-0.375\text{psi}$, $P_{\text{Liquid}}=0.24\text{psi}-0.375\text{psi}$.
Figure A5. Pressure measurements in the gas line of the bubble gate when the gate is exposed to liquid pressure perturbations at the liquid inlet and outlet when an additional gas control volume of 60cm$^3$ is added to the system (a) $P_{\text{Gas}}=0.24\text{psi}-0.375\text{psi}$, $P_{\text{Liquid}}=0.24\text{psi}-0.375\text{psi}$  (b) $P_{\text{Gas}}=0.24\text{psi}-0.375\text{psi}$, $P_{\text{Liquid}}=0.24\text{psi}-0.375\text{psi}$. 