Development of a Microfluidic Platform to Investigate Effect of Dissolved Gases on Small Blood Vessel Function

by

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Abstract

In this thesis I present a microfluidic platform developed to control dissolved gases and monitor dissolved oxygen concentrations within the microenvironment of isolated small blood vessels. Dissolved gas concentrations are controlled via permeation through the device substrate material using a 3D network of gas and liquid channels. Dissolved oxygen concentrations are measured on-chip via fluorescence quenching of an oxygen sensitive probe embedded in the device. Dissolved oxygen control was validated using the on-chip sensors as well as a 3D computational model. The platform was used in a series of preliminary experiments using olfactory resistance arteries from the mouse cerebral vascular bed. The presented platform provides the unique opportunity to control dissolved oxygen concentrations at high temporal resolutions (<1 min) and monitor dissolved oxygen concentrations in the microenvironment surrounding isolated blood vessels.
Acknowledgments

I would like to acknowledge all members of the Guenther and Bolz labs. Dr. Andrew Levy contributed significantly to this work by isolating cerebral vessels for almost all experiments. Meghan Sauve and Sascha Pinto also contributed by isolating vessels. Sanjesh Yasotharan assisted greatly by developing many of the techniques used in this work and assisting in their implementation. Manivasakan Vaheesar helped develop the fabrication protocol for embedding on-chip oxygen sensors as part of his undergraduate thesis. Arianna McAllister assisted with editing the thesis.

In addition I would like to thank my supervisor, Axel Guenther, our collaborating PI Steffen-Sebastian Bolz, and committee members Chris Yip, and Wolfgang Kuebler for their guidance throughout my project.

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List of Abbreviations

BSA  bouvine serum albumin
CO2  carbon dioxide
COC  cyclic olefin copolymer
DAG  diacylglycerol
DI   deionized
DO   dissolved oxygen
DRIE deep reactive ion etching
EC   endothelial cell
EDRF endothelium derived relaxing factor
EMCCD electron multiplying charge coupled device
FLIM fluorescence lifetime imaging
HB   hemoglobin
HIF  hypoxia inducible factor pathway
HPV  hypoxic pulmonary vasoconstriction
ICP  inductively coupled plasma
LED  light emitting diode
MFC  mass flow controller
MLCK myosin light chain kinase
MLCP myosin light chain phosphatase
MOPS 3-(N-morpholino)propanesulfonic acid
NO nitric oxide
PDMS polydimethyl(siloxane)
PE   phenylephrine
PKA  protein kinase A
PKG  protein kinase G
PS   polystyrene
PtOEPK platinum(II) octaethylporphyrin ketone
PU   polyurethane
RF   radio frequency
RIE  reactive ion etching
ROCs receptor-operated channels
ROS  reactive oxygen species
RTD  resistance temperature detector
RTDP ruthenium tris(2,20-dipyridyl) dichloride
erhenuium tris(2,20-dipyridyl) dichloride
erhenuium tris(2,20-dipyridyl) dichloride
S1P  Sphingosine-1-phosphate
SMC  smooth muscle cell
STP  standard temperature and pressure
UV   ultraviolet
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Chapter 1
Introduction and Background

1.1 Introduction

The microvasculature unit is an essential set of blood vessels consisting of terminal arterioles, capillaries and venules. The resistance arteries are located at the terminal part of the arterial network and consist of a single layer of endothelial cells (ECs) that is surrounded by a layer of smooth muscle cells (SMCs). These arteries contribute to the peripheral vascular resistance, protect the delicate capillaries, and distribute local blood flow according to metabolic demand. They are also involved in inflammatory responses and solute exchange. Resistance arteries are capable of adjusting their diameter (tone) in response to internal and environmental stimuli. Abnormalities in resistance artery function have been linked to prevalent conditions such as hypertension and atherosclerosis which increase risk factors for cardiovascular complications such as heart failure and stroke [1].

Dissolved gases play an important role in regulating resistance artery function. Hypoxia, a condition which is characterized by inadequate oxygen supply, contributes to metabolic hyperaemia (vasodilation in response to increased metabolic demand) and hypoxic pulmonary vasoconstriction (constriction of pulmonary arteries aimed at deflecting blood to more aerated regions of the lung) [2], [3]. Several other gases are known to have regulatory roles in artery function; carbon dioxide (CO₂) is an important metabolic factor which is critical in regulating the cerebral vasculature [4], and nitric oxide (NO) is a potent vasodilator previously known as endothelium derived relaxing factor (EDRF) [5]. Resistance arteries experience dynamically and spatially varying dissolved gas concentrations in their microenvironment from alterations in the blood gas supply, metabolic activity, and from endothelium derived factors, as illustrated in figure 1A [6]. Despite the significant interest in how these gases affect vascular function, our knowledge of underlying mechanisms through which vascular behaviour is altered is incomplete.

Progress toward understanding the mechanisms controlling vascular responses to hypoxia, and vascular function in general, has been hindered by conventional vascular investigation methods. Using myography systems, isolated vessels are mounted in a 5 mL bath by fitting them onto wires (isometric approach) or cannulas (isobaric approach). This technique allows for vessels to
be pressurized and maintained at physiologically relevant pH levels (7.35-7.45) and temperatures (37±5 °C) [7]. Although this technique has enabled microvascular research, it requires highly skilled personnel and is not scalable. The mass transport limitations associated with myography systems are evident in previously reported work on vessel segments exposed to hypoxic conditions [8], [9]. In these studies, the vessel organ bath solutions were bubbled with different N₂, CO₂, and O₂ mixtures. Both works report gas-liquid equilibration times of at least 10 minutes. This procedure clearly does not allow control over physiologically relevant timescales (<30 s) and physiologically relevant space (µm).

Figure 1. (A) Factors contributing to dynamic dissolved gas gradients in small blood vessel microenvironment (B) unknown effects of interactions between dissolved gas concentrations and other factors regulating the vasculature function. This schematic particularly plots the unclear relationship between vessel tone, dissolved oxygen (DO) and the vasoconstrictor phenylephrine (PE).

Microfluidic and lab-on-a-chip systems have provided a wide range of solutions for the on-chip culture and investigation of cells [10–13], tissue slices [14] and engineered tissue constructs [15] as well as embryos [16–18] and small organisms [17], [19]. Our group has previously developed a microfluidic strategy that allows mouse mesenteric artery segments (approximately 250 µm in diameter) to be reversibly loaded in a microfluidic channel and assessed under perfusion and superfusion with various solutions [20]. Although the device has demonstrated control over drug concentrations within the vessel microenvironment, it does not allow for quantitative control over dissolved gas concentrations as they are affected by uncontrolled permeation of gases through the device substrate material.
The objective of this project is to develop a platform that provides spatial and temporal control over dissolved gas concentrations within the microenvironment of an intact small blood vessel *in vitro*. Figure 1B illustrates how this platform expands the parameter space available for studying small blood vessels. In this work I present techniques developed to dynamically control and monitor dissolved oxygen (DO) concentrations within the microenvironment of isolated small blood vessels using a microfluidic platform. Chapter 1 describes the relevant physiological and technological background to the project. The DO control schemes are presented in chapter 2. Chapter 3 describes on-chip DO sensing schemes. Experiments conducted with the platform using mouse cerebral arteries are described in Chapter 4. Chapter 5 describes conclusions and recommendations for future work.

1.2 Resistance Artery Function and Dissolved Gases

*Resistance Vessel Function*

Resistance arteries regulate regional blood flow, arterial blood pressure, capillary filtration rate, and central venous pressure. Local blood flow through each tissue is regulated by resistance artery tone, as changes in vessel diameter scale by \( r^4 \) according to Poiseuille’s law. Therefore, resistance arteries can change their hydraulic resistance by up to 20 times in the skin and in skeletal muscle. Global arterial blood pressure is also regulated by resistance artery tone as global blood pressure is the product of cardiac output and vascular hydraulic resistance. Changes in resistance artery tone assist in maintaining blood pressure during a variety of activities such as standing up. Capillary perfusion and capillary pressure are also regulated by changes in resistance artery tone. Vasodilation increases blood flow to metabolically active tissues and affects fluid exchange between capillaries and surrounding tissues. Resistance artery tone is regulated intrinsically by the myogenic response, endothelial secretions, vasoactive metabolites, and temperature, and extrinsically by nerves and hormones [1].

*Dissolved Oxygen & Vascular Tone*

The pathways through which DO affects the vasculature have yet to be clearly determined [2], [3], [21–23]. It is known that hypoxia induces vasoconstriction in pulmonary arteries and vasodilation in systemic arteries [2], [3]. Hypoxic pulmonary vasoconstriction (HPV) is believed to preserve normal lung function and prevent right to left shunting of deoxygenated blood by...
deflecting blood away from poorly aerated regions in the lung [2]. Metabolic vasodilation, also known as metabolic hyperaemia, matches blood flow (oxygen supply) in systemic vessels to metabolic demand in tissues [21].

The role of DO as a signaling molecule itself has been questioned by Segal [21] and Allen et al. [23]. In a review on the regulation of blood flow in the microcirculation Segal argues that the skeletal muscle vasculature is not intrinsically sensitive to oxygen. Instead vasodilating metabolic factors such as interstitial K⁺, adenosine, and CO₂, diffuse toward arterioles. These metabolic factors induce vasodilation by decreasing cytosolic Ca²⁺ and decreasing myosin sensitivity to Ca²⁺ through interactions with the mechanisms described in Appendix A [21]. Another theory explaining metabolic vasodilation involves protected transport of NO by hemoglobin (Hb) [23]. In this theory, Hb releases NO as it transforms from the R-state (high pO₂) to the T-state (low pO₂). NO then diffuses to nearby arterioles from venules [23].

Intrinsic oxygen sensing and transduction mechanisms have also been proposed [3], [22]. These theories are based on changes in reactive oxygen species (ROS) as the cellular signal for hypoxia. ROS are generated by the mitochondria and NAD(P)H oxidase. There is still disagreement over whether hypoxia increases or decreases cytosolic ROS [3]. In the case of hypoxia induced elevation of ROS in the pulmonary vasculature, evidence suggests that ROS may induce constriction via several of the mechanisms described in Appendix A. The mechanisms by which hypoxia induced changes in ROS affect the systemic vasculature are less defined [22]. The source of hypoxia induced ROS generation has been identified as complex III of the mitochondria. Bicyclo sesterterpene terpestacin has been found to inhibit hypoxia induced ROS generation and stabilization of hypoxia inducible factor (HIF) pathway by interacting with UQCRB, a subunit component of complex III in the mitochondria [24].

**Dissolved Oxygen Gradients in the Microvasculature**

The Krogh cylinder model [25] proposed that oxygen transfer between the blood stream and tissues occurs through the capillaries. The model assumes that oxygen radially diffuses from capillaries into tissues with homogeneous oxygen consumption. In the model, oxygen tension in the blood stream remains at 100 mmHg until it reaches the capillaries. Advancement in measurement techniques, such as oxygen microelectrodes and phosphorescence quenching microscopy, have enabled the measurement of radial and longitudinal oxygen gradients in the
microcirculation [26]. These measurements have revealed substantial longitudinal oxygen gradients in precapillary arterioles indicating that oxygen is lost in these vessels. This loss can be attributed to diffusion to and consumption by nearby cells, diffusion to nearby capillaries, veins, and other arterioles, and oxygen consumption by the vessel wall [27]. Tsai et al. [28] measured significant radial gradients in oxygen tension across the arteriole wall in the rat mesentery (20-30 mmHg) and calculated the vessel wall oxygen consumption rate to be 3.9 mlO₂/(cm³ tissue min). This rate is 277.8 times the metabolic rate measured for tissue in their experiments. The calculation was based on an oxygen mass balance considering upstream and downstream intravascular oxygen measurements, perivascular oxygen gradients, and blood velocity. Other researchers have measured intravascular oxygen gradients on the order of 1-2 mmHg and have calculated vascular oxygen consumption to be on the same order as that of tissue [29]. Golub et al. [30] determined arteriole wall consumption by arresting flow in a rat mesentery arteriole and measuring the transient oxygen disappearance curve by phosphorescence quenching microscopy. The oxygen was consumed in 290 seconds and the vessel wall consumption rate was estimated to be 0.0018 mlO₂/(cm³ tissue min). Most of the uncertainties regarding vessel wall oxygen transport and consumption are directly related to experimental limitations. The above experiments were conducted on exteriorized tissue, making it difficult to differentiate between oxygen loss caused by tissue consumption, blood flow, and vessel wall consumption or the measurement technique itself.

*Scientific Aims*

The aims of this work are; 1) to probe changes in vascular tone response in response to hypoxic exposures, and 2) to map the oxygen profile surrounding isolated blood vessels in order to gain insight into oxygen transport and consumption in resistance arteries. Studying the effect of hypoxic exposure on the tone of vessels isolated from their surrounding tissues could provide insight into whether oxygen regulates the vasculature directly or indirectly via metabolites released by surrounding tissue. Studying vessels in isolation from their surrounding tissue also provides the opportunity to relate the measured oxygen profiles surrounding the vessel to transport and consumption in the vessel wall directly.
1.3 Artery-on-a-Chip Platform

The artery-on-a-chip device previously developed in the Guenther lab allows for mesenteric (250 µm) and cerebral (100 µm) artery segments from mice to be studied under physiological pressure (45 mmHg), physiological temperature (37°C), and controlled chemical microenvironments [20], [31]. Briefly, as illustrated in figure 2, artery segments are immersed in a loading well then loaded into the inspection area by manually withdrawing on a syringe connected to the loading channel. Once the artery is in place the top two fixation points are open to reservoirs applying a hydrostatic pressure of 45 mmHg below atmospheric pressure. The perfusion channel is then open to a reservoir applying a pressure of 45-60 mmHg above atmospheric. Once the vessel is slightly stretched and aligned with the perfusion channel, the bottom two fixation points are open to the -45 mmHg reservoirs. The loading well is then sealed and the superfusion channels are open. At this point the artery segments are gradually heated to 37°C before testing. A cerebral vessel loaded in the device is shown in figure 2A [31]. Typically changes in vessel tone were measured in response to increasing concentrations of the vasoconstrictor phenylephrine (PE) to validate vessel viability. The vessel is shown constricted in Fig. 2B in response to 10 µm PE.

Figure 2. Technique used to load vessel segments in artery-on-a-chip platform. Described in detail in [20], [31].
Figure 3. Mouse cerebral olfactory artery (outer diameter ~ 120 µm) loaded in artery-on-a-chip platform. (A) initial tone, exposed to 0 µm PE (B) constricted, exposed to 10 µm PE. Scale bars are 50 µm. Taken from [31].

1.4 Membrane-Based Gas Control

Microfluidic devices present a unique opportunity for dynamic control of dissolved gases within nanolitre liquid volumes. Polydimethyl(siloxane) (PDMS), an inexpensive substrate material commonly used to fabricate microfluidic devices, is also a functional membrane material for several gases. PDMS has a high permeability for O\(_2\) (7 times higher than water) and CO\(_2\), as summarized in table 1. Permeability defines the gas flux per area across the thickness of a membrane according to,

\[
N = \frac{P(p_2 - p_1)}{T} \quad (1)
\]

where \(N\) is the steady state gas flux through the membrane, \(T\) is the membrane thickness, \(P\) is the permeability of PDMS, and \(p_2 - p_1\) is the partial pressure difference across the membrane.

**Table 1: Solubility and diffusivity values of O\(_2\) and CO\(_2\) in water and PDMS**

<table>
<thead>
<tr>
<th></th>
<th>Solubility (\times 10^{-5} \frac{m^3(STP)}{m^2 \times Pa})</th>
<th>Diffusivity (\times 10^{-9} \frac{m^2}{s})</th>
<th>Permeability (\times 10^{-14} \frac{m^3(STP)}{m \times Pa \times s})</th>
</tr>
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<tbody>
<tr>
<td>(H_2O - O_2)</td>
<td>0.031 [32]</td>
<td>2.2 [33]</td>
<td>0.086</td>
</tr>
<tr>
<td>(PDMS - O_2)</td>
<td>-</td>
<td>0.18 [34]</td>
<td>0.60</td>
</tr>
<tr>
<td>(H_2O - CO_2)</td>
<td>0.82 [32]</td>
<td>1.94 [35]</td>
<td>1.8</td>
</tr>
<tr>
<td>(PDMS - CO_2)</td>
<td>-</td>
<td>1.3 [34]</td>
<td>2.8</td>
</tr>
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Solubility is defined as the equilibrium concentration of a solute across an interface. For gases, solubility is expressed as the concentration of gas in a medium (volume of gas at STP / volume of medium) per atmospheres of gas partial pressure. In the absence of convective transport, diffusivity (or the diffusion coefficient) relates the concentration gradient of a solute to its mass flux [36].

Permeation of gas molecules through a solid or static liquid medium is a two-step process in which the gas molecule concentrations equilibrate across the gas-solid or gas-liquid interface according to the solubility of the gas in that medium. Gas molecules then diffuse through the solid or liquid medium at a rate proportional to the diffusion coefficient in that medium. The permeability coefficient is a product of the solubility and diffusion coefficients between the gas and the solid or liquid medium, \( P = D \times S \), as calculated in table 1 [37]. Permeability is typically measured directly for solids by applying a known partial pressure gradient of the gas across a solid film with known thickness (T) and measuring the gas flux (N) across the film. For liquids solubility and diffusivity coefficients are typically determined separately. In this work all models and calculations are based on using the solubility and diffusion coefficients separately. The permeability coefficients in table 1 are included to indicate the significant difference in oxygen transfer rates through PDMS and water.

A typical gas concentration profile in a PDMS microfluidic device is shown in figure 4. In this profile discontinuities in the gas concentration occur at the gas-PDMS and PDMS-solution interfaces due to the different solubility coefficients in these mediums. The steady state gas concentration profile is linear across the PDMS as there is no convective transport. In the solution the concentration profile decreases as it approaches the center of the parabolic flow profile as the image depicts a region of the channel before the required equilibration length.
Figure 4. Schematic of oxygen profile across PDMS microfluidic device. An oxygen flux (N) is created through the PDMS membrane as oxygen gas (pO$_{2g}$) dissolves in the PDMS membrane (pO$_{2m}$), diffuses through the solid membrane thickness (T), and finally dissolves into the flowing aqueous solution (pO$_{2aq}$). Discontinuities occur at the interfaces due to the gradually decreasing oxygen solubilities between gas, PDMS, and water.

1.5 Fluorescence-Based Oxygen Sensing

DO has conventionally been sensed with Clark-type oxygen electrodes [38], [39] and more recently with optical detection techniques [40], [41]. Clark-type probes rely on the electrochemical reduction of oxygen at a cathode producing an electrical current proportional to DO. Electrochemical sensors are difficult to utilize in microfluidics since they consume oxygen and exhibit sensor drifts [42]. Optical DO sensors present an attractive alternative to electrochemical sensing within microfluidic devices as they rely on fluorescent quenching of a fluorescent or phosphorescent fluorophore by oxygen. Dynamic quenching is a process by which molecular collisions between quenching molecules and excited fluorophores reduce the intensity emitted by fluorophores. Molecular oxygen is one of the most well-known collisional quenchers and is able to quench most fluorophores [43]. As collisional quenching is a diffusive process, the volume in which oxygen molecules can interact with a fluorophore is determined by fluorophore’s lifetime. Fluorophores used to sense oxygen typically have long lifetimes (100 ns – 60 µs) which relate to oxygen concentrations according to the Stern-Volmer relation

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + K[Q]$$ (2)
where $F$ is the fluorescence intensity, $\tau$ is the fluorescence lifetime, the subscript '0' represents these values in the absence of the collisional quencher (Q), and $K$ is the Stern-Volmer quenching constant [44].

Quencher concentrations can be determined by measuring fluorescence intensity or fluorescence lifetimes of the quenched fluorophores. Intensity emitted by a fluorophore is a function of time, fluorophore lifetime, fluorophore concentration, fluorophore environment, and excitation conditions. For a fluorophore with a single exponential decay excited by an instantaneous pulse of light, the emitted intensity is described by

$$I(t) = I_0 e^{-t/\tau} \quad (3)$$

where $I(t)$ is the emitted intensity, $I_0$ is the initial intensity at excitation, and $\tau$ is the lifetime of the fluorophore. Under steady state excitation the measured intensity is the integral of Eqn. 3 from zero to infinity, giving;

$$I_{ss} = I_0 \tau \quad (4)$$

Eqn. 4 indicates that steady state fluorescence intensity measurements are related to the initial fluorescent emission and the lifetime of the fluorophore. The initial emission ($I_0$) is function of fluorophore concentration which is difficult to quantify due to uncertainties in fluorophore distribution and photobleaching. Fluorescent lifetime measurements represent a statistical average of the amount of time each fluorophore remains in an excited state. As dynamic quenching directly reduces fluorophore lifetime, fluorescence lifetime measurements are directly proportional to quencher concentrations. Fluorescent lifetime measurements are therefore independent of fluorophore concentrations or interference from background fluorescence [44].

Two common fluorophores used in oxygen sensing are a ruthenium complex, e.g. ruthenium tris(2,20-dipyridyl) dichloride hexahydrate (RTDP) [45], and a platinum complex, e.g. platinum(II) octaethylporphyrin ketone (PtOEPK) [46]. RTDP displays fluorescent lifetimes of 775 ns and 450 ns and PtOEPK displays lifetimes of 61.4 $\mu$s and 16.3 $\mu$s for oxygen concentrations of 0 % $O_2$ and 21% $O_2$ respectively [45], [46].
1.6 Previous Platforms for DO Control and Sensing

Several platforms have been developed to control [47–49] and measure [47, 49–51] micro scale DO concentration fields. DO concentrations have been measured in microfluidic devices by flowing fluorescent solutions through channels [48], [52], [53], immobilizing fluorophores within the device [47], [49], [50], or by adapting electrochemical detection schemes [51]. RTDP was used by several groups [48], [52], [53]. Imbedded sensors developed by Vollmer et al. [47] and Nock et al. [50] used PtOEPK suspended in polystyrene (PS). Vollmer et al. [47] embedded the PtOEPK-polystyrene matrix in a device by pipetting 1-2 µL of the dye solution into rectangular patches that were etched in a glass slide. Fluorescent lifetimes of the dye were measured at a single point using a custom LED based optical excitation and detection system. Nock et al. [50] spin coated the PtOEPK-polystyrene solution onto a glass slide. Sensing regions were patterned by reactive ion etching (RIE) using a PDMS stamp or a thin chrome layer [54] as a mask. Fluorescence microscopy was used to measure 2D intensity profiles of the dye.

Several microfluidic devices have taken advantage of the high oxygen permeation rates of PDMS with the goal of controlling dissolved oxygen concentrations in bioreactors. Vollmer et al. [47] developed a PDMS-based device with a gas channel layer that was positioned above a liquid channel layer separated by a 20 µm thick PDMS membrane. The time required for the dissolved gas concentration in the liquid to equilibrate with the gas channels varied from 30 seconds to 5 minutes. The platform did not provide spatially resolved oxygen measurements and was not validated with a biological application. In a similar approach, Adler et al. [48] developed a device capable of generating oxygen gradients with arbitrary shapes via diffusion through PDMS. Different computer controlled O2-N2 mixtures were perfused through nine closely spaced gas channels above a single perpendicular liquid channel. The device allowed for custom one dimensional oxygen profiles to develop in the liquid channel and for the profile to be altered in less than 30 seconds. Oxygen was sensed by flowing RTDP through the liquid channel, limiting the use of the device for biological applications due to phototoxicity concerns. A device developed by Park et al. [49] also took advantage of oxygen permeation through PDMS. In this device oxygen gradients were developed via an electrode array which generated precise oxygen gradients through electrolysis of water. The electrode array was separated from the culture chamber by an aqueous electrolyte and a 10 µm PDMS membrane. The device successfully generated two dimensional oxygen gradients within a 1 × 1 mm² area with a spatial resolution of
approximately 40 µm. The device was used to study cell migration and cell and tissue culture development under oxygen gradients. Operation of the device required the use of an electrolyte solution in close proximity to the cell culture chamber and a custom printed circuit board to interface with the on-chip electrodes, making the platform difficult to apply to other applications.

The previously developed strategies for DO control are not directly applicable to studies involving whole organs [55]. Integration of these control and measurement techniques into the previously developed platform for studying small blood vessels [20] required careful design and fabrication of PDMS based devices and development of integrated sensors compatible with the established fabrication protocol.
Chapter 2
On-chip Dissolved Gas Control

2.1 Microfluidic Device Fabrication

Microfluidic devices were fabricated using standard rapid prototyping techniques previously described [56]. The fabrication sequence is shown in figure B-1. Masters were fabricated by spin coating layers of SU-8 (Microchem, Newton, MA, USA) on 2”x3” glass slides (Corning Inc., Corning, NY, USA). Featureless seed layers were first coated by spin coating SU-8 25 at 2000 RPM for 30 s. These layers were then baked at 65°C for 5 min and 95°C for 10 min. They were then exposed using the UV mask aligner at 365nm (Model 200, OAI, San Jose, CA, USA) for 13 seconds. The slides were then hard baked at 65°C for 5 min and 95°C for 5 min. After allowing the slides to cool for 5 min the feature layers were coated by spin coating SU-8 2050 at 1950 RPM for 30 s with a 5 s ramp time. These layers were baked at 65°C for 10 min and 95°C for 15 min. After allowing the slides to cool, they were exposed under transparency masks with exposure energy of 150 mJ/cm² using a 360 nm long pass filter (PL-360LP, Omega Optical, VT, USA). Masks were designed using AutoCAD (AutoDesk, San Rafael, CA, USA) and were printed at a resolution of 20000 dpi (CAD/Art Services, Bandon, OR, USA). The mask designs are shown in figure B-2. These feature layers were then hard baked at 65°C for 5 min and 95°C for 10 min. Devices that required a single height were then developed in SU-8 Developer (Microchem) agitated for 10 min. These masters were then placed in an 80° oven for at least an hour prior to molding. The artery-on-a-chip device used for studying cerebral vessels required an additional feature layer of posts to allow the perfusing solution to flow around the vessel [31]. These posts were fabricated by spin coating an additional layer of SU-8 2050 at 4200 RPM for 30 s on hard baked master prior to development. These layers were baked at 65°C for 10 min and 95°C for 25 min. They were then aligned with a mask featuring the posts and exposed at and energy of 90 mJ/cm². They were hard baked at 65°C for 5 min and 95°C for 15 min and developed.

Microfluidic devices were fabricated by molding PDMS (Dow Corning) on SU-8 masters. The artery-on-a-chip devices used in this work required three SU-8 masters; one for the valve actuation and gas control layer, one for the channel layer, and one for layer of posts below the organ bath [31]. The PDMS fabrication sequence is shown in figure B-3. Devices were
fabricated by molding 25 g of PDMS (10:1 resin to curing agent ratio) onto the valve actuation/gas control layer, 23 g of PDMS onto the bottom post layer, and preparing 10 g of PDMS for spin coating. The prepared PDMS molds were then degassed in a -20 inHg vacuum for 1 hour. Once the PDMS was degassed, the valve actuation layer molds were cured in an oven at 80°C for approximately 15 min while the bottom post layer molds cured for 1 h. Simultaneously, the 10 g of degassed PDMS was spin coated onto the channel layer at 400 RPM for 30 s. These layers were then degassed again for 5 min. After the valve actuation layer molds were removed from the oven, the PDMS coated channel layer molds were placed in the oven for approximately 7 min to partially cure the PDMS [57]. The valve actuation layer molds were simultaneously cut and peeled from the valve actuation layer masters. Once the channel layer molds were ready for partial curing, the valve actuation layer molds were manually aligned above them using a stereomicroscope and the two layers were carefully brought into contact. They were then cured together for at least 10 min. The two bonded layers were then carefully peeled off of the channel layer master. Holes for fluidic inlets/outlets were punched and CYTOP (Asahi Chemical Company, Japan) was selectively spotted to prevent on-chip PDMS valves [58] from permanently sealing. The devices were then cured for 6 min to evaporate the applied CYTOP. These layers were finally bonded to bottom post layer mold via O₂ plasma (Harrick Plasma, Ithaca, NY, USA) and cut to fit on a 1”x3” slide.

Gold coated (100 nm thick) 1”x3” glass slides (EMF, Ithaca, NY, USA) were etched to create a resistance temperature detector (RTD) used to locally measure the temperature at the glass surface [31]. A thin layer of PDMS was spin coated onto the etched gold layer at 2000 RPM for 30 s to facilitate bonding to PDMS devices. The thin layer of PDMS was then cured and bonded to the sealed PDMS devices via O₂ plasma (Harrick Plasma, Ithaca, NY, USA).

2.2 Design and Fabrication for On-chip Dissolved Gas Control

To control DO in a microfluidic context, the permeability of PDMS to oxygen was utilized. Devices were designed with PDMS membranes (~ 100 µm) separating gas channels from liquid channels. The DO concentration in the solution flowing through the liquid channel (i.e. buffer or de-ionized water) was controlled by applying varying partial pressures of oxygen in the gas channels. Oxygen molecules migrate through the membrane (toward or away from the liquid) according to Eqn. 1.
Assuming a flux of oxygen through PDMS based on Eqn. 1, the concentration of DO in the flowing solution is governed by the convection-diffusion equation,

$$\frac{\partial c}{\partial t} = D \nabla^2 c - \vec{v} \cdot \nabla c \quad (5)$$

where $c$ is the DO concentration field in the solution, $\vec{v}$ is the solution velocity field and $D$ is the diffusion coefficient of oxygen in the solution.

Analytical and computation mass transfer models were developed and used as essential tools for chip design. The mass transfer problem involves convective and diffusive transport across interfaces of different mediums. The governing equation is the convection-diffusion equation (Eqn. 5). The boundary conditions are summarized in figure 5. The model assumes a constant initial concentration in the solution ($C_0$), a symmetrical concentration profile across the middle of the channel at $y=0$, a constant concentration at the PDMS-gas interface ($C_{\text{sat}}$), a laminar flow field in the solution ($V_z$), and flux continuity at the PDMS-solution interface.

Figure 5. Mass transfer model boundary conditions; constant applied concentration ($c_{\text{sat}}$) at gas-PDMS interface, flux continuity at PDMS-solution interface, constant inlet concentration ($C_0$), symmetric parabolic flow profile ($V_z$) in solution channel, symmetric concentration profile ($c$) across center of channel. $D_L$-diffusion coefficient in liquid, $D_m$-diffusion coefficient in membrane, $T$-PDMS membrane thickness, $U$-mean flow velocity, $b$-half of solution channel height.

An analytical solution was adapted from Tang et al. [59] solving for mass transfer of dissolved gases through a tubular membrane. A MATLAB (Mathworks, MA, USA) script was written to
solve for the steady state concentration profile of the non-dimensional problem using the Crank-Nicolson numerical method [60] (Appendix G). A 3D computational model (Comsol 4.2, Burlington, MA, USA) was solved for transient conditions and different cross sectional geometries. Mesh independence for the model is shown in table F-1. Figure 6 summarizes the resulting non-dimensional mid channel concentration from the two models across a 75 µm by 60 µm channel with a flow rate of 10 µL/min. In the models, the PDMS membrane separating the gas and liquid mediums is 100 µm thick. As can be seen, the computational solution and the analytical solution adapted from Tang et al. [59] were in very close agreement. Both models estimated the required equilibration length to be less than 25 cm, as estimated by the Einstein diffusion equation. Different channel cross sections were evaluated with the 3D computational model. It was found that amount and location of control gas channels affects the dissolved gas concentration profile in the solution channel. The estimated equilibration time from the 3D model is ~30 s.

Figure 6. Summary of analytical and computation models of gas transfer in device. Different cross sections in the 3D model are shown at the bottom of the plot. G- gas channel, B- buffer channel.
Devices were fabricated with multilayer soft lithography using PDMS as described in Section 2.1. A cross section of one of the devices is shown in figure 7A. The fabrication strategy produced devices in which the gas control channels were in a completely separate channel network than the solution channels. Figures 7B and 7C show a real device in which the gas channels are indicated with green food colouring while the solution channels are indicated with red food colouring.

Figure 7. (A) Cross section of device showing buffer and gas channels separated by 100 µm PDMS membranes. Scale bar is 100 µm. (B,C) Real device with buffer channels indicated with red food colouring surrounded by gas channels indicated with green food colouring. Scale bars are 500 µm.
Chapter 3
On-chip Dissolved Oxygen Sensing

3.1 Commercial Fiber Optic Oxygen Sensors

Two methods were pursued for on-chip DO sensing. The first approach, aimed at attaining preliminary measurements, involved integrating implantable fiber optic oxygen microsensors (PreSens, Germany) (Fig. 8A) into the microfluidic device. These sensors are based on the dynamic quenching of a fluorescent dye by oxygen at the tip of the fiber. The sensors measure the changes in the fluorescent lifetime of the dye and correlate these measurements with the oxygen concentration according to the Stern-Volmer relation (Eqn. 2).

Figure 8. (A) Presens fiber optic sensor. Scale bar 1 mm (B) Integration through side channel. Scale bar 1 mm (C) Integration through top of device. Scale bars are 3 cm.

These sensors were integrated into PDMS microfluidic devices laterally via open side channels (Fig. 8B) and vertically via holes punched through to the top of the devices (Fig. 8C). Devices prepared for lateral integration of the fiber had a dedicated open channel fabricated to a height of 300 µm. The fibers were carefully inserted into the channel under a stereomicroscope. Paraffin wax (Sigma Aldrich) was used to seal the channel. Devices prepared for vertical integration of the fiber had a 0.5 mm hole punched through to the top of the device. The fibers were carefully inserted into short lengths of FEP tubing (0.040” id, 1/16” od, Upchurch) which were epoxied to the punched hole. The tubing was sealed to the outer cladding of the fiber using a PEEK union (Upchurch).
In later experimental setups the fiber optic sensors were used to validate the oxygen concentration of the gas flowing into the PDMS devices. The sensors were sealed in T connections (Upchurch) connecting the incoming gas line with the PDMS devices.

3.2 On-chip Oxygen Sensor Fabrication

Several fabrication approaches were pursued in an effort to develop reliable on-chip DO sensors. The oxygen sensitive probe used was PtOEPK due to its long fluorescence lifetime (16-60 µs) and high sensitivity to oxygen [46]. The probe was embedded in polystyrene because it has a high gas permeability and good mechanical stability [61]. As previously reported [47], [50], 1 mg/mL of Pt(II)OEPK (Frontier Scientific, Inc. Utah, USA) was dissolved in a 7% w/w solution of polystyrene (M_w 280000, Sigma) dissolved in toluene (Sigma). The prepared solution was used in the patterning techniques described in the following subsections.

3.2.1 Patterning by Spin Coating and RIE

The protocol defined in Nock et al. [50] was adapted for this fabrication technique. The fabrication sequence is outline in figure B-3. 200 µL of the PtOEPK solution was spin coated on 1” X 3” glass slides at 4000 RPM with a ramp of 0.1 s for 30 s. The slides were then annealed on a 100°C hot plate for 90 s. Following this step, the sensor films were patterned by RIE with oxygen plasma using a chrome layer or a PDMS stamp as a mask. PDMS stamps were fabricated by soft lithography using SU-8 masters as described in Section 2.1. Chrome layers (100 nm) were deposited using e-beam evaporation (BOC Edwards A306 Evaporator). Chrome layers were then patterned by wet etching (Transene Company, MA, USA) using positive photoresist (S1818, Shipley, MA, USA) as a masking layer. Using a patterned chrome layer or PDMS stamp as a masking layer, the Pt(II)OEPK films were patterned in a RIE (Phantom Etcher, Triontech) with a 0.55 Torr etch pressure, 50 sccm O_2 flow rate, 200 W radio frequency (RF) power, with a 5 min etch time. The RIE step removed the photoresist protecting the patterned chrome layer. The remaining chrome was removed by wet etching, revealing the patterned Pt(II)OEPK film.

After characterizing the sensors off-chip, an attempt was made to bond them to the PDMS devices by oxygen plasma bonding. Significant challenges arose at this step due to incomplete etching of the polystyrene film from the glass slide. Incomplete etching can be seen when measuring the fluorescent signal of the Pt(II)OEPK dye in non-patterned regions that are
supposed to be etched during the RIE step. To overcome this challenge, the RIE process was optimized and a thin PDMS layer was coated above the sensor pattern to facilitate bonding.

3.2.2 Patterning by Flow

An attempt was made to pattern the PS/Pt(II)OEPK sensor by flowing the sensor solution through a PDMS microfluidic channel and subsequently purging with N₂ gas. Two different configurations were used in this approach. In the first attempt the channel used for coating was permanently bonded to the glass microscope slide via O₂ plasma bonding. In the second attempt the coated channel was reversibly sealed to the microscope slide by clamping.

3.2.3 Patterning by µ-Contact Printing

µ-contact printing is a common patterning technique in which an “ink” is deposited on a stamp with micro-scale relief structures which is subsequently stamped on the substrate material [62]. In this configuration approximately 2 µL of the PS/Pt(II)OEPK/toluene mixture was pipetted onto the PDMS based stamp. Air was blown on the drop to remove excess dye. The stamp was then carefully brought into contact with a clean glass slide for approximately 1 minute and subsequently removed from the slide.

3.2.4 Patterning by Dissolving Pt(II)OEPK in PDMS

Oxygen sensitive films have previously been fabricated by dissolving a fluorescent dye directly into PDMS [63]. In this configuration 2 µL of the Pt(II)OEPK/toluene solution was pipetted onto the PDMS posts which elevate the vessel. This PDMS layer was then aligned and plasma bonded to the rest of the device.

3.3 Experimental Set-Up

The experimental setup is shown in figure 9. Devices were fabricated with on-chip valves controlling six different pressurized wells. Devices were interfaced with a custom made aluminum manifold (Fig. C-1) [31] equipped with nine 300 µL wells and six magnetic solenoid valves (Lee Company). In my configuration, six wells were pressurized with a single digital pressure regulator (MarshBellofram, WV, USA), typically set to 5 mmHg of air. PDMS membrane valves were actuated by switching the control pressure ($P_{\text{control}}$, Fig. C-2) between the open position ($P_{\text{VAC}} = -20 \text{ inHg}$) and the closed position ($P_{\text{HI}} = 15 \text{ psi air}$). When the applied
pressure \( (P_s) \) from the pressure regulator is greater than the control pressure, the well is activated within milliseconds and the solution in the well flows into the device at flow rate determined by the applied pressure and the fluidic resistance on-chip. The six wells in my configuration were used to quickly switch between phenylephrine \((PE, \text{Sigma Aldrich})\) concentrations premixed in each well, specifically 0, 0.1, 1, 2.5, 5, and 10 \( \mu \text{M} \) PE in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer. These solutions flow around the abluminal side of the vessel via the superfusion line. In addition to the wells, five threaded connections are built into the manifold; two for the vessel fixation lines, one for the perfusion line, one for the superfusion outlet, and one for the vacuum connection to the on-chip bubble trap [20], [64].

Dissolved gas control experiments were conducted both on and off-chip. Gas compositions were controlled by flowing supply gases (nitrogen, compressed air; Linde) through mass flow controllers (MFCs) (EL-FLOW, Bronkhorst; Holland) that were separately operated. Total gas flow of the controlled mixture was set to 15 mL/min or 0.67 mL/min at STP. DO concentrations were measured with an implantable fiber optic oxygen sensor (PreSens). Off-chip measurements were made by bubbling the controlled gas mixture through 5 mL of DI water. During on-chip measurements the fibers were sealed into a T-connection connecting the MFCs and the manifold.

Figure 9. Experimental Setup; MFCs used to control composition of gas. Presens sensor used to validate DO concentration. Pt(II)OEPK patterned below organ bath.
Bright field and fluorescent images were taken on an inverted fluorescence microscope (model TI Eclipse, Nikon, Japan) using the Nikon NIS Elements software. The devices were typically imaged using a 20x objective with a 0.45 numerical aperture and an 8.2-6.9 mm working distance (CFI S Plan Fluor ELWD 20X, Nikon, Japan). Images were captured with an electron multiplying charge coupled device (EMCCD) camera (Evolve512, Photometrics, Tuscon, AZ) using a 175 W Xenon lamp source and a typical gain of 500. The Evolve512 features a 512 x 512 imaging array with 16 µm x 16 µm pixel size. Fluorescent lifetime measurements were taken on the LIFA-X Fluorescence and Phosphorescence Lifetime imaging system (Lambert Instruments, Holland). A custom made filter cube (Chroma, US) with a 590 nm (+/- 15 nm) excitation filter and a 760 nm (+/- 35nm) emission filter was used for fluorescent intensity and fluorescent lifetime measurements of the Pt(II)OEPK patterns.

3.4 On-chip Sensing Results

3.4.1 Commercial Fiber Optic Oxygen Sensors

Attaining quantitative DO measurements on-chip by integrating the commercial fiber optic sensors into the PDMS devices proved to be challenging due to the size and fragility of the sensor tips. The fluorescent probe-containing polymer matrix on the tip of the fibers has a diameter of approximately 200 µm, and easily detached when brushed against PDMS. The fibers were initially integrated through the top of the device by punching relatively large holes (2 mm) and inserting a stainless steel tube to guide the fiber to detection region. Although measurements could be made with this configuration they required long times (~1 hour) to reach equilibrium. After comparing the measurements with a computational model as well as with an analytical solution, the slow response was attributed to the sensor tip being located 1-2 mm above the flowing channel. This was a reasonable result as the channel height was 60 µm, while the length of the fiber tip is 375 µm and there was limited control over the vertical position of the fiber tip.

The fiber was then integrated through the side of the device by designing an enlarged open channel for fiber insertion fabricated with a multi-height master. Once the fiber was inserted, the open end of the channel was sealed with paraffin wax. The on-chip DO measurements shown in figure 10 were based on this configuration. The time response of the on-chip dissolved gas control was 1 minute while the 5 mL cylinder bubbled with the control gas required 10 minutes to equilibrate. Due to the challenges and limitations associated with the commercial sensors, integrating them into the PDMS devices was abandoned as an on-chip sensing technique.
3.4.2 Patterning by Spin Coating and RIE

The patterned sensors were characterized with fluorescence lifetime imaging (FLIM) and fluorescence intensity. A 5 mL cylinder was fixed above the sensor pattern and filled with DI water. Controlled oxygen concentrations were then bubbled into the reservoir by adjusting relative flow rate of N₂ and air using MFCs. DO within the reservoir was measured with a commercial fiber optic oxygen sensor. A frequency domain FLIM system (Lambert Instruments) was used to characterize the DO sensing accuracy of the film. The lifetime of the Pt(II)OEPK varied between 59 µs in a solution with sodium sulfite (0% O₂) and 19 µs when bubbled with air. A plot was generated of the lifetime ratio (τ₀/τ) in which τ₀ is the lifetime in sodium sulfite (0% O₂) versus measured DO concentrations (Fig. 11). Intensity characterization (Fig. 12) was conducted in the same fashion. After characterizing the sensors off-chip significant oxygen plasma bonding challenges arose. To overcome these challenges the RIE process was optimized and a thin PDMS film was coated above the sensor pattern to facilitate bonding.

Figure 10. On-chip measurements from integrating Presens fiber optic sensors in PDMS devices. n=3, standard deviation=0.4% O₂
Figure 11. Fluorescence lifetime measurements from patterned Pt(II)OEPK film. n=3, sd=0.156

Figure 12. Fluorescence intensity measurements from patterned films
Optimizing the RIE Patterning Process

The etching process was optimized by modifying the RIE parameters (RF power and etching time). Figure 13 compares patterns produced by different etching times. Longer etch times as well as including RF power through inductively coupled plasma (ICP) in addition to RIE produced cleaner patterns. However, these sensor layers still failed to bond to the PDMS devices by oxygen plasma bonding.

![Figure 13. Optimization of RIE patterning process, (A) 7 min RIE etch (B) 9 min RIE etch with ICP. Scale bars are 200 µm.](image)

PDMS Film Coated on PS/Pt(II)OEPK Sensor

To circumvent the bonding issue, a thin layer of PDMS was spin coated onto the patterned PS/Pt(II)OEPK sensor layer. The thickness of the spin coated PDMS film was characterized in order to minimize its effect on the sensitivity of the sensor spots (Fig. D-1). A PDMS thickness of 22 µm can be produced by spinning at 4000 RPM for 30 seconds. Thinner films can be fabricated by spinning at the same speed for longer time periods (~ 5 minutes). The sensitivity of the PDMS coated sensors were evaluated in comparison to sensor films not coated by PDMS. The intensity ratio between 0% O₂ and 19% O₂ was 4.39 for the non-coated film and 4.54 for the coated film. Figure D-2 shows off chip calibration curves for the PDMS coated PS/Pt(II)OEPK film measured by adjusting dissolved oxygen concentrations in a 5 mL reservoir.

These devices were characterized and used in a set of vessel experiments. Figure 14A shows the fluorescent sensor pattern overlaid with a vessel loaded in the artery-chip. An algorithm was written in MATLAB to analyze the fluorescence images of the sensor patterns (Appendix H). The algorithm filters out pixels that are not sensitive to oxygen and measures the intensity ratio
(I₀/I) of the selected pixels versus their intensity under 0% oxygen (I₀). This ratio is then used to calculate oxygen concentration based on the Stern-Volmer equation and produce a 2D concentration map of oxygen (Fig. 14B,C). A set of calibration measurements were conducted to validate the Pt(II)OEPK sensors on chip (Fig. 15). In these measurements, the fluorescence intensity was averaged over all the sensor spots while flowing a gas mixture of known composition in the gas control channel for at least 2 minutes. The sensors were calculated to have a sensitivity of $0.096 \frac{I_0/I}{\%O_2}$ and a sensing resolution of $0.7 \%O_2$.

Figure 14. (A) Fluorescence from Pt(II)OEPK sensor spots overlaid with loaded vessel; 2D Oxygen maps calculated by Stern-Volmer equation in MATLAB script. (B) 100% air applied (C) 20% air applied. Scale bars are 100 µm.

Figure 15. Characterization of Pt(II)OEPK sensor spots integrated into artery device. Intensity measurements were averaged over all sensor spots. n=5, sd=0.441 \%O_2
Sensor Characterization

In addition to characterizing the correlation between the applied %O₂ and the %O₂ calculated by averaging across all of the sensor spots, the deviation between sensor spots was also analyzed. Figure 16 shows the average intensity measured at each spot (99 spots detected) when exposed to nitrogen for 3 separate tests. The standard deviation between the mean intensities at each spot ranged from 5982-7034, while the average intensity ranged from 17674-20808 (based on 16 bit data, max intensity=65536). The deviation between spots corresponds to approximately 34% of measured average intensity for each image. A Bland-Altman analysis [65] (Fig. 17) was also conducted to assess potential biases and the limits of agreement using this sensing strategy. The analysis revealed a bias of -1.03 between the applied and measured O₂ concentration. This could be corrected by subtracting 1.03 from the measured O₂ concentrations and by calibrating the sensors more accurately (i.e. with sodium sulfite based calibration solution). The 95% limits of agreement are approximately 0.5 %O₂ and -2.5 %O₂. After correcting for the bias this analysis indicates that the sensors have an accuracy of ±1.5 %O₂.

![Figure 16. Intensity variation between 99 sensor spots when exposed to nitrogen. n=3, average intensity = 19444, average standard deviation = 6548.](image-url)
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Figure 17. Bland-Altman analysis of sensor agreement. The plot indicates a bias of -1.03 between the measured and applied %O₂. The 95% limits of agreement are -2.54 %O₂ and 0.4 %O₂. All measurements fall within these limits.

Photobleaching Analysis

Photobleaching is the loss of fluorescence by a fluorophore due to photon-induced damage and covalent modification. Transitions between excitation states provide fluorophores with the opportunity to undergo irreversible chemical reactions with other molecules. Photo-stable oxygen sensors based on PtOEPK have been previously developed [47], [50]. Photostability of the PtOEPK dye was analyzed by [46], [47]. Vollmer et al. [47] illuminated the PtOEPK/PS sensor film continuously for 3 hours by exposing the film to a 590 nm LED pulsed at 20 KHz. Over this period an 8 mV change was measured by the Si photodiode used in the custom optical detection set-up; corresponding to 2.75% of the full scale sensing range. Papkovsky et al. [46] compared the photodegradation of Pt porphyrin films with PtOEPK/PS films. After continuous illumination under polychromatic UV light for 18 hours in 22°C air, the PtOEPK films recovered 88% of their initial fluorescence while the Pt porphyrin films recovered only 10%. Therefore the PtOEPK films are 10 times more stable than the Pt porphyrin films.

Optical oxygen sensors were fabricated as described in section 3.2.1. The sensors were validated by measuring the average sensor intensity while changing the DO concentration in steps of approximately 4% O₂ by changing the supply air between 0% and 100% air. 5 consecutive step change measurements were made. In these measurements, 2 frames were imaged per second with a 4 ms exposure time using a 20x objective. Each step increase measurement lasted 12 minutes.
Table 2 shows the intensity averaged over all sensor spots for exposure to air and nitrogen over 3 consecutive step increase measurements. The average intensity decreased by 5.7% (when exposed to air) and 10.9% (exposed to nitrogen) between the last step increase measurement and the first measurement. These values represent a higher photodegradation rate than previously reported for these sensors. The decreased photostability may have been caused by interactions with thin PDMS layer coated above the PtOEPK/PS layer or sensor damage during the RIE patterning process.

Table 2: Intensity measured over 3 consecutive step increases. Intensity averaged over all sensor spots for exposure to air and nitrogen. Measurements are based on 16 bit intensity data (ranges from 0 – 65536).

<table>
<thead>
<tr>
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<th>Step increase 1</th>
<th>Step increase 2</th>
<th>Step increase 1</th>
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<tr>
<td>Air</td>
<td>10949</td>
<td>10594</td>
<td>10325</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>19288</td>
<td>18594</td>
<td>17185</td>
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A challenge with this configuration is that the PS/Pt(II)OEPK sensors cannot detect the oxygen concentration in the flowing solution directly. This was determined by flowing a calibration solution which chemically reduces the oxygen concentration to 0% O₂ (sodium sulfite) over the sensor pattern. Under this condition, the sensors continued to measure the oxygen concentration in the PDMS surrounding the channel, which wasn’t affected by the calibration solution. This observation can be explained by the high solubility of oxygen in PDMS relative to water. Due to this limitation additional fabrication techniques were developed in an attempt to interface the sensors directly with the solution.

3.4.3 Patterning by Flow

The first configuration (permanent bonding) produced uneven coating of the PS/Pt(II)OEPK on all surfaces of the channel (Fig. 18A). The sensitivity of the coated sensor was characterized by measuring the intensity ratio between equilibration with nitrogen (I₀) and air (I₂₀), I₀/I₂₀=5.4. Although this indicates high sensitivity, this configuration could not be used with the artery-chip since the uneven coating (particularly at channel intersections) would damage vessels during loading.

The second configuration is intended to overcome this challenge by reversibly sealing a channel used for coating only. The artery device could then be plasma bonded directly to the coated glass.
slide. Figure 18B outlines the mask designs used for this process. The black design represents the channel used for coating while the green outline represents the artery device subsequently permanently bonded. Figure 18C shows the resulting fluorescence and bright field overlay of the PS/Pt(II)OEPK film coated on the glass slide. From the image it is clear that a layer of PS was coated on the glass slide, however the Pt(II)OEPK dye is not evenly distributed in the film. This was most likely caused by the toluene/Pt(II)OEPK solution being absorbed into the PDMS, depositing mostly PS on the glass slide, as toluene has been found to swell PDMS by a factor of 1.31 [66].

Figure 18. (A) Pt(II)OEPK patterned by flow in permanently bonded device (scale bar 100 µm) (B) Mask design for patterning by flow; green-artery channel network, black-channel reversibly bonded for patterning (scale bar 1 mm) (C) PS/Pt(II)OEPK film unevenly patterned by flow with reversibly sealed channel (scale bar 100 µm)

3.4.4 Patterning by µ-Contact Printing

The resulting fluorescent and bright field dye patterns for µ-contact printing patterns are shown in figure 19A, B. Once again, it was observed that only a small amount of the Pt(II)OEPK dye was deposited on the glass slide. When measuring the fluorescence of the PDMS stamp used in the printing process, it was evident that much of the dye was absorbed by the stamp itself (Fig. 19C). The intensity ratio between exposing the stamp to nitrogen and air was measured to be $I_0/I_{air}=5.70$. 
3.4.5   Patterning by Dissolving Pt(II)OEPK in PDMS

Figure 20A shows the fluorescence image of the posts after the dye is applied before bonding to the rest of the device. The measured intensity ratio for this configuration was $I_0/I_{air}=4.9$. These layers were then plasma bonded to the channel layers of the artery device, immersed in DI water, and placed in the vacuum chamber in order to liquid fill the channels in preparation for artery experiments. When these devices were characterized 3 days later, the Pt(II)OEPK dye appeared to diffuse and spread through the rest of the PDMS device (Fig. 20B). One of these devices was characterized on three separate days (one day apart and one week apart). The resulting calibration measurements are shown in figure 21. This sensor integration technique proved to be unstable and inaccurate as the dye signal decreased with time due to diffusion within the PDMS. Each time the device was used, the exposure had to be increased significantly to sense the Pt(II)OEPK dye (i.e. 100 ms, 200 ms, 800 ms). This effect also reduced the intensity ratio with time (i.e. decreased sensitivity) ($I_0/I_{air}=4.50$ day 1; $I_0/I_{air}=3.81$ day 2; $I_0/I_{air}=2.16$ day 7).
Figure 21. Characterization of artery devices doped with Pt(II)OEPK. n=3, sd=0.696 %O₂.
Chapter 4
Cerebral Vessel Experiments

4.1 Protocol

The protocol used for vessel experiments was adapted from previously developed techniques [20], [31]. Olfactory artery segments were isolated from C57 black 6 mice (Charles River, Montreal Canada) by collaborating researchers in the Department of Physiology (primarily by Dr. Andrew Levy as well as Meghan Sauve). Microfluidic devices were loaded into the manifold described in Section 3.3. Devices were then flushed with 1% Bovine serum albumin (BSA) followed by MOPS buffer. Artery segments were loaded into the devices as described in [20]. Transmural pressure was maintained at 45 mmHg via a hydrostatic head applied to the perfusion line. The superfusion flow rate was maintained at 0.5 µL/min, by applying a constant pressure of 5 mmHg to the 6 manifold wells via a digital pressure regulator (MarshBellofram). The vessels were stabilized at the organ bath via 8 fixation points, each at 45 mmHg below atmospheric pressure. Once pressurized on-chip, vessels were gradually heated to 37.5°C in steps of 3°C over 5 minutes. PE dose response curves were measured from heated vessels to evaluate vessel viability. Different PE concentrations in MOPS buffer (0, 0.1, 1, 2.5, 5, and 10 µM) were premixed into each manifold well to perform the dose response curves. Artery diameter was automatically measured via custom MATLAB software as previously reported [20].

Vessels exhibiting healthy PE dose response curves would have been exposed graded levels of hypoxia by adjusting the ratio of air to N₂ supplied to the gas control line. After each healthy dose response the oxygen concentration supplied would be lowered by 5% O₂ and allowed to equilibrate with the buffer solution for 5 minutes. Unfortunately, due to difficulties routinely reproducing healthy PE dose response curves, this protocol was not performed.

4.2 Results

The characterized devices described in Chapters 2 and 3 were used in a series of vessel experiments over a six month period. Between February and July 2012 approximately 45 days of vessel experiments were conducted with 2-3 vessels loaded per day of experiments. Throughout all the experiments only 4 healthy PE dose response curves were observed (from measurements
taken in mid-March and early April). Vessels that did not show any visible constriction when exposed to 10 µM PE were excluded from the analysis. Figure 22 compares the PE dose response curves measured in these devices with dose response curves previously measured with the same platform [31]. The yield for PE dose responses measured in this work is approximately 9% (over all experiments) while in Sanjesh Yasotharan’s work it was approximately 88% (after 1 month training period) [31]. The difference in yield can be attributed to the manual skill level required to perform the experiments and fabricate the devices. The primary challenge throughout the vessel testing period was loading and pressurizing the artery segments on-chip. Many arteries were not loaded smoothly; they were damaged by stretching and bending during loading or not pressurized to 45 mmHg (not held open by fixation points). The design changes made during the testing period only affected the control gas channels, so should not have had any effect on the vessel loading technique. Due to the low experimental yield during this period the following steps were taken to improve the experimental technique.

Figure 22. PE dose response curves for cerebral vessels in the artery-on-a-chip platform. Chip design shown in Fig. A-2.
4.2.1 Validating Channel Height

The exact height of the loading channel has been found to significantly affect the ability to load vessels smoothly in the artery-chip. Based on experience, the ideal channel height for loading cerebral vessels is 65-75 µm. In channels below 65 µm, the vessels tend to get trapped before the organ bath and require a relatively large force (applied manually by withdrawing on a 3 mL syringe) to pull them farther up the channel. This usually results in the vessel being damaged by shear forces or by being pulled past the organ bath. In the case for which the channel height is above 75 µm, the vessels tend to move too easily past the fixation points. This makes it difficult to localize the vessels in the organ bath manually.

After a series of unsuccessful loading attempts, the heights of the masters being used for molding were measured. Figure E-1 shows the number of masters measured within each height range. Unfortunately the masters used to mold devices in earlier experiments were approximately 60 µm. This was unexpected, as these masters were fabricated using recipes that were validated to produce heights within the 65 µm to 75 µm range. Factors which can affect the reproducibility of the SU-8 fabrication recipes include temperature and humidity in the cleanroom, as well as how long the bottle of SU-8 photoresist has been open and exposed to ambient air. Since it is difficult to control these environmental factors in our facility, a series of fabrication rounds were necessary to produce multi-height masters that were well aligned and fabricated to the required channel height. Vessels loaded more smoothly into artery devices molded with these masters.

4.2.2 Incorporating on-chip temperature electrodes

Local temperature has been found to significantly affect vessel responsiveness to PE once loaded in the artery chip. In several experiments, it was found that the vessel would respond to a 10 µM PE “wake up” dose at a certain temperature but not at others. If the temperature at the sapphire disk was raised too high (usually above 39°C), the vessel would slightly constrict and not respond to PE. At temperatures that were too low (usually below 37°C), the vessel would not respond or weakly respond to PE. The artery chip configuration originally used did not include on-chip temperature sensors to validate the vessel temperature. Temperature in this configuration is controlled with a heating element and thermistor that are attached to a sapphire disk, which is placed on top of the PDMS device above the organ bath. Due to the low thermal conductivity of PDMS (0.15 W/m K) the temperature at the organ bath has been found to be 1-5°C below the
temperature of the sapphire disk after a 20 minute equilibration time. A computational model previously developed displaying the temperature profile in the PDMS device is shown in figure E-2A [20]. The exact temperature at the organ bath depends on the thickness of the molded PDMS, the orientation of the sapphire disk and heating element around the organ bath, and the ambient temperature.

To circumvent the uncertainty regarding vessel temperature, previously developed on-chip gold micro-electrodes were integrated into the fabrication process for on-chip sensing [31]. An image of the electrodes in a similar device is shown in figure E-2B. Temperature is sensed by measuring the resistance in the electrodes, which linearly relates to temperature. Resistance is determined by applying a constant current through the micro-resistors (5.085 mA) and simultaneously measuring the voltage drop across them. In the control scheme, the input temperature is read from the on-chip sensor instead of the sapphire disk temperature. Figure E-2C shows a graph comparing the sapphire temperature with the temperature measured by the on-chip electrodes.

4.2.3 Evaluation of PDMS Deflection

The only major difference between the devices used in this work and the devices used in previous work on cerebral vessels [31] is the additional gas channel network. The gas network was used to control dissolved oxygen concentrations in the buffer solution as explained in Chapter 2. This gas channel network covers the vessel inspection area, creating a thin PDMS membrane above the organ bath. The PDMS membrane is intended to maximize mass transfer between the gas channel and the organ bath. Its dimensions are 170 μm x 730 μm, with a thickness of 150 μm. It was suggested that the deflection of this membrane may be a factor affecting the loading technique. An analytical model was used to estimate the deflection of the PDMS membrane above the organ bath [67]. Figure E-3A shows the estimated PDMS deflection of the membrane. The negative pressure applied by withdrawing on the perfusion syringe during loading is estimated to apply a negative pressure at the organ bath of 5 – 10 kPa. Based on the model this would deflect the PDMS membrane by 5-15 μm into the organ bath. A design was developed without the PDMS membrane above the organ bath. In this design the control gas channel separates before the organ bath figure E3B. This design was fabricated and used in the last round of vessel experiments. Unfortunately the vessel loading yield did not immediately
improve with this design change. However, further experiments with this design may produce improved experimental yields.
Chapter 5
Conclusions and Recommendations

5.1 Summary of Developed Platform

A microfluidic platform for controlling and measuring dissolved oxygen concentrations within the microenvironment of an isolated small artery segment was developed. Dissolved gas concentrations in the platform are controlled by permeation through the substrate material, PDMS, as described in Section 2.2. Since PDMS is permeable to a variety of gases the methodology can be extended to control dissolved gas concentrations of other physiologically relevant gases such as CO$_2$ and NO. 3D numerical models (COMSOL) were developed to validate the dissolved gas control scheme. The estimated required equilibration length was 2.5 cm and the estimated equilibration time was 30 s. These parameters can be further optimized by reducing the thickness of the PDMS membranes separating the gas control channels and the buffer channels.

The on-chip dissolved gas control scheme was validated with on-chip oxygen sensors integrated into the microfluidic device. Oxygen sensors were based on fluorescence quenching of Pt(II)OEPK embedded in PS. Several integration techniques were pursued including patterning by RIE, µ-contact printing, flow through a microfluidic channel, and dissolving the dye in PDMS. Patterning by RIE produced the most favourable results. When the other techniques were attempted, it was observed that most of the Pt(II)OEPK dye absorbed into the PDMS. This effect made patterning using a PDMS substrate not feasible. Patterning by RIE produced clean Pt(II)OEPK/PS patterns on glass slides that could be reliably used for oxygen sensing. The main challenge using these patterns is that they did not bond to PDMS devices due incomplete etching of the PS layer. To overcome this challenge, a thin layer of PDMS was spin coated onto the patterned sensors. This was the only configuration for which the oxygen sensors could be integrated into the artery device. These devices were used to validate the on-chip gas control scheme as described in Section 3.4.2. The spin coated PDMS layer used to facilitate bonding limits the ability of the sensors to spatially resolve the dissolved oxygen concentration in the buffer channel. A direct interface between the patterned oxygen sensors and the buffer solution is required to accurately and spatially resolve the oxygen concentration field in the organ bath.
The developed platform is capable of exposing isolated vessels segment to controlled dissolved gas concentrations by manipulating the dissolved gas concentration field on the abluminal side of the vessel within approximately 1 minute. This configuration is significantly different from the physiological configuration in which oxygen is supplied via the blood supply on the luminal side of the vessel and diffuses outward to metabolically active tissues. Active tissues can decrease the oxygen concentration surrounding the microvasculature instantaneously [1]. The blood supply also consists of erythrocytes (approximately 45% by volume) which greatly increase the oxygen carrying capacity in blood. In the blood plasma oxygen solubility is \(0.023 \times 10^{-5} \text{ m}^3_\text{O}_2 / (\text{m}^3_\text{blood Pa})\) while the hemoglobin oxygen binding capacity is \(1.37 \text{ mLO}_2 / (g_\text{hemoglobin})\), increasing the blood oxygen carrying capacity by approximately 70 times. In the experimental configuration used in this work no oxygen carriers were used. The buffer solution used had an oxygen solubility of \(0.027 \times 10^{-5} \text{ m}^3_\text{O}_2 / (\text{m}^3_\text{water Pa})\), similar to that of plasma and extracellular fluid. The developed platform does not completely mimic the in vivo conditions. However it is comparable to conventional myography based vessel investigation techniques [8], [9] described in Section 1.1.

5.2 Further Studies with Platform

To validate the ability of the device to expose viable microvascular segments to hypoxia in vitro, PE dose response curves can be measured for cerebral olfactory arteries from mice under graded hypoxic exposures. Vessels will be exposed to increasing PE concentrations (0.1, 1, 2.5, 5, 10 \(\mu\)M PE in MOPS) while under normoxia (21% \(O_2\)) and graded hypoxia (5% - 15% \(O_2\)) followed by another dose response under normoxia. The second normoxic dose response is intended to show recovery of the initial dose response of the vessel. In a similar procedure, Bartlett et al. [52] demonstrated that the PE response is attenuated during graded hypoxic exposures in a wire myograph.

Other potential studies with the platform include hypoxic preconditioning experiments [68], exploring the hypoxia inducible factor (HIF) pathway [69], and analyzing oxygen transport and consumption within the vascular wall [26], [70]. The developed platform provides the unique opportunity to study entire isolated functional blood vessels in vitro under dynamic hypoxic conditions. The advantage of the platform for hypoxic preconditioning experiments is that vessel can be quickly exposed to different dissolved gas concentrations and vessel viability parameters
can be tested at any point in the experiment. For HIF studies, a recently developed staining technique [31] would allow for vessel segments to be stained for proteins related to HIF. These proteins could then be tracked as the vessel segment is exposed to dynamic hypoxic conditions. The advantage of the platform for studying dissolved oxygen transport and consumption is that the vessel is isolated from surrounding tissue and studied in a completely controlled microenvironment.

5.3 FEA Simulation to Analyze Feasibility of Measuring Vessel Metabolism On-chip

One of the initial goals of this project was to use the developed platform to study oxygen transport and consumption in the vascular wall. A finite element model was developed in COMSOL to predict the feasibility of measuring physiological vessel oxygen consumption within a PDMS based artery-on-a-chip device. The model incorporates the different diffusivities and solubilities of oxygen in the substrate material (PDMS), MOPS buffer solution, and the vascular wall. Mesh independence for the model is shown in table F-2. Vascular oxygen transport properties were taken from measurements previously reported in literature (table F-3). The literature values for estimated oxygen consumption varied greatly, between 4.92×10⁻⁴ and 3.9 mlO₂·cm⁻³ tissue⁻¹ min⁻¹ [28], [71]. Table F-4 summarizes the measured values from literature including vascular bed measured and technique used. The average of the reviewed consumption rates is 0.709 mlO₂·cm⁻³ tissue⁻¹ min⁻¹ while the standard deviation is 1.416 mlO₂·cm⁻³ tissue⁻¹ min⁻¹. This deviation was found to significantly affect the feasibility of measuring oxygen consumption rates in a PDMS device.

The other model parameters studied were the boundary conditions, geometry of the vessel and the device, and the velocity field of the superfusing solution. Parametric sweeps were used to study the effects of different consumption rates, superfusion flow rates, vessel locations and geometries, and fabrication techniques on the oxygen concentration profile surrounding the vessel. The values used in the model are summarized in table F-5. Of the parameters studied, only the oxygen consumption rate significantly affects the feasibility of measuring oxygen consumption in a PDMS chip.

The 3D oxygen profile surrounding the vessel is shown in figure 23A. The oxygen profiles across the center of the channel and along the bottom of the channel (sensor layer) are shown in
Fig. F-1 and Fig. 23A respectively. Based on this configuration, consumption values in the range of 0.01-0.51 mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$ can be detected in a PDMS microfluidic device. Consumption rates below 0.01 mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$ cannot be detected in PDMS devices since the diffusive oxygen flux from the substrate material resupplies oxygen to the vessel wall at much higher rate than it is consumed. Consumption rates higher than 0.51 mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$ create severe hypoxic conditions (<5% O$_2$) below the vessel that will impact viability.

Unfortunately, this consumption range does not include a large set of consumption rates measured in the literature (i.e. < 0.01 mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$). A second model was then developed beyond the current fabrication constraints to determine a configuration for which lower consumption rates could be measured directly (Fig. 23B). In this model the vessel segment is subjected to a diffusive environment with no flux at the boundaries. The transient oxygen profile is used to determine the consumption rate as the vessel is the only source/sink in the model. These conditions resemble an in vitro configuration of the experiments conducted by Golub et al. [30] in which transient trans-luminal oxygen profiles were measured in arterioles with arrested blood flow. Potential non-gas permeable substrate materials for such a device are silicon, glass, or polystyrene, and other non-permeable polymers. Potential fabrication techniques include wet and dry etching, and hot embossing. This configuration theoretically allows consumption rates on the order of 0.001-0.011 mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$ to be measured in approximately 3 minutes.
Figure 23. Model of vessel consumption on-chip. 3D profile of oxygen surrounding vessel followed by consumption rate sweep (mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$) of oxygen profile below vessel. (A) Steady state model in PDMS device (B) Transient diffusive model with no flux boundary condition.
5.4 Conclusions

A microfluidic platform was developed to control and measure dissolved oxygen within the microenvironment of isolated blood vessels. Oxygen was controlled by permeation through the substrate material, PDMS, via a 3D gas and liquid channel network. On-chip sensors based on collisional quenching of Pt(II)OEPK embedded in PS were patterned by RIE and integrated into the devices by O₂ plasma bonding. DO control in the device was validated by a 3D computational model and on-chip fluorescence intensity measurements using the integrated sensors. Approximately 45 days of vessel experiments were performed with this platform. Due to challenges achieving healthy PE dose responses from cerebral vessels, experiments with the platform did not produce physiologically relevant information. Further experiments with the platform require optimization of the technique for studying small blood vessels previously developed [31], [72] and more time to be invested in gaining the skills required for using the technique. For studying oxygen transport and consumption of small blood vessels a separate platform should be developed using a non-permeating substrate material and a sensing technique compatible with the fabrication of this platform should be developed.

Recommendations for Studying Oxygen Transport and Consumption of Small Blood Vessels

Potential substrate materials include stiff polymers such as cyclic olefin copolymer (COC), polyurethane (PU), and polystyrene (PS), as well as silicon wafers. All of these materials are at least 360 times less permeable to oxygen than PDMS [73]. Stiff polymer microfluidic devices have previously been fabricated by hot-embossing [74–76]. A challenge with hot-embossing for this specific project is reliably fabricating devices with dense features and the required aspect ratio (smallest feature in the design is 25 μm wide by 75 μm tall) [77]. Silicon microfluidic devices have previous been fabricated by deep reactive ion etching (DRIE) [78]. Significant challenges implementing a silicon based approach to the artery project include the opacity of the devices and the number of devices that can be made accessible. As silicon is opaque, a custom optical configuration would need to be developed to image and load vessels in these devices. Also, due to the fabrication cost and the fabrication equipment not being directly accessible at the university, only a limited number of devices could be fabricated over extended periods of time (approximately 1-2 devices over six months), limiting the ability to modify designs or replace damaged devices.
Moving forward, the first steps are to develop an artery-chip platform using an oxygen impermeable substrate material (stiff polymer or silicon) which can reproduce vessel viability responses measured in PDMS devices. In conjunction with showing vessel viability in such a device, an accurate oxygen sensing strategy should be developed that is compatible with the device fabrication. The sensors developed in this work based on RIE patterning of PtOEPK/PS films are a good starting point for such sensors. The patterned films worked well when characterized outside of PDMS devices. A bonding technique that allows for the patterned sensors to directly contact the solution in the vessel microenvironment must be developed.

**Potential Uses for the Device**

A micro device capable of measuring oxygen transport and consumption in small blood vessels (specifically resistance arteries) could be an important tool in drug screening and development. Many pharmaceuticals administered daily to treat ischemia, inflammation, and various vascular disorders have vasoactive effects. Two recent studies have shown that vasodilators (prostaglandin E1[79] and Verapamil [80]) increase tissue oxygenation not only by dilating arteries but also by decreasing vessel wall O$_2$ consumption. A device capable of measuring vessel O$_2$ consumption *in vitro* could provide more accurate measurements of vessel oxygen consumption and make such screens more widely available in industry and clinical settings. Clinically such a device could be used as a diagnosis tool or to optimize treatment options on a patient by patient basis by analyzing resistance arteries isolated from patient biopsies.
References


Appendices

Appendix A  Regulation of Vascular Tone

Vascular tone is modulated by changes in intracellular \( \text{Ca}^{2+} \) and changes in contractile protein sensitivity to \( \text{Ca}^{2+} \). Cytosolic \( \text{Ca}^{2+} \) ions bind to calmodulin forming the \( \text{Ca}^{2+} \)-calmodulin complex which activates the myosin light chain kinase (MLCK) enzyme. MLCK phosphorylates the light chain component of the myosin head creating a crossbridge formation between myosin and actin. The myosin head then rotates generating prolonged tension in the vascular smooth muscle (VSM) cell. VSM cells relax by the removal of the phosphate group from the light chain by myosin light chain phosphatase (MLCP). Decreasing intracellular \( \text{Ca}^{2+} \) concentrations lead to vasodilation by MLCP activity dominating over MLCK activity. Activation and inhibition of MLCP and MLCK also provide a \( \text{Ca}^{2+} \) independent pathway for regulating vascular tone [1].

Intracellular \( \text{Ca}^{2+} \) is determined by vascular ion channels. Potassium channels decrease membrane potential while calcium channels depolarize VSM cells. The four primary types of \( K^+ \) channels in VSM cells are inward rectifier (\( K_{ir} \)), ATP- dependant (\( K_{ATP} \)), voltage dependent (\( K_V \)), and calcium dependent (\( K_{Ca} \)) channels. \( K_{ir} \) and \( K_{ATP} \) channels play a role in metabolic hyperaemia as \( K_{ir} \) channel open probability increases with extracellular \( K^+ \) (a function of tissue metabolic rate) and \( K_{ATP} \) open probability increases with low ATP concentrations (a function of severe ischemia). \( K_{Ca} \) and \( K_V \) repolarize the VSM cell after depolarization and prevent excessive activation. Voltage sensitive calcium channels (VSCC) and calcium conducting TRP channels are responsible for \( \text{Ca}^{2+} \) influx into the VSM cell. The open probability of VSCC increases with increasing membrane potential regardless of whether an action potential is initiated. Vessel tone is therefore a graded function of membrane potential in many vessels. TRP channels are receptor-operated channels (ROCs) that conduct \( \text{Ca}^{2+} \) and \( Na^+ \) when activated by diacylglycerol (DAG). The cation influx contributes to membrane depolarization and increase cytosolic \( \text{Ca}^{2+} \). Cytosolic \( \text{Ca}^{2+} \) is also modulated by \( \text{Ca}^{2+} \) release from the sarcoplasmic reticulum (SR) through \( IP_3 \)- \( \text{Ca}^{2+} \) release channels and \( \text{Ca}^{2+} \) sensitive ryanodine receptors [1].

Myosin light chain sensitivity to \( \text{Ca}^{2+} \) is modulated by inhibiting or activating MLCP and MLCK. Protein kinase A (PKA) and protein kinase G (PKG) are two downstream vasodilating signaling molecules that inhibit MLCK thereby desensitizing the myosin light chain to \( \text{Ca}^{2+} \) [1].
Similarly S1P has been found to play a role in the myogenic response by inhibiting MLCP leading to contraction [81].
Appendix B Fabrication Sequence

Figure B-1. Master fabrication sequence
Figure B-2. Mask designs used in master fabrication
Figure B-3. PDMS device fabrication sequence
Appendix C  Experimental Setup

Figure  C-1. Custom aluminum manifold. Adapted from [31]

Figure  C-2. Valve actuation schematic. Adapted from [31]
Appendix D  Characterization of PDMS Film Coated on Pt(II)OEPK Patterns

Figure D-1. Thickness of PDMS spin coated onto patterned Pt(II)OEPK sensor layer

Figure D-2. Characterization of Pt(II)OEPK patterns coated with PDMS film
Appendix E  Steps Taken to Improve Experimental Yield

Figure E-1. Height distribution of fabricated masters

Figure E-2. Temperature control adapted from [31], (A) Computational model of temperature field in artery chip (B) Gold electrodes embedded in artery chip for temperature sensing (scale bar 1 mm) (C) Temperatures measured on sapphire disk and on-chip
Figure E-3. Evaluation of PDMS deflection. (A) Analytical estimation of PDMS membrane deflection vs. applied pressure [57] (B) Design without PDMS membrane above organ bath (green - control gas, black - artery channel)
**Appendix F  COMSOL Modeling**

**Table 1: Mesh independence- 3D gas transport model**

<table>
<thead>
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<th>property</th>
<th>mesh1</th>
<th>mesh2</th>
<th>% difference</th>
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<tr>
<td>mesh elements</td>
<td>264650</td>
<td>1792500</td>
<td>-</td>
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<tr>
<td>velocity (m/s)</td>
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<td>0.016341615</td>
<td>0.562774112</td>
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<tr>
<td>non-dimensional concentration</td>
<td>0.647577646</td>
<td>0.650972553</td>
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**Table 2: Mesh independence- Consumption model**

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<td>mesh elements</td>
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<td>1473480</td>
<td>-</td>
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<td>velocity (m/s)</td>
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<td>oxygen concentration %O₂</td>
<td>2.736692249</td>
<td>2.699162925</td>
<td>1.371338864</td>
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**Table 3. Literature values for oxygen diffusivity and solubility in vascular wall**

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<tr>
<th>Reference</th>
<th>Diffusivity ($D_{O_2}$) $cm^2/s$</th>
<th>Solubility $mlO_2/cm^3_{tissue} \times mmHg$</th>
<th>Vessel type</th>
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</thead>
<tbody>
<tr>
<td><em>Yaegashi et al.</em> [71]</td>
<td>0.0000104</td>
<td></td>
<td>Rat mesentery</td>
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<tr>
<td><em>Tsai et al.</em> [28]</td>
<td>0.000017</td>
<td>0.0000214</td>
<td>Rat mesentery</td>
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<tr>
<td><em>Vadapalli et al.</em> [82]</td>
<td>0.0000242</td>
<td>0.000033</td>
<td>Hamster retractor muscle</td>
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### Table 4. Review of reported vascular oxygen consumption rates

<table>
<thead>
<tr>
<th>Reference</th>
<th>Consumption rate ( \frac{mlO_2}{cm^3_{tissue} \times min} )</th>
<th>Vascular Bed</th>
<th>Technique</th>
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</thead>
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<tr>
<td>Duling et al. [83]</td>
<td>1.68</td>
<td>cat pial microvessels</td>
<td>Recessed tip oxygen microelectodes</td>
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<td>Yaegashi et al. [71]</td>
<td>0.000492</td>
<td>Rat mesentery</td>
<td>Oxygen quenching of ruthenium based dye in exteriorized tissue</td>
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<td>Tsai et al. [28]</td>
<td>3.9</td>
<td>Rat mesentery</td>
<td>Oxygen quenching of palladium based dye in exteriorized tissue</td>
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<tr>
<td>Vadapalli et al. [82]</td>
<td>High limit - 0.096 Low limit – 0.000078</td>
<td>Estimated based on previously published in vivo measurements</td>
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<td>Shiabata et al. [84]</td>
<td>1.08 (10^{-4}) M papaverine- 0.54</td>
<td>Rat cremaster muscle</td>
<td>Phosphorescence quenching microscopy</td>
</tr>
<tr>
<td>Ye et al. [85]</td>
<td>Control – 0.0468 Constricted – 0.0820 (3 nM noradrenaline or 0.1 nM vasopressin)</td>
<td>Rat hindlimb, kidney, intestine, and mesentery</td>
<td>Oxygen uptake rates were calculated from atriovenous oxygen differences measured by clark type electrodes in perfusing solution</td>
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<tr>
<td>Barron et al. [86]</td>
<td>Control – 0.0303 Constricted – 0.0315 (80 mM KCl, 100 µM norepinephrine)</td>
<td>Porcine carotid artery</td>
<td>Oxygen depletion measured in sealed chamber over time</td>
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<tr>
<td>Golub et al.[87]</td>
<td>0.0036</td>
<td>Rat mesentery</td>
<td>Phosphorescence quenching microscopy of exteriorized tissue</td>
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<td>Golub et al. [30]</td>
<td>0.001179 Max possible – 0.0178</td>
<td>Rat mesentery</td>
<td>Calculated the oxygen disappearance curve measured in an arteriole after rapid arrest of blood flow</td>
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Table 5. Parameters used in finite element model

<table>
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<th>Parameter</th>
<th>Value</th>
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</thead>
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<tr>
<td>Superfusion flow rate</td>
<td>0-0.3 µL/min</td>
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<tr>
<td>Diffusivity in vessel</td>
<td>$1.7 \times 10^{-5}$ cm$^2$/s</td>
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<tr>
<td>Solubility in Vessel Wall</td>
<td>$2.14 \times 10^{-5}$ mlO$_2$ x cm$^{-3}$ x mmHg$^{-1}$</td>
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<td>Vessel position in channel</td>
<td>2-14 µm from bottom of channel</td>
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<tr>
<td>Vessel geometry (constriction, constant cross</td>
<td>130-110 µm diameter;</td>
</tr>
<tr>
<td>sectional area)</td>
<td>10-12 µm wall thickness</td>
</tr>
<tr>
<td>Consumption sweep</td>
<td>0.001-0.51 mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$</td>
</tr>
<tr>
<td>Channel height</td>
<td>100 µm</td>
</tr>
</tbody>
</table>

Figure F-1. Steady state consumption rate sweep of oxygen profile across middle of vessel in PDMS device (mlO$_2$·cm$^{-3}$·tissue·min$^{-1}$).
Appendix G  MATLAB Code for Analytical Mass Transfer Solution

% code for solving PDE in tubular membrane
% mass transfer of dissolved gasses through tubular membrane tang 1976 [59]
% PDE is \( \frac{d^2}{dR^2}U + \frac{1}{R}\frac{d}{dR}U = (1-R^2)\frac{d}{dX}U \)
% B.C. 1 : U=1 @ X=0
% B.C. 2 : dU/dR=0 @ R=0
% B.C. 3 : dU/dR=-EU @ R=1
% where E=(DmSm)/(DlSl*ln(ro/ri))
% pde sovled by crank nicolson [60]

% problem parameters
% Dm = membrane diffusivity
% Sm = membrane solubility
% Dl = liquid diffusivity
% Sl = liquid solubility
% ro = outer radius
% ri = inner radius
% E=(DmSm)/(DlSl*ln(ro/ri))

% parameters in numerical solution
% h = mesh size in R
% k = mesh size in X
% Xmax= maximum range of X; 0<X<Xmax
% Rmax= 1 maximum range of R; 0<rR<1
% M= Rsteps = number of steps in R (coloumns)
% Xsteps = number of steps in X (rows)
% VECTORS
% R(M) radius vector
% Am parameter vector
% Bm parameter vector
% Cm parameter vector
% Dm parameter vector

% physical parameters

diffm = 0.000034;
solm = 0.18;
diffl = 0.0000197;
soll = 0.031;
ri=0.015 %cm
ro=0.025 %cm
Q = 7.5; %mL/hour
height = 0.015; %cm
width = 0.015; %cm
Vave = Q/(60*60*height*width); %cm/s

% model parameters

Xmax=1;
Rmax=1;
Rsteps=100;
Xsteps=100;

M=Rsteps;
N=Xsteps;

h=Rmax/Rsteps;
k=Xmax/Xsteps;

Am=zeros(M+1,1);
Bm=zeros(M+1,1);
Cm=zeros(M+1,1);
Dm=zeros(M+1,N+1);
beta=zeros(M+1,1);
alpha=zeros(M+1,N+1);
R=zeros(M+1,1);
X=zeros(N+1,1);

E=(diffm*solm)/(diffl*soll*log(ro/ri))

U = zeros(M+1,N+1);
Umean = zeros(1,N+1); % Um in paper
sigmam = zeros(1,N+1); % cup mixing partial pressure
output1 = zeros(M+1,2);

for i = 0 : M
R(i+1)= h*i;
end
R;
for i = 0 : N
X(i+1)= k*i;
end

% BOUNDARY CONDITIONS

% B.C. 1 (feed condition)
U(:,1)=1;
for n=1 : N

% B.C. 2
Bm(1)=(1/k)+2/(h^2);
Cm(1)=-2/(h^2);
Dm(1,n)=(1/k-2/(h^2))*U(1,n)+(2/(h^2))*U(2,n);

% B.C. 3
Am(M+1)=-1.0;
Bm(M+1)=1+E*h+E*(h^2)/2;
Dm(M+1,n)=0;

% algorithm parameters
% based on Crank-Nicolson method
% refer to "Methods for the numerical solutions of
% partial differential equations", Von Rosenberg, 1969 (pp 22)

for i = 2 : M
%p=R(i);
Am(i)= 1/(2*(h^2))-1/(4*R(i)*h);
Bm(i) = -1/(h^2)-(1-(R(i)^2))/k;
Cm(i) = 1/(2*(h^2)) + 1/(4*R(i)*h);
Dm(i,n) = (1/(4*R(i)*h)-1/(2*(h^2)))*U(i-1,n)+(1/(h^2)-(1-(R(i)^2))/k)*U(i,n)-(1/(2*(h^2))+1/(4*R(i)*h))*U(i+1,n));
end
Am;
Bm;
Cm;
Dm;

% algorithm
% based on thomas algorithm for tridiagonal matrix
% refer to appendix A in [60]

beta(1)=Bm(1);
alpha(1,n)=Dm(1,n)/Bm(1);
for i=2 : M+1
    beta(i)=Bm(i)-(Am(i)*Cm(i-1))/beta(i-1);
    alpha(i,n)=(Dm(i,n)-Am(i)*alpha(i-1,n))/beta(i);
end
U(M+1,n+1)=alpha(M+1,n);
for i=M : -1 : 1
    U(i,n+1)=alpha(i,n)-(Cm(i)*U(i+1,n+1))/beta(i);
    % i, n, alpha(i,n), Cm(i), U(i+1,n+1), beta(i)
end
end

xreal=(X*2*Vave*ri^2)/(diffl);  % real x, depends on how reynolds # is defined
X;
R;
U;

% calculates mean partial pressure
for i = 1 ; N + 1
    sum1=0;
    for j = 2 : M
        sum1 = sum1 + U(j,i)*(R(j)-R(j)^3);
    end
    Umean(i)= 4*(h/2)*(U(1,i)*(R(1)-R(1)^3)+2*sum1+U(M+1,i)*(R(M+1)-R(M+1)^3));
end
Umean;
sigmam = 1-Umean;  % cup mixing partial pressure
figure
plot(xreal,sigmam);  % To check plots from paper use this function

figure
%surf(R,X,U);  % surface plot of U as a function of R and X
%figure;
%surf(xreal,R,U); %surface plot of U vs. real x values (based off X)

middle=U(1,:);
%figure;
%plot(xreal,1-middle); %plots U in the center of channel vs xreal

%outputs 'output1' to text file
output1(:,1)=xreal;
output1(:,2)=1-middle;
dlmwrite('output1.txt', output1, 'delimiter', '	', 'newline', 'pc');

output2(:,1)=xreal;
output2(:,2)=sigmam;
dlmwrite('output2top.txt', output2, 'delimiter', '	', 'newline', 'pc');

%calculates average U across R
av=zeros(M+1,1);
for n=1 : N+1
    sum = 0;
    for i=1 : M+1
        sum = sum+U(i,n);
    end
    av(n)=sum/(M+1);
end

%figure;
%plot(xreal,av) %plots average U across R vs xreal
Appendix H  MATLAB Code for Calculating Oxygen Profiles from Pt(II)OEPK Intensity Images

**Pixel Map Function**

```matlab
function [pixel_map, I0, I20, Ksv] = calibrate( cal0, cal20, diff );
%pixelmap : determines pixel map of pixels that change intensity between
%(cal0) 0% and (cal20) 20% O2 with a minimum difference defined by diff
% returns pixel map, I0, I20, and Ksv

max_intensity=2^16; % # of bits in data
pixel_map=find((cal0-cal20)>diff); % finds pixel map for which
diff between cal0 and cal20 is significant

map_size=size(pixel_map) % # of pixels used in map
figure
imshow(cal0), title('cal0'),
figure
imshow(cal20), title('cal20'),

image_size=size(cal0) % # of pixels in original
image

selected_pixels=zeros(size(cal0));
selected_pixels(pixel_map)=max_intensity-1; % image showing selected
pixels

figure
imshow(selected_pixels), title('selectedpixels'),
figure
imhist(cal0(pixel_map)), title('histcal0'),
figure
imhist(cal20(pixel_map)), title('histcal20'),

I0=mean(cal0(pixel_map)) % calculates I0
I20=mean(cal20(pixel_map)) % calculates I20
Ksv=(I0/I20-1)/20.5 % calculates Ksv
I0_vs_I20=I0/I20 % calculates I0/I20

end
```
Analysis Function

function [ data ] = analysis1( pixel_map, I0, Ksv )
% determines average intensity, and perO2 for pixel map of data set
% given pixel_map, I0, Ksv from calibration image

for k=1:1441 %k loops from 1 to max # of frames
temp=num2str(k);

while(size(temp)<4)
    temp=strcat('0',temp);
end

file=strcat('step Increase3_seq_ap3_nd_cropt',temp,'c1.tif'); % filename
current_image=imread(file);

I0vsI_image=zeros(256);
I0vsI_image(pixel_map)=I0./current_image(pixel_map);

ave(k)=mean(current_image(pixel_map)); % calc ave intensity

perO2=((I0./ave)-1)./Ksv; % calc perO2

j=1:1441;
data=[j ; j.*12./1441 ; ave ; perO2];
data=data'; %returns one matrix with I and perO2 averages indexed by frame#
xlswrite('data.xls',data);
end

O2 Image Function

function [ perO2_image ] = O2image( file, pixel_map, I0, Ksv )
% determines spatially resolved O2 image based on input file, pixel_map, %I0 and ksv

current_image=imread(file); % opens file
perO2_image=zeros(256);
perO2_image(pixel_map)=(I0./current_image(pixel_map)-1)./Ksv; % calculates O2 image

figure
colormap(hot) % color map
imagesc(perO2_image), title('O2 image') % displays image

axis image
axis off
end